

ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE
FISCAL YEAR 1979
VOLUME II

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

ANNUAL REPORT

OF

PROGRAM ACTIVITIES

00 NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE

FISCAL YEAR 1979

Volume II

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ANNUAL REPORT

October 1, 1978 through September 30, 1979

National Institute of Neurological and Communicative Disorders and Stroke

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National Institute of Neurological and
Communicative Disorders and Stroke
October 1, 1978 through September 30, 1979

Neurosciences research, ranging from basic neurobiologic studies to clinical applications of new therapeutic agents, continued to flourish within the Institute's Intramural Research Program during FY 1979. As evidenced by the record of scientific publications or the considered judgement of peer evaluators, IRP made important contributions to the quickening pace of research into the prevention and cure of neurologic and communicative disorders. Nevertheless, problems now looming on the horizon threaten the Program's creative vigor and ultimately its very existence. Before taking note of the important scientific accomplishments of IRP scientists, it may be of value to review certain of the more exigent administrative matters now confronting the Program.

Changes in several key positions occurred during the past year. Dr. Cosimo Ajmone Marsan retired to an academic post after 25 years of distinguished service as Chief of the Electroencephalography and Clinical Neurophysiology Branch, and later the Clinical Neurosciences Branch. The simultaneous departure of Dr. J. Kiffen Penry, who in IRP headed the Epilepsy Section of the Experimental Therapeutics Branch, has seriously depleted the Program's research in convulsive disorders and efforts to recruit a new leadership in this area have now begun. Intensive recruitment activities for the post of Chief, Laboratory of Neurophysiology, have yet to attract any of the highly qualified candidates identified by an external search committee. Low, non-competitive NIH salaries, now compounded by the complexities and uncertainties surrounding implementation of the Senior Executive Service, were major determinants for this failure. The position of Chief, Medical Neurology Branch, also became vacant during the past year when its long-time incumbent, Dr. King Engel, stepped down. A search committee for this important post has already submitted its recommendations, and negotiations with designated individuals are now taking place.

Several important shifts in Program operations have occurred during the past twelve months. Direct research activities in the Laboratory of Experimental Neurology, which recently focused on the delayed nervous system effects of ionizing radiation and on the propagation of focal motor seizures, have now been concluded. Dr. William Caveness, who provided such competent direction to this work for many years, will continue contract studies of the neurologic sequelae of head injury. The Section on Neuroepidemiology, led by Dr. Bruce Schoenberg, has been transferred from the Office of Biometry and Epidemiology to the IRP. Studies of the distribution and dynamics of neurologic disorders, especially those major illnesses in which diagnostic criteria are reasonably well established, will be the major investigative interest of the Section. A Section on Neurotoxicology, headed by Dr. Ellen Silbergeld, has been organized within the Office of the Director, IRP, and should fill an important gap in the investigative activities of the Program.

Finally, the installation of the NINCDS purchased ORTEC ECAT scanner signals the beginning of a major new IRP effort in positron emission tomography. Under the guidance of the Section on Neuroradiology and Computed Tomography, initial studies will utilize ^{18}F labelled fluoro-deoxyglucose to evaluate regional neuronal function in patients with various neurologic and psychiatric disorders. In order to use important isotopes having much shorter half lives (for example: ^{11}C , ^{13}N , ^{15}O), IRP is now concluding purchase arrangements for a negative ion cyclotron to be installed in an underground extension of the Ambulatory Care Research Facility.

Despite these promising new initiatives, a variety of external administrative actions seriously threaten the Program's ability to maintain scientific productivity. Continuing fluctuations and uncertainties in resource allocations, especially in personnel ceilings, remain a major problem. The number of full-time permanent positions allocated IRP was cut from 295 to 287 in April 1979, and to 281 three months later. This number compares with a ceiling of 302 in 1975. While the percentage reduction is relatively small, its impact is quite considerable. Since no effective mechanisms exist to rid unproductive yet tenured investigators or support personnel from government service, ceiling reductions can only be attained through the process of normal attrition. This situation inevitably creates shortages in such high turnover categories as nontenured scientists and certain support positions. As a partial response to this problem, the Program has continued efforts to recruit ceiling-free personnel. As a result the number of doctoral level scientists increased during the past 12 months from 246 to 261, through expansion of such non-budgeted categories as guest workers, visiting fellows, and university faculty members temporarily hired under the Intergovernmental Personnel Act. These appointments are largely filled by talented young investigators, and provide much needed flexibility and creativity to the Program.

A more insidious danger to the Program's research integrity arises from the mounting burden of new procedural complexities and constraints, proliferating reporting requirements, and the increasing centralization of decision-making authority. It is essential that public funds be spent here as elsewhere only on research of the highest scientific and ethical quality. In the nominal service of this goal, the past few years have witnessed the development of a spiraling bureaucratic tangle, which substantially increases the time and expense of administering the program and unquestionably delays or diminishes its scientific output. A great need now exists to find a more reasonable balance between the acknowledged requirement for public accountability and the real costs of procedures designed to serve this purpose.

Budgetary allocations during FY 1979 were adequate to sustain existing direct and contract research activities. The apportionment for direct research operations totaled \$9.5 million in comparison with \$8.0 million in FY 1978. This relatively modest increase in purchasing power enabled IRP to maintain reasonable per capita support for its scientific staff and to launch some new research initiatives. Research contracts, which

continued to provide an important extension to the Program's in-house investigative activities, again amounted to about \$3.0 million. Much of this outlay supported primate facilities for long term virologic studies.

The amount of research space available to the Program, both on the NIH campus and at remote sites, also remained essentially unchanged. Some relief from this chronically over-crowded situation should occur with the opening of the Ambulatory Care Research Facility in mid 1981. NINCDS has been assigned laboratory and clinic space on the 5th floor of the ACRF, as well as both fifth floor Building 10 in-patient wards and adjoining 4th and 5th floor laboratory and office modules. These changes will provide a net increment of approximately 6,000 square feet of much needed and conveniently located space.

During the past year IRP has continued various activities in general support of the neurosciences. For example, in response to a recommendation by the Huntington's Disease Commission, IRP will manage contract studies of a unique Huntington's focus in Venezuela. In collaboration with the National Institute of Mental Health, IRP has continued to operate two neurospecimen banks, and has sponsored an international consensus exercise to standardize dissection techniques and nomenclature for the human central nervous system. IRP has also contributed to the Institute's technology transfer and medical information dissemination objectives through the organization of several major conferences and symposia. Furthermore, IRP continues to provide an important training function for the neurosciences. This year 156 young investigators worked in IRP laboratories under temporary Staff Fellow, Research-Clinical Associate, Visiting, or Guest Worker appointments. Most of these individuals go on to distinguished careers in academic or industrial research both in this country and abroad.

Despite the widely acknowledged accomplishments of NIH intramural research programs, the adequacy of quality assurance procedures applied to these activities has been a matter of increasing misunderstanding and concern in the outside scientific community. The NINCDS Board of Scientific Counselors, augmented by ad hoc members who are selected on the basis of their specialized knowledge of the field under evaluation, conducts searching reviews of every tenured investigator on a triannual basis. Resource allocations to IRP investigators reflect the relative merits of their research as determined by the Counselors' reviews as well as by the continuing assessments of Program management. NINCDS intramural scientists can expect support only so long as the quality of their research equals or exceeds that which is funded by the Institute extramurally. Two recently published studies, using modern bibliometric techniques, appear to support the generally high regard with which IRP scientists are held by their peer reviewers. Narin found that NIH intramural scientists produce more biomedical research papers per year than any other group in the United States, accounting for approximately 3.4 per cent of all U. S. biomedical research articles. These papers largely appear in the most influential journals and their influence generally exceeds that of publications from most other U. S. institutions. Indeed, an independent analysis by Garfield revealed that of the 300

most cited authors, 45 were affiliated with NIH, 19 with Harvard, 11 with Rockefeller University, and successively fewer from other institutions. Even when these data are corrected for the preponderance of research scientists at the NIH Campus, they clearly document the leadership of intramural research investigators among all American biomedical research institutions.

The appended Summaries and Individual Project Reports from each of the Programs' 16 Laboratories and Branches detail the outstanding contributions made during the past year. It may, nevertheless, be of interest to call brief attention to certain of these accomplishments here.

The Laboratory of Central Nervous System Studies has continued to explore various chronic, progressive, non-inflammatory central nervous system disorders (such as Creutzfeldt Jakob disease) which now appear to be slow infections caused by transmissible virus-like agents. Their unusual resistance to ultraviolet and ionizing radiation, formaldehyde or heat place them in a group unique among viruses. Their ability to produce fatal CNS disease without eliciting inflammatory responses, the inability of immunosuppression to alter the course of the disease, the failure to demonstrate any antigenicity in high titer infective virus preparations or to find any evidence of humoral or delayed hypersensitivity reactions in the diseases constitute some of the emerging atypical biological properties which further differentiate these agents from any other group of microorganisms. On the other hand, such classical viral properties as adaption to new hosts, dependence on host genetic breed, the presence of strains of differing virulence in wild stock viruses selected by limiting detection, and the interference of fast-growing by slow-growing strains, all indicate a complex host-virus genetic interaction characteristic of more classical viruses. Attempts are now being made to delineate the chemical nature of these replicating agents.

The Laboratory of Molecular Biology has continued studies of the regulation and mode of replication of viruses capable of producing encephalitis or meningitis. Many such viruses produce defective interfering particles which inhibit replication of the infectious virus and favor the persistence of non-infective yet destructive viral components. In the case of vesicular stomatitis virus RNA sequencing now indicates that the ends of the interfering particle genome are complementary, a property not shared by the parent virus genome. Different interfering particles have the same complementary regions for a distance of 45-48 nucleotides from the ends, suggesting that the particles were formed by a replicative event in which the viral replicase detaches from the template and resumes synthesis at a specific recognition site, near position 45-48 from the 5' end of the viral genome. The fact that defective interfering particles have the same attachment sites as the regular virus genome explains how they can compete for attachment to RNA polymerase and thus limit infection (less infective virus made).

The Infectious Diseases Branch has been examining antibody effects on rhabdovirus infection of neurons. Disassociated mice neuron cultures inoculated with vesicular stomatitis virus and subsequently exposed to

a medium containing sufficient antiviral antibody to neutralize all free virus survive 1 to 2 weeks in contrast to the 1 or 2 day survival of untreated cultures. With antibody treatment viral maturation sites tend to be grouped along the membrane of the neuronal body and postsynaptic dendrites. Antiviral antibody probably crosslinks membrane viral antigen on the neuron surface, causing a redistribution of viral assembly sites and clearing of antigen-antibody complexes from the cell surface as a result of macrophage activation.

Investigations of the biological role of cell membrane gangliosides have continued in the Developmental and Metabolic Neurology Branch. Gangliosides serve as transducers for external signals that influence cell metabolism and growth. They appear well suited to this function, since their carbohydrate chains protrude from the cell surface while the remainder of the molecule is anchored in the lipid bilayer of cell membranes. The rate and extent of responses of cells to external stimuli is a function of the type and number of ganglioside molecules in their membranes and the efficiency of ganglioside coupling to cytoskeletal components. An important function of gangliosides is their interaction with fibronectin, an external cell protein. Certain higher ganglioside homologues now appear to act as connecting links between the collagen-fibronectin complex and the cell membrane. These particular gangliosides are lacking in the membranes of tumorigenic virus-transformed cells, which might explain such properties as their lack of adhesion to substrata, increased motility, and their tendency to grow to an unusually high cell density.

In the Laboratory of Neurophysiology studies of disassociated cultures of mammalian neurons have yielded important new insights into the actions of central transmitters. All spinal cord cells grown in culture have been found to respond to glycine, B-alanine, GABA, and glutamate by changes in conductance mechanisms. Some (like glutamate) appear to activate sodium and potassium conductances, while others (like glycine) activate chloride conductance mechanisms. The membrane distribution of responsive areas is non-uniform, suggesting that the receptors may be clustered. Benzodiazepine and barbiturate derivatives induce complex changes in conductance, particularly involving chloride conductance. It is clear from these studies that a variety of clinically important drugs influence receptor coupled changes in excitability, possibly by engaging receptors normally occupied by endogenous ligands.

The Experimental Therapeutics Branch has gathered increasing evidence favoring the existence of pharmacologically distinct categories of dopamine receptors in the mammalian central nervous system. D-1 receptors are linked to adenylate cyclase, while a second category of dopamine receptor, designated D-2, does not influence the activity of this enzyme. This dichotomy may have important pharmacologic implications. For example, dopaminergic ergot derivatives such as lisuride or lergotriple, which are effective antiparkinson agents, block D-1 receptors but are among the most potent agonists of D-2 dopamine receptors. Moreover, some dopamine agonists such as apomorphine act indirectly at dopamine autoreceptors to inhibit impulse activity in

dopaminergic neurons, while others such as lisuride, appear to inhibit dopamine cell activity in the substantia nigra indirectly through some neuronal projection from the striatum.

The Laboratory of Neuro-otolaryngology has advanced its search for the primary afferent transmitter of the mammalian auditory nerve. Although glutamate, aspartate, alanine, and taurine are present in the cat cochlear nucleus, only levels of glutamate and aspartate decrease substantially following primary afferent degeneration. Moreover, glutamate and, to a lesser extent, aspartate are released endogenously and this release is reduced in the presence of a cochlear lesion. Furthermore, kainic acid injections produce a rate and extent of degeneration that correlates with the distribution of primary auditory nerve fibers, thus providing additional evidence that glutamate is a transmitter for this nerve. In a related study α -bungarotoxin reversibly blocked efferent inhibition of auditory activity in the cat cochlea, strengthening the hypothesis that cochlear efferent receptors are cholinergic and suggesting that these receptors are different from cholinergic receptors at most other vertebrate synapses.

Studies in the Laboratory of Neurochemistry have added to our understanding of the effects of anticonvulsants on brain metabolic changes occurring during electrically- or chemically-induced seizures. In mice it was found that the accumulation of cerebellar cyclic nucleotides which normally follows electroshock was selectively inhibited by phenytoin, suggesting that a locus of action for this drug is in the cerebellum. Sodium valproate not only prevented isoniazid-induced seizures, but also blocked the elevation of cyclic GMP and depression of GABA produced by isoniazid. This effect did not appear to be localized to a given brain region. It was also found that electroshock has a relatively small effect on energy depletion in cerebellar Purkinje cells. Presumably, these neurons are not stimulated to fire. Since the low levels of inhibitory output of the Purkinje cells probably would not offset the massive hyperexcitability resulting from the seizures, this condition may be permissive to epileptic activity.

Research efforts in the Laboratory of Neural Control remain focused on mechanisms by which the central nervous system controls movement. Some studies of neuronal activity during voluntary motor behavior in awake, intact Rhesus monkeys have utilized a novel electrode system, developed by the Laboratory, that permits stable, long-term recording from single neurons, thus allowing the examination of activity patterns under a variety of conditions including operant conditioning of cell firing. With careful monitoring of resulting movements, and additional data on the sensory receptive fields and effects of intracortical microstimulation through the recording electrode, a detailed picture of the specificity of association between particular cortical cell groups and particular muscles or muscle groups can be assessed. These studies now indicate that the association between cell discharge properties and "best" movements appears to be stable over prolonged periods of time. Ongoing work continues to explore the issue of spatial specificity for cortical cell "colonies" using conventional (movable) microelectrodes to

search for cortical cell groups that are temporally associated with activity in specific muscle groups.

The Laboratory of Biophysics has continued investigations into the neural basis for learning using the mollusc Hermissenda crassicornis. Movement of Hermissenda toward light is diminished by repeated pairing of a light stimulus with rotation. This behavioural change persists for several days, increases with practice, and shows evidence of stimulus specificity. Various neural changes have been observed in Hermissenda subjected to this associative learning paradigm. For example, hair cells receive less excitatory input from ipsilateral Type A photoreceptors after repeated stimulus pairing, but not after control training paradigms. Current and voltage clamp recordings from Type B cells in the eye show a prolonged voltage-dependent calcium current during and after light steps. The voltage-dependence of this calcium current and a calcium dependent potassium current provide a neural basis for the contingency necessary to the associative learning model. Moreover, excitation of hair cells by Type A photoreceptor impulses persists during synaptic blockade, possibly due to the accumulation of potassium around photoreceptor and hair cell axonal membranes. As such, this represents one of the few documented cases of neurons exchanging significant information without electrical or chemical synapses.

The Laboratory of Neuropathology and Neuroanatomical Sciences has found that hypothermia significantly enhances the survival of Mongolian gerbils with cerebral ischemia due to bilateral carotid artery occlusion. Almost 100 percent survival could be achieved when hypothermia was induced during or shortly before carotid occlusion. Cooling was ineffective, however, when applied more than one hour after the ischemic insult. An increased survival rate of gerbils subjected to carotid occlusion was also produced by various central nervous system depressants such as γ -hydroxybutyrate or γ butyrolactone. In contrast to hypothermia, both drugs were also effective when administered in the late postischemic period. Although the mechanism of this action is uncertain, both agents, like hypothermia, have a sparing effect on brain energy metabolism. These drugs also benefited the cerebral edema produced by ischemia. Their administration almost completely restored brain specific gravity values to normal within one week of the reestablishment of circulation.

The Neuroimmunology Branch has continued basic and clinical investigations related to multiple sclerosis. Studies of antibody formation to myelin basic protein now indicate that various strains of mice differ in their capacity to produce a humoral response and that this difference is related to histocompatibility background. Results obtained with recombinant and congenic strains of mice indicate the genes which regulate this immune response map to the K region of the chromosome encoding for the H-2 complex. Recent clinical results confirm the increased representation of HLA-A3, HLA-B7 and HLA-DW2 in multiple sclerosis patients. The best correlation occurs with B-cell antigens, which are believed to be analogous to murine Ia antigens. An evaluation of monozygotic and dizygotic twins, either concordant or discordant for multiple sclerosis,

now indicates that there is high degree of concordance in both monozygotic and dizygotic twin pairs. In a high percentage of the monozygotic, clinically discordant twin sets, the clinically unaffected member has a spinal fluid immunoglobulin abnormality suggesting the occurrence of a subclinical immune reaction.

Research in the Medical Neurology Branch has attempted to elucidate the basic causes and therapeutic approaches to peripheral neuropathies. In a presumably dysimmune relapsing neuropathy it was observed that localized immunoglobulin complexes containing IgG, IgM and C₃ were localized in sural nerves suggesting the complexes to be of pathogenetic significance. Moreover, the finding of a serum IgM factor which binds specifically to Schwann cells in rat peripheral nerve cultures in four of five patients studied suggests a new tissue culture test of pathogenetic mechanisms. The finding of unfluctuating monoclonal IgG bands in the CSF of patients with chronic relapsing but not the acute "Guillain-Barre" form of neuropathy, suggests that the monoclonal bands may be a predictor of chronicity.

The Laboratory of Experimental Neurology has continued to explore structural and functional sequelae of penetrating head injuries based on data from over 1200 cases arising from military combat in Viet Nam. Analysis of this material has yielded a body of consistent findings relating to the phenomena of post-traumatic epilepsy. The incidence of convulsions has remained essentially unchanged from one war to another, despite improvements in medical care and the use of prophylactic anticonvulsants. Onset of seizures is related to the location and severity of brain destruction. Convulsions cease in half the cases within 5 to 10 years regardless of therapy; half of the remainder have intractable seizures. A constitutional tendency towards epilepsy, as well as brain damage, appear to be the principal determinates for post-traumatic epilepsy.

The Surgical Neurology Branch has launched a program of biological, immunological, and chemotherapeutic investigations of human brain tumors. Tissue culture systems provide a model for studies of most brain tumors, since tumor cells in culture retain many of their in vivo characteristics. Observations made on these cultures often permit characterization of the cell of origin and degree of malignancy, information which can be useful clinically in assessing prognosis and planning therapy. There appears to be a direct correlation between the response of cultured tumor cells to a given chemotherapeutic agent and the clinical response to the same drug. Immunologic studies using individual patient's tumor cells as targets now suggest that the fraction responsible for the immune adherence response is largely IgM, while both IgG and IgM are involved in the cytotoxic responses. These studies indicate the feasibility of determining the system of antitumor immune responses in which the role of specific antibody fractions can be delineated. It may be that the immune response is not only one of blocking lymphocyte interaction with target cells but that the humoral response may in certain instances be helpful. Such a conclusion is supported by the observation that the presence of cytotoxic antibody in the serum correlates with a longer duration of survival.

ANNUAL REPORT

October 1, 1978 through September 30, 1979

Technical Development Section

National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report of the Section on Technical Development
National Institute of Neurological and Communicative Disorders and Stroke
and the
Research Services Branch
National Institute of Mental Health
October 1, 1978 - September 30, 1979

The Section on Technical Development provides broad technical support for the Intramural Research Programs of NIMH and NINCDS through (1) research and development in advanced biomedical instrumentation techniques and systems; (2) evaluation, specification and management of computer systems; (3) direction of a program of laboratory animal medicine and care (NIMH only); and (4) provision of other technical services in support of the research program.

The Branch is comprised of two sections:

Section on Instrumentation and Computers provides technical support for investigators 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.

Section on Laboratory Animal Medicine and Care provides professional advice and assistance to intramural scientists on all aspects of animal health, medical care, and testing and surgical protocols; manages a central primate holding facility; and conducts a program of laboratory animal care.

Additional services provided by the Branch 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 Branch personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Branch 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 Branch, he first meets with the Branch Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether RSB 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, RSB 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 Branch Chief or the Assistant to the Chief agree to accept a project, the investigator submits a standard work request form (available from RSB), 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 RSB 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 Branch's CAN.

INSTRUMENTATION

The following are selected instrumentation projects undertaken during the past year. These are chosen from a total of 281 projects, and are representative of the types of electronic instruments and systems developed by the Branch:

(1) Patient Activity Monitoring System. The Branch has continued to develop a system for monitoring the activity patterns of ambulatory subjects. The development this year was divided into two major areas. First, the continued production of the standard activity monitor, following its third re-design last year, was paced to meet the increased need. Twenty-five new monitors were produced; about half were used to phase-out older monitors with reliability problems and the other half went to expanded or new research programs. The advantages of the newer design (better calibration consistency and longer battery life, using an off-the-shelf battery) are sufficient for the Branch to plan to gradually replace all the older models. Eighty monitors are now distributed among twelve research groups, with approximately 65-70 monitors in active use at any given time.

The second major effort this year was, and continues to be, the development of a more compact monitor (approximately two-thirds the present volume) with a four-fold increase in memory capacity. To accomplish this, a contract was let for the production of miniaturized electronic circuits using thick-film hybrid technology. The first samples of these hybrid circuits will be delivered in the near future, and will undergo checkout using electronic instrumentation developed by the Branch. Once correct function is verified, subsequent deliveries should follow quickly so that production of the complete "hybrid" monitors can progress rapidly. With the capacity for 10 days of continuous recording at 15 minute resolution, the majority of the expected 50 monitors produced from this initial contract are aimed for long-term studies of manic-depressive outpatients.

A new calibration device with improved stability and repeatability of movement has been developed for quantitative characterization of each monitor's sensitivity to simulated patient movement. Up to eight monitors can be attached to a circular platter which is then rotated through + 10 degrees

at varying magnitudes of angular acceleration. The platter is driven by a brushless DC torque motor arranged in a closed-loop position control system. The motor commands are under programmed logical control so that acceleration sequences can be accurately repeated, and coordinated with the internal timing of the activity monitors. This device will also see general use as a calibrator for other devices employing accelerometers, such as the tri-axial accelerometer of the Neurological Function Testing Station, described below.

(2) Large Screen Display System. A large screen CRT display system has been developed to present complex spatial and temporal visual patterns to monkeys during single unit recording. In addition to the large format (16" x 12"), the display has high resolution (1024 raster scan lines/frame) with no detectable flicker (120 frames/second). Under control of a PDP LSI-11 microprocessor, the display circuitry can (1) generate arbitrary spatial frequency patterns, either fixed or drifting; (2) instantaneously rotate and/or change position of the visual pattern; (3) limit the area of the pattern without changing intensity; and (4) change from a light-on-dark visual target to a dark-on-light target. These capabilities allow a much greater range of visual experiments than previously possible.

(3) Control Electronics For Tissue Freezing Apparatus. An electromechanical apparatus for very rapid freezing of tissue samples is being refined by the Laboratory of Neuropathology and Neuroanatomical Sciences, NINCDS, with the assistance of RSB. Successful and safe operation of this device depends on activation of gas valves, solenoids, and an electromagnet in a precisely timed sequence. An electronic timer-controller has been developed to insure that this sequence is correctly generated with high repeatability over many experimental runs. This automation has greatly reduced equipment and operator error, which in turn, has reduced repeat experiments and wasted time.

(4) Dual Discriminator For Post-Synaptic Potentials. This instrument incorporates a standard RSB window discriminator for pre-synaptic spike detection and a second modified discriminator for preprocessing post-synaptic potentials (PSP). In addition to generating a multiplexed signal for the investigator's display scope, the PSP discriminator provides sampling time and amplitude limit information to a PDP-11 minicomputer which then generates a variety of on-line histogram displays.

(5) Neurological Function Testing Station. A neurological function testing station has been developed to measure the various parameters associated with Parkinson's disease. Three separate tests will be administered by the equipment to obtain data relating to the three major symptoms of the disease: tremor, akinesia, and rigidity. The hardware developed for the system interfaces to a small laboratory computer, the Plessey LSI 11/03. The computer controls the experiment, records and analyzes the data.

The electronics developed for this system cover three areas: measurement of tremor, gait, and reaction and movement time. Tremor is recorded with a tri-axial accelerometer with circuitry incorporated to allow a measure of tremor frequency and amplitude. The gait of the subject is measured with a 22 foot long treadway with pressure sensitive switches every 4 inches,

enabling the step length and elapsed time to be recorded. The last test performed is for reaction and movement time. This test is done by means of two capacitive-sensitive touch pads. The pads are designed with three circular "zones" that allow a measure of accuracy during the test.

Although designed initially for use in Parkinson's disease studies, the system will eventually be used in other studies involving recording of similar neurological parameters.

(6) Hamster Activity Recording System. An expanded system for measuring and recording the activity of 32 animal exercise wheels was developed. A photoelectric detector on each wheel and interface circuitry allows an LSI-11 computer to monitor each of the 32 input lines and record each rotation. The total number of counts for each cage is recorded every 15 minutes on a cartridge tape system, and displayed on a screen. Periodically the data recorded on the cartridge recorder can be transferred to a larger computer system for plotting and further analysis.

(7) Muscle Rigidity Measurement. A system was developed to measure resistance to passive stretch of a rat's hindfoot. Such resistive force is a measure of muscle rigidity, which is used when studying the pathophysiological mechanisms underlying akinesia and Parkinsonian rigidity. Resistance is induced by extending a metal beam against the lower part of a rat's hindfoot while the leg is held fixed. The foot is dorsiflexed and held for a given period and then the beam is retracted. A second time period elapses before the beam is again extended. Stretch resistance is measured by determining the strain in the metal beam used to dorsiflex the animal's limb. Resistive force can accurately resolve 1 gram-force over a 500 gram-force range using semiconductor strain gages and an integrated circuit strain gage conditioner.

Beam position is controlled by a digital linear stepping motor under command of a microprocessor which takes inputs from thumbwheel switches; 0.1 to 9.9 mm stepping distance; 1-99 sec. extension time; 10-990 sec. waiting time; and 1-99 trials per test run. An internally stored program calls the input values and operates the stepping motor as prescribed. Such microprocessor control offers accurate and reliable beam positioning and test run timing.

(8) Rat Sleep Scorer. Efforts are continuing to develop a device to categorize rat sleep state by analysis of bifrontal and frontal-occipital electrocortical activity and electromyographic activity. The desired device would convert such activities in real-time and provide a hard-copy record of total time in each sleep state, as well as a chronological record of 10-sec. epochs. It is hoped to improve an achieved accuracy rate of approximately 80% by better understanding the current human-scorer methods and also by analysis of spectral power of the brain waves.

(9) Strain Gage System. A strain gage system has been developed to measure finger pressure on the handle of a torque motor position control system used for research on the mechanisms which produce tremor of Parkinson's disease. The output is a voltage proportional to the force applied

by the primate's fingers; accurate and reliable resolution to 0.1 gram-force over a 250 gram-force range has been achieved. The circuitry was implemented with a new, integrated circuit strain gage conditioner. It consists of a high performance instrumentation amplifier with 120 db common-mode rejection and adjustable gain to 1000 V/V; a three-pole, low-pass filter having 60 db/decade roll-off and an adjustable corner frequency; and an adjustable excitation voltage stage.

(10) Rat Rotation Monitor. This previously reported device transduces and counts the clockwise/counterclockwise rotations of a rat. It is used in pharmacology studies. During the past year a modified design was implemented to expand memory storage of total counts per interval to service eight animals for 32 intervals. Available time intervals range from 5 to 60 min. in 5 min. increments. The device has been used on a more widespread basis in NIMH and NINCDS, and details of the device were published in the literature.

(11) Rat Activity Monitoring Cage. The activity cage developed by the Branch has been modified to decrease inter-cage variations in the measurement of activity. The cage electronically senses the location and rearing movements of an unrestricted rat located within the cage. This is accomplished through the measurement of capacitance changes induced by the rat's positioning on four large plates placed one on each of the four sides of the cage. The modifications included upgrading some electronic components to newly developed devices with better specifications. Other, more sensitive, methods of capacitance measurement are being studied to further improve the performance of the cages.

(12) Electromagnetic Muscle Stretch Device. A technique for stretching individual muscles in intact behaving animals via chronic intramuscular implantation of a magnetically permeable slug and use of an external electromagnet to apply force to the slug, has been developed for use in the study of the role of muscle sensory receptors in skilled motor activity. The slug is surgically implanted in the musculotendinous junction in the forearm. The arm is placed inside a solenoidally wound electromagnet; current through the coil produces a magnetic field which exerts a force on the slug, and thus on the muscle. The placement of the slug in relation to the coil and the coil current determine the direction, amplitude, and frequency of the force on the muscle, and thus determine which receptors are stimulated. Developments this year included a comprehensive analysis of the theory and practical considerations of the interaction between coil and slug, to be published in the engineering literature.

(13) Torque Motor Position Control System. A system was developed for research on the mechanisms which produce the tremor of Parkinson's disease. The system, consisting of brushless DC torque motor, handle, housing, power amplifier, and control electronics, was designed to give controlled mechanical stimuli to the wrist joint. The control system operates in an open loop torque mode or a closed loop position control mode. Switching is done electronically under experiment control. Two systems have been developed: one for human subjects, and a smaller version for use by monkeys.

(14) Remote Audio Cassette Data Entry Logger. This project configured a DEC VT40 computer terminal and an analog cassette recorder as a stand-alone data entry point for observational comments regarding monkey behavior. Facilities were also provided for cassette play-back at a later time at a DEC PDP-12 for correlation of observational comments with other physiological records recorded on another media. The VT50 TTY serial output is fed back to the TTY serial input in order to provide the operator a display of the text going onto tape. Then a portion of the TTY serial output was tapped and conditioned for analog recording. At play-back the analog data is re-formatted into a serial data stream and recovered at a PDP-12 A/D input channel.

COMPUTERS

The Research Services Branch continues to support the use of the computer as a laboratory instrument. Small computers are used in the individual laboratories for on-line, real-time interaction, process control and data acquisition. RSB maintains support computers in Buildings 10 and 36. These systems provide means for program preparation, bulk storage, printing and plotting capabilities, and minor mathematical and statistical processing. Experimental data may be transmitted from the laboratory computers, via these systems, to the DCRT facilities for further processing. The support computers also serve to develop prototype systems and to test the feasibility of the use of a computer in specific laboratory applications. The latter capability allows an investigator, once he determines that the computer will do the job, to purchase an efficient system at minimal cost.

The system for automated analysis of autoradiographs that was developed for the Laboratory of Cerebral Metabolism (LCM), proved so successful that the facility was soon overloaded. Preliminary studies demonstrated that the usefulness of an interactive system for the graphic analysis of photographic images extends far beyond autoradiographs. Examples are the study of neural processes, results from a PET scanner and two-dimensional electrophoretic gels. A systems study was conducted to develop an improved configuration for such analysis. Two systems were purchased: an additional system for the LCM to increase their production; and a system to be maintained by the Research Services Branch to provide image scanning services to NIMH and NINCDS. This system will be used to continue developmental studies aimed at extending the usefulness of such systems.

The Branch provides software support for the individual investigators. A library of procedures has been developed that are tailored to the needs of the Intramural Program. Individual training is available for beginning investigators. Computer specialists are available for consultation for all areas of computer use, programming, interfacing, real-time applications, time series analysis, data presentation, systems configuration and computer procurement. Although RSB does not provide an applications programming service, systems have been developed in collaboration with individual laboratories. Examples are included in the list of computer related projects.

An increasing amount of time is devoted to program maintenance. Computer programs, especially in research related services, are not static and often require development, improvement or modifications. In addition to the software library and research related projects developed by the Branch, much work is caused by the turnover of scientific and support personnel. Many systems developed by these persons prove useful to the laboratory. After they leave, maintenance of such systems often falls to the personnel of the Branch. The Branch has investigated the use of structured programming techniques and programming languages in an attempt to define software standards. PASCAL was chosen as a standard language. As the problem of program maintenance continues to grow, use of these techniques will be necessary to enable the Branch to furnish this service without an increase in personnel.

One of the major functions of the Branch is to provide systems studies for the use of the computer as a laboratory instrument. The concept of the micro-computer as an integral part of laboratory instrumentation for process control, data logging, timing and coordination of instruments has proven to be both cost effective and efficient for laboratory applications. Mini- and micro-computer based systems provide a wide spectrum of tools for research ranging from lower cost data loggers to sophisticated systems which interact with the on-going experiment. These systems have resulted in a further integration of the engineering and computer functions of the Branch and have enabled the Branch to offer a wide range of computer related services. Projects such as the Neurological Function Testing Station are the result of close coordination between the scientist, the electronic engineers, and the computer scientist and will provide a service not feasible a few years ago.

Examples of computer-related projects include:

(1) Membrane Activity of Neurosecretory Cells. This system was developed in collaboration with the Laboratory of Neurophysiology (NINCDS) to study the conductance of cell membranes that often exhibit bursting activity during clamping. The system is being upgraded to record and monitor the tissue culture neurons on-line and to allow the iontophoretic or pressure injection of neuroactive substances onto the external surfaces of the cells. The purpose is to discover the nature of the action of such substances on the properties of the cell membrane.

(2) Cell Culture Analysis. This system is designed to provide an on-line analysis of tissue culture neurons. The first phase, to study the excitatory or inhibitory post-synaptic potentials of these cells, has been completed. A unique feature of this system is the on-line control of artifacts introduced by the measurement system and the properties of tissues in culture and to control the threshold levels and amplification level as the experiment is in progress. Visual displays of amplitude, integral and latency are available, as well as averaged evoked response. In addition, on-line monitoring of post-synaptic potentials elicited by stimuli presented in pairs or in trains of pulses are available. The system also studies spontaneously occurring miniature potentials. This system will be extended to allow analysis of the cells by other techniques such as voltage clamping and the iontophoretic injection of neuroactive substances on the surface of the cell.

(3) Rat Activity Monitoring System. The system designed to measure gross movements and rearing activity of white rats was extended to differentiate between two levels of gross movements and to monitor the amount of time the animal spends in each quadrant of the cage.

(4) Membrane Channel Analysis. Acetylcholine molecules outside neural membranes open and close channels allowing the passage of Na⁺ and K⁺ ions. Current fluctuations resulting from single channels opening and closing have a high signal-to-noise ratio. This program catalogues and identifies the duration and amplitude of channel activity.

(5) Cytofluorographic Analysis of Single Cells. The system designed for cytofluorographic cell sorting by the Computer Systems Laboratory, DCRT, is being modified for the cytofluorographic analysis of cells in immunological studies conducted by the Immunochemistry and Clinical Investigations Section, Infectious Diseases Branch, NINCDS.

(6) Data Acquisition System For Rat Vocalization. A data acquisition and display program has been written for a PDP-11/04 to sample and store the video signal of a Tektronix 5L4N spectrum analyzer. The audio transducer of this device is placed within the 'rat universe' of the Laboratory of Brain Evolution and Behavior, NIMH. The program selectively stores only those vocalizations within a frequency range specified by the experimenter, and records the time of the vocalizations. The results can be displayed on a video terminal or line printer. Further analysis of this data is yet to be done.

(7) Digital Filter Design and Applications. A package of FORTRAN programs has been implemented on the PDP-11/40 which is used to design digital filters for use in off-line signal processing applications. Although the original algorithms were obtained from the open literature, this system implements the algorithms in an interactive way and contains support programs which display and use the filters in a convenient manner. The results of this package have been used in various other programming systems.

ANNUAL REPORT

October 1, 1978 through September 30, 1979

Section on Neuroepidemiology, ODIR

National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report
for Period October 1, 1978 through September 30, 1979
Section on Neuroepidemiology
Office of the Director
Intramural Research Program
National Institute of Neurological and Communicative
Disorders and Stroke

The Section on Neuroepidemiology is responsible for the development and implementation of epidemiologic 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 first priority of the Section when it was created four years ago was the successful completion of already ongoing field studies supported by research contracts. The most important of these was a population-based, case-control study of multiple sclerosis (MS) in the Shetland and Orkney Islands. This area has the highest reported prevalence of the disease anywhere in the world. Analysis of the results have been completed in the past year, and these are currently being reported. The investigation revealed 1) a "heritability" of MS of 31%; 2) no predominant HLA type among the cases compared to the controls; and 3) no predominant viral exposures as demonstrated by antibody titers among the cases as compared to the controls. Furthermore, the high MS prevalence figures for the islands were not the result of patterns of population movements. There was evidence of space-time clustering of MS at two points in time:
1) Twenty-one years before onset, and 2) at the time of onset.

The severe shortage of available manpower in neuroepidemiology necessitated development of an educational program which was initiated by the Section. A series of four videotapes were produced by the Section; they are distributed on a loan basis without charge by the National Medical Audiovisual Center, National Library of Medicine. A textbook, entitled NEUROLOGICAL EPIDEMIOLOGY: PRINCIPLES AND CLINICAL APPLICATIONS, was published this past year by Raven Press. A symposium on the solutions to methodologic problems in neuroepidemiology was held in conjunction with the Society for Epidemiologic Research and the World Federation of Neurology. A future course is planned in collaboration with the American Academy of Neurology. Finally, current individual and institutional research training grant programs have recently been expanded to include neuroepidemiology. With the completion of ongoing, contract-supported studies and the initiation of an educational program, the Section has focused on research investigations.

With regards to neurologic problems in children, the Section 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 Section has conducted extensive investigations of primary intracranial neoplasms. First, problems with nomenclature and disease definition were resolved. After this, two patterns of age-specific incidence emerged. Analyses of most population-based data worldwide revealed 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 are currently underway 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. An exhaustive, critical review of a survey strategy to measure the national incidence and prevalence of intracranial neoplasms has just 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.

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 both transient ischemic attacks and completed stroke. Data editing has been completed during the present fiscal year and analysis of these data should be finalized within six months. In addition, certain unusual patterns of cerebrovascular disease (e.g., more than 20 TIA's/day) are being studied.

Alzheimer's disease-senile dementia, despite its high apparent clinical frequency among the elderly, has not been well studied in a U.S. population. Because of this, we have launched three investigations. One is derived from a review of detailed clinical records utilizing a population-based, records-linkage system; the second utilizes a two-stage survey consisting of a questionnaire and clinical examination; and the third (in collaboration with the National Institute on Aging) is based on a questionnaire survey. These investigations should provide better estimates of the magnitude of the disease and its distribution in the population. These will likely yield etiologic hypotheses which can be further studied using the case-control approach, and will provide the opportunity to investigate dementia occurring with other neurologic disorders such as Parkinson's disease.

A case-control study of the relationship between cigarette smoking and Parkinson's disease confirmed previous reports of an inverse relationship (i.e., a low rate of smoking among those with Parkinson's disease as compared to the smoking rate among controls).

The Section 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 re-investigated (in conjunction with the Section on Disease Statistics Surveys) 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, 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 99% 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. Similar strategies are being developed for application in developing countries, in collaboration with the World Health Organization.

We currently have very little information on the patterns of medical care received by all individuals with neurologic disease in a given community. The Section 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.

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.

A number of other collaborative projects include the investigation of space/time clusters of neurologic disease (with the Center for Disease Control), 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 description of lymphocyte ultrastructure in Batten's disease (with the Clinical Investigations and Therapeutics Section, DMN, NINCDS and the University of Kentucky), and a study of the relationship between headache and hypertension (with the University of Kentucky). Finally, extensive reviews have been prepared on the epidemiologic aspects of Huntington's disease, otitis media, Alzheimer's disease, and cerebrovascular disease.

CONTRACT NARRATIVE
Section on Neuroepidemiology
Office of the Director, Intramural Research Program, NINCDS
Fiscal Year 1979

THE UNIVERSITY OF NEWCASTLE UPON TYNE (N01-NS-6-2337)

Title: Genetic Study of Multiple Sclerosis in the Orkney and Shetland Islands

Contractor's Project Director: Dr. D.F. Roberts, Professor
Dept. of Human Genetics

Current Annual Level: None from the 1979 budget. A sum of \$39,179 was allotted to this contract out of the 1976 budget. To date, Dr. Roberts received \$31,056.64 of this money.

Objective: (See contract N01-NS-4-2321 for an introductory statement)

Family pedigrees of multiple sclerosis patients and their controls were established for Shetland and Orkney. Blood groups, red cell enzymes, and serum proteins on specimens of blood obtained during the March 1976 field trip to the Orkney Islands were determined. The pedigree as well as all serology data for Orkney and Shetland were analyzed in a study of the genetic aspects of multiple sclerosis among the inhabitants of the Orkney and Shetland Islands.

Major Findings: Three manuscripts were recently received:

- 1) "Genetic Analysis of Multiple Sclerosis in Orkney"
(In a family study of all patients with multiple sclerosis in Orkney, the number of inbred among patients, though high for Britain, is not elevated over the number of controls.)
- 2) "Polymorphic Variants and Multiple Sclerosis in Orkney"
(Study of the blood group, isoenzyme, and serum protein systems representing polymorphic variants at 23 loci, in the population of 53 multiple sclerosis patients in Orkney, their relatives, and control series, shows that patients are neither more homozygous nor more inbred than controls.)
- 3) "Serum Immunoglobulin Levels in Multiple Sclerosis in Orkney"
(Serum levels of immunoglobulins A, G, and M in the population of multiple sclerosis patients in Orkney were generally similar to those in a series

of contiguous and discontinuous controls, and in the normal first-degree relatives both of patients and controls.) Thus, in summary the findings do not support heredity as an etiologic factor.

The investigators anticipated publication of these manuscripts in the British Journal of Preventive and Social Medicine in 1978, but to our knowledge to date, none of them have as yet been published.

Significance to the NINCDS Program and Biomedical Research:
(See this item as stated for contract N01-NS-4-2321.)

Proposed Course of the Project: The contract expired on June 26, 1977. The following reports required by the contract were received to date:

- 1) Church burial data to complete Shetland pedigrees.
- 2) Three manuscripts pertaining to genetic analysis of multiple sclerosis in Orkney as listed under "Major Findings".
- 3) Shetland pedigree analysis.
- 4) A final report on the genetic aspects of multiple sclerosis among the inhabitants of the Shetland and Orkney Islands.

CONTRACT NARRATIVE
Section on Neuroepidemiology
Office of the Director, Intramural Research Program, NINCDS
Fiscal Year 1979

MASSACHUSETTS GENERAL HOSPITAL (N01-NS-4-2321)

Title: Multiple Sclerosis in the Shetland and Orkney Islands
and Caithness, Scotland

Contractor's Project Director: Dr. Raymond D. Adams
Professor, Dept. of Neurology

Contractor's Co-director: Dr. David C. Poskanzer
Associate Professor
Dept. of Neurology

Current Annual Level: None - Contract expired in October
1977.

Objective: An epidemiologic, virologic, and immunologic study of multiple sclerosis was undertaken in the Orkney and Shetland Islands, Scotland, where the rates of the disease are 309 per 100,000 and 184 per 100,000, respectively, as compared with the estimated prevalence rate of 40 per 100,000 in Boston. A search for exogenous etiologic factors and factors which might implicate heredity was undertaken.

All patients with multiple sclerosis in the Shetland and Orkney Islands have been identified, and two sets of appropriate controls were selected for each patient. For such individuals (both patients and controls), as well as certain family members of these individuals, previous history of infection (confirmed by serology), dietary history, sanitation history, history of exposure to animals, occupational history, travel history, and history of allergic diatheses were obtained, and family pedigrees were traced to 1776. Blood samples were obtained from patients, their two age- and sex-matched controls, and family members for (1) the histocompatibility determinants HLA, MLC, and B-cell alloantigen Ag7a; (2) blood group typing, red cell enzymes and serum proteins; and (3) viral antibody titers (rubeola, rubella, mumps, varicella, cytomegalovirus, herpes 1 and 2, Coxsackie B3 and B4, parainfluenza 1, 2, and 3, poliomyelitis 1, 2, and 3, echo 4 and 9, and EB virus). The blood samples were shipped to appropriate laboratories for study.

The establishment of family pedigrees and determination of blood groups, as well as their analysis, was sub-contracted (see contract N01-NS-6-2337). The principal investigators of contract N01-NS-4-2321 and contract N01-NS-6-2337 are cooperating in the interpretation and publication of results.

Major Findings: Findings determined to date were reported during the American Academy of Neurology meetings in April 1977 and 1979. The findings reported in the 1977 meeting were as follows:

(1) A bimodal age at onset curve was observed for Orkney Islands with two distinct peaks at ages 20 to 25 and 35 to 40. From this observation it became apparent that there is a subgroup of patients who share the following characteristics: same sex, early age at onset, temporal course of exacerbating-remitting disease, and the occurrence of HLA-B7. This group of patients, referred to as Multiple Sclerosis Type I, can be quite clearly distinguished from other forms of multiple sclerosis and may account for the disparities and variations in tissue typing present when the disease is perceived as if it were homogeneous, rather than at least two clinical, epidemiologic, laboratory, and possibly etiologic entities.

(2) The absence of optic neuritis as an isolated entity without subsequent evidence of other lesions of the nervous system in these islands is remarkable.

(3) Female Orkney patients who are HLA-B7 positive appear to have a lower titer response to several of the viruses studied, especially measles, as compared to female controls with HLA-B7 and other Orkney patients without this specificity.

(4) No virus of those studied is clearly associated with multiple sclerosis, either by antibody titer or the presence or absence of previous exposure by history to each virus.

The findings reported in the 1979 American Academy of Neurology meeting are as follows:

(1) Genetic analysis in collaboration with Roberts demonstrated a "heritability" of MS of 31 percent.

(2) HLA analysis in collaboration with Terasaki did not show a predominant type or trend.

(3) Studies for a long series of common viruses in collaboration with Sever did not demonstrate the putative agent.

(4) Demographic analyses in collaboration with Taylor and Illsley indicated that the high prevalence in the Islands was not the result of patterns of population movement.

The Orkney and Shetland Islands were shown to have the highest rates of MS recorded, though increases in the

prevalence over time were largely a function of increasing life expectancy of patients. Availability of controls born in the same year and in the same parish as the patient, and lifetime information about residence and travel of all subjects provided clear evidence of temporal-spatial clustering of MS patients in Orkney at two points in time: (1) about 21 years prior to onset, and (2) at the time of onset. The data suggest that not one, but two, environmental factors play a role in the etiology of MS. They may represent two discrete exposures or one exposure occurring twice in the lifetime of patients.

Significance to the NINCDS Program and Biomedical Research:

To date research on the etiology of multiple sclerosis follows a divergent path; the quests in search of environmental and genetic factors continue at comparable rates. It was anticipated that a study of genetic and environmental factors in a defined population with the world's highest reported prevalence of multiple sclerosis would provide guidelines for future research, justifying emphasis on the pursuit of either genetic or environmental factors.

Proposed Course of the Project: The contract expired on October 15, 1977. According to contract specification, the five manuscripts resulting from this study were submitted to the Institute. Publication plans for any of these manuscripts are uncertain.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02240-03 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Epidemiology of Dementia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: B.S. Schoenberg Chief Section on Neuroepidemiology ODIR NINCDS J.E. Hogg Neurologist Section on Neuroepidemiology ODIR NINCDS Other: R.J. Baumann Neurologist Section on Neuroepidemiology ODIR NINCDS J.A. Brody Associate Director EDBP NIA		
COOPERATING UNITS (if any) Epidemiology, Demography, and Biometry, NIA; W. Massey, M.D., Duke University; L.T. Kurland, M.D. and J.P. Whisnant, M.D., Mayo Clinic; B. Jordan, Harvard Medical School		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.61	PROFESSIONAL: 0.61	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) A number of different approaches are being utilized to estimate the <u>mortality</u> and <u>morbidity of Alzheimer's disease-senile dementia</u> in several population groups in the U.S. and to measure the distribution of this disease in segments of the population.		

Project Description:

Objectives: To obtain estimates of the magnitude and distribution of Alzheimer's disease-senile dementia in segments of the U.S. population.

Methods Employed: Mortality information is obtained from death certificate data for the U.S. Three morbidity studies are also currently underway. One is derived from a review of detailed clinical records utilizing a population-based, records-linkage system; the second utilizes a two-stage survey consisting of a questionnaire and clinical examination; and the third (in collaboration with the National Institute on Aging) is based on a questionnaire survey.

Major Findings: Data are currently being collected and have yet to be analyzed.

Significance: Alzheimer's disease-senile dementia, despite its high apparent clinical frequency among the elderly, has not been well-studied in a U.S. population. These descriptive studies will likely yield etiologic hypotheses which can be further investigated using the case-control approach, and will provide the opportunity to investigate dementia occurring with other neurologic disorders, such as Parkinson's disease.

Proposed Course of Project: Data collection will continue during the coming year. Two of the studies are at a stage in which data analysis may be expected to begin during the coming fiscal year.

Publications:

Schoenberg, B.S.: Neuroepidemiologic Considerations in Studies of Alzheimer's Disease -- Senile Dementia. In Katzman, R., Terry, R.D., and Bick, K.L. (Eds.): Aging: Alzheimer's Disease -- Senile Dementia, and Related Disorders. New York, Raven Press, 1978, pp. 327-335

Schoenberg, B.S.: Methodologies for studying the epidemiologic aspects of dementia. In: Epidemiology of Dementia. New York, Raven Press, in press

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02241-03 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) The Epidemiology of Cerebrovascular Disease in Adults		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Bruce S. Schoenberg Chief Section on Neuroepidemiology ODIR NINCDS		
COOPERATING UNITS (if any) J.P. Whisnant, M.D., Mayo Clinic; D.G. Schoenberg, M.S., Bethesda, Maryland; A. Lilienfeld, Johns Hopkins University		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.6	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This investigation is aimed (1) at evaluating the effect of <u>heart disease</u> and <u>hypertension</u> as potentially treatable <u>precursors</u> of <u>completed stroke</u> and <u>transient ischemic attacks</u> and (2) at documenting unusual patterns of cerebrovascular disease.		

Project Description:

Objectives: To determine the following: (1) the risk of stroke and transient ischemic attacks in individuals with heart disease and/or hypertension as compared to the risk in individuals without these conditions; (2) whether the existence of pre-existing heart disease and/or hypertension affects the type of stroke and whether it affects survival following stroke; and (3) whether there is a particular time interval following the onset of heart disease or hypertension during which an individual is at high risk for stroke.

Methods Employed: This study involves a non-concurrent prospective approach evaluating a cohort of 2,000 elderly individuals. The type of analysis follows the person-years strategy and utilizes life-table methods.

Major Findings: The data are currently being analyzed.

Significance: 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.

Proposed Course of Project: The forthcoming year will be devoted to data analysis and publication of the results.

Publications:

Schoenberg, B.S.: Risk Factors for Cerebrovascular Disease. In Rose, C.F. (Ed.): Clinical Neuro-Epidemiology. Tunbridge, England, Pitman Medical Publishing Co., in press

Note: This project number includes the previous projects: Z01 NS 02241-02 OBE, and Z01 NS 02244-02 OBE.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02243-03 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (90 characters or less) Pediatric Neuroepidemiology		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Bruce S. Schoenberg Chief Section on Neuroepidemiology ODIR NINCDS		
COOPERATING UNITS (if any) D. Schoenberg, M.S., Research Epidemiologist, Bethesda, Maryland; J.F. Mellinger, M.D., and M.R. Gomez, M.D., Department of Neurology, Mayo Clinic; B.W. Christine, M.D., M.P.H., Connecticut State Department of Health		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The project documented the frequency of <u>primary intracranial neoplasms</u> in the <u>pediatric populations</u> of Rochester, Minnesota, and the State of Connecticut. In addition, we investigated the magnitude and risk factors for <u>cerebrovascular disease</u> in <u>infants</u> and <u>children</u> in the Rochester, Minnesota population.		

Project Description:

Objectives: To document the frequency of primary neoplasms and cerebrovascular disease in pediatric populations. This had never been done before in a well-defined population group.

Methods Employed: Descriptive epidemiologic studies were carried out utilizing the resources of the Connecticut Tumor Registry and the Rochester, MN records-linkage system. In addition, a case-control study was undertaken to determine risk factors for perinatal intracranial hemorrhage.

Major Findings: The brain tumor incidence rate in children (<15 years of age) varied from 2.5-5.0 cases/100,000/year. 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.

Significance: This study represents the first time that the magnitude of either brain tumors or cerebrovascular disease has been documented in a well-defined pediatric population.

Proposed Course of Project: Additional diseases will be studied using similar methodology. The problem of cerebrovascular disease in children will be re-evaluated following the introduction of computerized tomography as a diagnostic tool.

Publications:

Schoenberg, B.S., Mellinger, J.F., and Schoenberg, D.G.: Cerebrovascular disease in infants and children: a study of incidence, clinical features, and survival. Neurology 28: 763-768, 1978.

Schoenberg, B.S.: Risk Factors for Stroke in Infants and Children. In Goldstein, M., Bolis, L., Fieschi, C., Gorini, S., and Millikan, C.H. (Eds.): Cerebrovascular Disorders and Stroke. New York, Raven Press, in press

Serial No. Z01 NS 02243-03 ODIR

Schoenberg, B.S., and Schoenberg, D.G.: The Spectrum of Pediatric Cerebrovascular Disease. In Rose, C.F., (Ed.): Clinical Neuro-Epidemiology. Tunbridge, England, Pitman Medical Publishing Co., in press

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02297-03 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Mortality from Neurologic Disorders: National and International Comparisons		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: B.S. Schoenberg Chief Section on Neuroepidemiology ODIR NINCDS Judith E. Hogg Neurologist Section on Neuroepidemiology ODIR NINCDS		
COOPERATING UNITS (if any) W. Massey, M.D., Duke University; D.G. Schoenberg, M.S., Bethesda, Maryland		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) 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 <u>mortality data for selected neurologic disorders</u> 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.		

Project Description:

Objectives: To analyze available mortality data by country and by county in the U.S.; to measure secular trends and formulate etiologic hypotheses.

Methods Employed: Age-adjusted death rates for selected neurologic disorders were calculated for 33 countries and for each U.S. county. Rates were ranked and patterns of mortality are being illustrated graphically and with maps.

Major Findings: The data are currently being analyzed, and no findings are yet available.

Significance: Such detailed data are not available through sources of morbidity information. Consequently, we must utilize death certificate information to evaluate secular trends and patterns worldwide and by county within the U.S.

Proposed Course of Project: Analysis of these data is continuing, and publications will be prepared.

Publications:

Hogg, J.E., Massey, E.W., and Schoenberg, B.S.: Mortality from Huntington's Disease in the United States. In: Chase, T.N., Wexler, N., and Barbeau, A. (Eds.): Huntington's Chorea: 1972-1978. New York, Raven Press, in press

Note: This project number includes the previous projects: Z01 NS 02297-02 OBE, and Z01 NS 02298-02 OBE.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02299-03 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Reviews of Epidemiologic Aspects of Neurologic Disease		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: B.S. Schoenberg Chief Section on Neuroepidemiology ODIR NINCDS J.E. Hogg Neurologist Section on Neuroepidemiology ODIR NINCDS T. Kudrjavcev Neurologist Section on Neuroepidemiology ODIR NINCDS		
COOPERATING UNITS (if any) W. Massey, M.D., Duke University; D. Schoenberg, M.S., Bethesda, Maryland		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Development of new neurologic studies requires thorough historic and <u>methodologic reviews</u> of prior investigations. These yield important unexplored etiologic clues that may be investigated using current technology. Major emphasis has been given to <u>cerebrovascular disease</u> , <u>otitis media</u> , <u>inherited ataxias</u> , and <u>Huntington's disease</u> .		

Project Description:

Objectives: To review comprehensively previous studies dealing with neurologic disorders in human populations, as well as other diseases with neurologic manifestations.

Methods Employed: Pertinent literature is critically reviewed for etiologic clues and unresolved issues which are answerable through properly designed neuroepidemiologic studies.

Major Findings: Suggested studies have been designed for cerebrovascular disease, otitis media, inherited ataxias, and Huntington's disease. Some of these investigations are being pursued by the Section, as well as extramural scientists.

Significance: It is only through careful and critical review of previous efforts that a productive research program can be launched. These critical reviews are generally published so that both intramural and extramural investigators have access to this information.

Proposed Course of Project: These review efforts will continue and will generally focus on major neurologic diseases (e.g., dementia).

Publications:

Schoenberg, B.S.: Epidemiology of the Inherited Ataxias. In Kark, P., Rosenberg, R., and Schut, L. (Eds.): Advances in Neurology: The Inherited Ataxias (Biochemical, Viral and Pathological Studies). New York, Raven Press, 1978, pp. 15-32

Schoenberg, B.S.: The Epidemiologic Approach to Huntington's Disease. In Chase, T., Wexler, N., and Barbeau, A. (Eds.): Huntington's Disease. New York, Raven Press, in press

Kudrjavcev, T., and Schoenberg, B.S.: Otitis media and developmental disability: Epidemiologic considerations. Annals of Otolaryngology and Rhinology. Accepted for publication.

Baumann, R.J.: Stroke. In Wekstein, D.R. (Ed.): Keeping Fit After Sixty. Dutton and Company. In press

Schoenberg, B.S., and Schoenberg, D.G.: Refsum's disease. Southern Medical Journal 71: 715-716, 1978.

Schoenberg, D.G., and Schoenberg, B.S.: Eponym: William Stokes. Southern Medical Journal 71: 956-957, 1978.

Schoenberg, D.G., and Schoenberg, B.S.: Eponym: Sir William Gowers. Southern Medical Journal 71: 1148-1149, 1978.

Schoenberg, D.G., and Schoenberg, B.S.: Eponym: William John Little. Southern Medical Journal 71: 1296-1297, 1978.

Schoenberg, D.G., and Schoenberg, B.S.: Eponym: Golgi, Cajal, Nissl, and Weigert. Southern Medical Journal 72: 44-46, 1979.

Schoenberg, B.S., and Schoenberg, D.G.: Eponym: Pancoast's syndrome. Southern Medical Journal 72: 219-220, 1979.

Schoenberg, B.S.: The epidemiology of cerebrovascular disease. Southern Medical Journal 72: 331-336, 1979.

Schoenberg, D.G., and Schoenberg, B.S.: Eponym: William Alexander Hammond. Southern Medical Journal 72: 346-347, 1979.

Schoenberg, B.S., and Massey, E.W.: Tapia's syndrome. Archives of Neurology 36: 257-260, 1979.

Schoenberg, D.G., and Schoenberg, B.S.: Eponym: Sir Astley Paston Cooper. Accepted for publication by the Southern Medical Journal.

Schoenberg, B.S., and Schoenberg, D.G.: Eponym: John Abernethy. Accepted for publication by the Southern Medical Journal.

Schoenberg, D.G., and Schoenberg, B.S.: Eponym: Theodor Billroth. Accepted for publication by the Southern Medical Journal.

Schoenberg, D.G., and Schoenberg, B.S.: Eponym: Henry Bence Jones. Accepted for publication by the Southern Medical Journal.

Schoenberg, D.G., and Schoenberg, B.S.: Eponym: Leo Buerger. Accepted for publication by the Southern Medical Journal.

Schoenberg, D.G., and Schoenberg, B.S.: Eponym: Paget's disease. Accepted for publication by the Southern Medical Journal.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02300-03 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Clinical Course and Medical Care for Neurologic Disorders		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: B.S. Schoenberg Chief Section on Neuroepidemiology ODIR NINCDS Other: R.J. Baumann Neurologist Section on Neuroepidemiology ODIR NINCDS		
COOPERATING UNITS (if any) Ms. Karin Rosenblatt, Johns Hopkins University; Mayo Clinic, Rochester, Minnesota; University of Kentucky		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The study uses a review and abstraction of data from records for a selected group of <u>neurological disorders</u> . It obtains the items of data necessary to determine onset of the disorder, duration, date and cause of death, or current status. These data will be used to construct <u>modified life tables</u> to estimate the <u>expectation of life after diagnosis</u> , the survival curve and morbidity and severity estimates. It will also include analysis of type and duration of <u>medical care</u> received by patients with neurologic disorders derived from a well-defined population. Another aspect of the project involves the evaluation of a plan to extend pediatric neurologic care in a rural setting.		

Project Description:

Objectives: To determine the clinical course and patterns of medical care for all individuals with major neurologic disease in a given community. In addition, the program also involves an evaluation of a program to extend pediatric neurologic care in rural settings.

Methods Employed: Cases of neurologic disease among residents of Rochester, MN have already been identified through previous studies. Medical encounters are documented through a records-linkage resource and this information has been abstracted.

Major Findings: The data for Rochester, Minnesota are currently being collected and have not yet been analyzed. The extension of neurologic care to children in rural Kentucky appears to be successful.

Significance: 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 Kentucky experience may serve as a model for similar programs elsewhere in the U.S.

Proposed Course of Project: Data analysis and publication will follow.

Publications:

Baumann, R.J. and Leonidakis, M.G.: Extending Neurologic Services to Rural Children. Accepted for publication by Neurology.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02301-03 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Collaborative Studies of Less Common or Less Debilitating Neurologic Disorders		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: B.S. Schoenberg Chief Section on Neuroepidemiology ODIR NINCDS Other: J.E. Hogg Neurologist Section on Neuroepidemiology ODIR NINCDS T. Kudrjavcev Neurologist Section on Neuroepidemiology ODIR NINCDS R.J. Baumann Neurologist Section on Neuroepidemiology ODIR NINCDS		
COOPERATING UNITS (if any) R. Kaslow, M.D., Atlanta, Georgia; Neurosciences Program, WHO, Geneva, Switzerland; D. Duane, M.D., B. Sandok, M.D., Mayo Clinic; University of Kentucky; D.E. Kobrin, M.D., Stockton, California		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.4	OTHER: 0.9
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) 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 <u>space/time clusters of neurologic disorders</u> may provide leads to etiology or therapy. These may be tested through more formal approaches.		

Project Description:

Objectives: To investigate and characterize unusual associations, patterns, or phenomena associated with neurologic diseases.

Methods Employed: In collaboration with other governmental agencies (Center for Disease Control), international organizations (World Health Organization), and universities (Mayo Medical School, University of Kentucky) methods have been developed to investigate less common neurologic disorders. Several such studies have been completed. Current projects include investigation of space/time clusters of neurologic disease (with the Center for Disease Control), 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 description of lymphocyte ultrastructure in Batten's disease (with the Clinical Investigations and Therapeutics Section, DMN, NINCDS and the University of Kentucky), and a study of the relationship between headache and hypertension (with the University of Kentucky).

Major Findings: Lymphocyte fingerprint profiles have been described in neuronal ceroid-lipofuscinosis. An unusual manifestation of aqueductal stenosis was reported. The magnitude of the Guillain-Barré syndrome was investigated in a well-defined population. This is only the second such study in the United States and provides a baseline for evaluating future phenomena (such as the increased occurrence with immunization against influenza A/New Jersey).

Proposed Course of Project: Several such ongoing studies will be analyzed and reported. New investigations are undertaken on an ad-hoc basis.

Publications:

Baumann, R.J. and Markesbery, W.R.: Juvenile Amaurotic Idiocy (Neuronal Ceroid-Lipofuscinosis) and Lymphocyte Fingerprint Profiles. Annals of Neurology 4: 531-536, 1978.

Robinson, R.O. and Baumann, R.J.: Late Cerebral Relapse of Congenital Toxoplasmosis. Accepted for publication by Arch. Dis. Child.

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Hogg, J.E., and Schoenberg, B.S.: Paralysis of divergence in an adult with aqueductal stenosis: case report. Accepted for publication by Archives of Neurology.

Hogg, J.E., Kobrin, D.E., and Schoenberg, B.S.: The Guillain Barré Syndrome; Epidemiologic and Clinical Features. J. Chronic Dis. 32: 227-231, 1979.

Note: This project number includes the previous projects: Z01 NS 02301-02 OBE; Z01 NS 02302-02 OBE; Z01 NS 02242 OBE; Z01 NS 02245-02 OBE; and Z01 NS 02346-01 OBE.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02305-03 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) The Epidemiology of Intracranial Neoplasms		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: B.S. Schoenberg Chief Section on Neuroepidemiology ODIR NINCDS Other: T. Kudrjavcev Neurologist Section on Neuroepidemiology ODIR NINCDS		
COOPERATING UNITS (if any) B.W. Christine, M.D., M.P.H., Connecticut State Dept. 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 Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.67	PROFESSIONAL: 0.67	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The Section has conducted extensive investigations on the descriptive epidemiology of primary intracranial neoplasms using data derived 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.		

Project Description:

Objectives: To resolve problems in nomenclature, disease definitions, and survey strategies; to define the magnitude and distribution of primary intracranial neoplasms; and to investigate the relationship between intra- and extracranial tumors.

Methods Employed: Descriptive epidemiologic techniques were applied to data obtained from tumor registries around the world. Wherever possible, cases were reviewed by the Section's staff. New analytic techniques for studies of multiple primary cancers were devised.

Major Findings: On the basis of the descriptive studies, two patterns of age-specific incidence emerged. Analyses of most population-based data worldwide revealed 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 are currently underway 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 child-bearing age. 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.

Significance: Previous epidemiologic studies regarded all brain tumors as a single disease. The set of studies described above provided overwhelming evidence that the individual histologic types of primary intracranial neoplasms represent distinct disease entities. The analytic studies for the first time began to define risk factors for these tumors. Possible role of hormones in the etiology or growth of meningiomas may have therapeutic significance.

Proposed Course of Project: Several of these findings must still be prepared in a form suitable for publication.

Publications:

Schoenberg, B.S., Christine, B.W., and Whisnant, J.P.: The resolution of discrepancies in the reported incidence of primary brain tumors. Neurology 28: 817-823, 1978.

Schoenberg, B.S.: Cancer of Specific Tissues: Nervous System. In Schottenfeld, D., and Fraumeni Jr., J.F. (Eds.): Cancer Epidemiology and Prevention. Philadelphia, W.B. Saunders Co., in press

Annegers, J.A., Schoenberg, B.S., Okazaki, H., and Kurland, L.T.: Intracranial Neoplasms in Rochester, Minnesota, 1935-1977. In Rose, C.F., (Ed.): Clinical Neuro-Epidemiology. Tunbridge, England, Pitman Medical Publishing Co., in press

Note: This project number includes the previous projects: Z01 NS 02306-02 OBE; Z01 NS 02304-02 OBE; Z01 NS 20305-02 OBE; Z01 NS 02303-02 OBE.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02307-03 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Educational Resources in Neurological Epidemiology		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Bruce S. Schoenberg Chief Section on Neuroepidemiology ODIR NINCDS		
COOPERATING UNITS (if any) D. Schoenberg, M.S., Research Epidemiologist, Bethesda, Maryland		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCOS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) A series of four videotapes on the principles of neuroepidemiology were produced by the Section. A two-day international conference on neuro-epidemiology was held in 1977; a one-day <u>course</u> was held in 1977; a one-day symposium was held in 1979; and a one-day course is planned for 1981. A textbook entitled <u>Neurological Epidemiology: Principles and Clinical Applications</u> was published during 1978.		

Project Description:

Objectives: The severe shortage of available manpower in neuroepidemiology necessitated development of an educational program which was initiated by the Section.

Methods Employed: A series of four videotapes were produced by the Section. They are distributed on a loan-basis without charge by the National Medical Audiovisual Center, National Library of Medicine - the 1977 course and conference were held in cooperation with Georgetown University. The 1979 Symposium was held in conjunction with Yale University. A textbook on neurological epidemiology was published by Raven Press in 1978.

Major Findings: Attendance at the conferences included over 250 representatives from Asia, Africa, Europe, Latin America, and the U.S. Approximately 2000 copies of the textbook have been requested.

Significance: With such a limited supply of expertise in neurological epidemiology, these educational resources fill an important need in the neurosciences.

Proposed Course of the Project: A further course and additional videotapes have been requested.

Publications:

Schoenberg, B.S.: "General Concepts of Descriptive Epidemiology." In: PRINCIPLES OF NEUROEPIDEMIOLOGY. Produced by the National Institute of Neurological and Communicative Disorders and Stroke, 1978.

Schoenberg, B.S.: "Investigating an Epidemic; Cohort Analysis in Descriptive Epidemiology." In: PRINCIPLES OF NEUROEPIDEMIOLOGY. Produced by the National Institute of Neurological and Communicative Disorders and Stroke, 1978.

Schoenberg, B.S.: "General Concepts of Analytic Epidemiology." In: PRINCIPLES OF NEUROEPIDEMIOLOGY. Produced by the National Institute of Neurological and Communicative Disorders and Stroke, 1978.

Schoenberg, B.S.: "General Concepts of Experimental and Theoretical Epidemiology; Review." In: PRINCIPLES OF, NEUROEPIDEMIOLOGY. Produced by the National Institute of Neurological and Communicative Disorders and Stroke, 1978.

Schoenberg, B.S. (Ed.): Advances in Neurology: Neurological Epidemiology (Principles and Clinical Applications). New York, Raven Press, 1978.

Schoenberg, B.S.: Principles of Neurological Epidemiology: General Considerations. In Schoenberg, B.S. (Ed.): Advances in Neurology: Neurological Epidemiology (Principles and Clinical Applications). New York, Raven Press, 1978, pp. 11-16

Schoenberg, B.S.: Principles of Neurological Epidemiology: Descriptive Epidemiology. In Schoenberg, B.S. (Ed.): Advances in Neurology: Neurological Epidemiology (Principles and Clinical Applications). New York, Raven Press, 1978, pp. 17-42

Schoenberg, B.S.: Principles of Neurological Epidemiology: Analytic, Experimental, and Theoretical Epidemiology. In Schoenberg, B.S. (Ed.): Advances in Neurology: Neurological Epidemiology (Principles and Clinical Applications). New York, Raven Press, 1978, pp. 43-54

Schoenberg, B.S.: The Epidemiology of the Guillain-Barré Syndrome. In Schoenberg, B.S. (Ed.): Advances in Neurology: Neurological Epidemiology (Principles and Clinical Applications). New York, Raven Press, 1978, pp. 249-260

Schoenberg, B.S.: The Epidemiology of Primary Nervous System Neoplasms. In Schoenberg, B.S. (Ed.): Advances in Neurology: Neurological Epidemiology (Principles and Clinical Applications). New York, Raven Press, 1978, pp. 475-496

Kudrjavcev, T.: Neurologic complications of thyroid dysfunction. In Schoenberg, B.S. (Ed.): Advances in Neurology: Neurological Epidemiology (Principles and Clinical Applications). New York, Raven Press, 1978, pp. 619-636.

Hogg, J.E.: Neurological Complications of Acute and Chronic Renal Failure. In Schoenberg, B.S. (Ed.): Advances in Neurology: Neurological Epidemiology (Principles and Clinical Applications). New York, Raven Press, 1978, pp. 637-644.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02369-01 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Cigarette Smoking and Parkinson's Disease		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Robert J. Baumann Neurologist Section on Neuroepidemiology ODIR NINCDS		
COOPERATING UNITS (if any) University of Kentucky		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.5	PROFESSIONAL: 0.5	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This is a case-control study of cigarette smoking and other factors associated with <u>Parkinson's disease</u> .		

Project Description:

Objectives: To re-investigate the negative association between cigarette smoking and Parkinson's disease reported by others.

Methods Employed: A case-control study was employed.

Major Findings: The investigation confirmed previous reports of an inverse relationship (i.e., a low rate of smoking among those with Parkinson's disease as compared to the smoking rate among controls).

Significance: Previous studies found fewer cigarette smokers among persons with Parkinson's disease than among other patients. This study reconfirmed this finding using non-patient controls. Possible explanations include selective mortality, premorbid behavioral or constitutional attributes, the presence of an anti-parkinsonian chemical in cigarette smoke, or some combination of these factors.

Proposed Course of Project: The results have been prepared for publication during this fiscal year.

Project Description:

Objectives: To accurately document possible racial differentials in the prevalence of major neurologic disorders in a well-defined biracial population.

Methods Employed: A strategy was developed which eliminated the requirement that persons must have entered the health care system for detection of disease. 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 99% 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.

Major Findings: The data are currently being analyzed, and no findings are yet available.

Significance: A number of early investigations suggested possible differences in neurologic disease frequency by race, but were based on hospital or clinic experience and these studies 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. The present study should eliminate these potential sources of bias.

Proposed Course of Project: The interviews and examinations have been completed, and the data are being edited and analyzed. Similar strategies are being developed for application in developing countries, in collaboration with the World Health Organization.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02246-03 ODIR
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PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (30 characters or less)

Alzheimer's Disease Questionnaire and Case-Control Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bruce S. Schoenberg, M.D., M.P.H., Chief, Section on Neuroepidemiology,
ODIR, NINCDS
OTHER: Judith E. Hogg, M.D., Neurologist, Section of Neuroepidemiology,
ODIR, NINCDS

COOPERATING UNITS (if any)

Dr. Albert Heyman, Professor, Department of Neurology, Duke University

LAB/BRANCH

Office of the Director, Intramural Research Program

SECTION

Neuroepidemiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER
- (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Development of a suitable epidemiologic protocol and questionnaire for a case-control study of Alzheimer's disease. This project has been completed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02295-03 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) ALS Registry & Questionnaire		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Bruce S. Schoenberg, M.D., M.P.H., Chief, Section on Neuroepidemiology, ODIR, NINCDS OTHER: Judith E. Hogg, M.D., Neurologist, Section on Neuroepidemiology, ODIR, NINCDS		
COOPERATING UNITS (if any) NONE		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.05	PROFESSIONAL: 0.05	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Development of operational systems and a series of questionnaires to be used in an <u>ALS registry</u> being implemented by the ALS Society of America. This project has been completed.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02344-02 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Otitis Media and Developmental Delay -- Epidemiologic Factors in Research Design		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Tatiana Kudrjavcev, M.D., Neurologist, Section on Neuroepidemiology, ODIR, NINCDS OTHER: Bruce S. Schoenberg, M.D., M.P.H., Chief, Section on Neuroepidemiology, ODIR, NINCDS		
COOPERATING UNITS (if any) Dr. David Hanson, Otolaryngology, Communicative Disorders Program, NINCDS		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: .25	PROFESSIONAL: .25	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This research was presented at a NINCDS-sponsored <u>Workshop on Otitis Media and Child Development</u> held in March 1978. It consists of a critical assessment of available literature on the epidemiology of otitis media, discussion of critical factors in the design of research to investigate the possible association of <u>otitis media</u> with <u>developmental disability</u> , and a proposal for a retrospective study of this association in a defined population. This project has been completed. Publications: Kudrjavcev, T. and Schoenberg, B. S.: Otitis Media and Developmental Disability: Epidemiologic Considerations. <u>The Annals of Otolology, Rhinology & Laryngology</u> , Supplement 60 -- Vol. 88, September - October 1979, No. 5, Part 2, pp.88-98.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02296-03 ODIR
PERIOD COVERED October 1, 1978 though September 30, 1979		
TITLE OF PROJECT (80 characters or less) Registry for Cruetzfeldt-Jakob Disease		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Bruce S. Schoenberg, M.D., M.P.H., Chief, Section on Neuroepidemiology, ODIR, NINCDS		
COOPERATING UNITS (if any) Charles Poser, Department of Neurology, University of Vermont		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.01	PROFESSIONAL: 0.01	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Development of a protocol for the implementation of a registry for <u>Cruetzfeldt-Jakob disease</u> to include epidemiologic pathologic, and clinical information. This project has been terminated.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02345-02 ODIR
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Neurologic Complications of Graves' Disease		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Tatiana Kudrjavcev, M.D., Neurologist, Section on Neuroepidemiology, ODIR, NINCDS OTHER: Bruce S. Schoenberg, M.D., M.P.H., Chief, Section on Neuroepidemiology, ODIR, NINCDS		
COOPERATING UNITS (if any) Mayo Clinic, Rochester, Minnesota		
LAB/BRANCH Office of the Director, Intramural Research Program		
SECTION Neuroepidemiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Records of all cases of Graves' disease diagnosed at the Mayo Clinic during years 1935-1967 will be abstracted in search of neurologic manifestation in an effort to determine epidemiologic parameters of <u>neurologic manifestations of Graves' disease</u> in a defined population. This project has been terminated.		

ANNUAL REPORT

October 1, 1978 through September 30, 1979

Developmental Brain Pathology Section, ODIR
National Institute of Neurological and Communicative Disorders and Stroke

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Pathogenesis of Brain Injury Produced by Cardiovascular and Pulmonary Diseases Z01 NS 02020-07 ODIR	81

ANNUAL REPORT
October 1, 1978 through September 30, 1979
Developmental Brain Pathology Section, IRP
National Institute of Neurological and Communicative
Disorders and Stroke

Ronald E. Myers, M.D., Ph.D., Chief

Previous studies have incriminated accumulation of lactic acid at concentrations greater than 18 to 20 μ moles/g as the critical tissue change leading to brain edema and necrosis following exposure to oxygen deficiency states. An animal's carbohydrate state reflecting recent food intake or glucose infusion significantly and often dramatically determines whether brain pathology will develop, what its distribution will be and whether the animal will survive in response to anoxia or hypoxia. A high serum glucose concentration leading to an increased brain carbohydrate content results in severe brain devastation while food deprivation markedly protects the brain from exposure to circulatory arrest. Food-deprived monkeys exposed to more than 14 and up to 24 minutes of circulatory arrest show damage restricted to specific nuclei in brain stem while animals pretreated with glucose infusions or normally fed develop brain edema and widespread tissue destruction and generally fail to survive long term following exposure to circulatory arrest. Studies this past year have demonstrated the vulnerable brain stem nuclei accumulate lactic acid at concentrations greater than 18 to 20 μ moles/g while other brain structures not injured accumulate it at lesser concentrations during exposure to circulatory arrest. Thus, still another study shows, a relation between lactic acid accumulation at high concentrations and brain tissue injury. The basis for lactic acid accumulation at high concentrations in the vulnerable brain stem nuclei lies in their normal high glycogen contents. Glucose infusion or normal feeding leads to lactic acid accumulation throughout all brain structures at concentrations higher than is observed in food-deprived animals.

We have demonstrated marked species differences in response to exposure to anoxia or hypoxia both with respect to brain metabolic and biochemical response and development and distribution of brain pathology. Current studies with different animal species have demonstrated wide differences in brain carbohydrate content and lactic acid accumulation during exposure to circulatory arrest while a close correlation exists between these metabolic and biochemical parameters and the brain pathologic response to oxygen deprivation.

Mode of death or whether animals or man die suddenly or experience a prolonged terminus critically determines the

brain's response to placement in physiologic solutions. The brains of animals that experience sudden death imbibe large quantities of water and show marked weight gains during the early hours and, at the same time, they show marked organellar and cytoplasmic swelling and advanced "autolytic" changes. In contrast, the brains of animals that experience a prolonged terminus associated with marked hypoxia imbibe only slight quantities of fluid and gain minimally in weight during incubation in physiologic solutions. The brains of these animals also show minimal changes in cellular morphology. This and other studies show the central effect of lactic acid accumulation at high concentrations in tissue in the alteration of the morphology and function of the membranes of cells and all their organelles. We are presently developing in vitro models using mitochondria that should provide critical answers as to the nature and time course of the changes in membrane characteristics that result from exposure of animals to different types of oxygen deficiency states and also from a more direct exposure of these organelles to lactic acid in the medium at various concentrations.

We have initiated a number of studies on the pathophysiology and pathogenesis of intraventricular hemorrhage in the premature infant. This disease process serves as a major cause of death in premature infants taking place two to three days following delivery. Intraventricular hemorrhage is generally believed to result from asphyxia at birth or during the newborn period. However, the evidence from both clinical and experimental studies is far from convincing while current studies in our own laboratory with sheep fetuses also have failed to demonstrate any relation between exposure to prolonged asphyxia and intraventricular hemorrhage. Rather, sheep fetuses exposed to two hours of marked hypoxia have exhibited widespread symmetrical hemispherical necrosis. Furthermore, production of marked hypotension for prolonged periods by rapid blood withdrawal and the rapid reintroduction of this and additional blood leading to periods of marked arterial and nervous hypertension all taking place during marked hypoxia has failed to improve the effectiveness of the asphyxic model in leading to any intracranial hemorrhage. These studies reveal no correlation between exposure to marked hypoxia either alone or in combination with arterial and venous hypertension and occurrence of intracranial hemorrhages. In contrast to these negative studies, experiments with infusion or injection of hyperosmolar solutions intravenously into midgestational sheep fetuses have demonstrated a direct correlation between an increased osmotic pressure of the circulating blood beyond a certain limit and the occurrence of clinically significant intracranial hemorrhages usually affecting the subdural rather than the germinal matrix region. These latter studies implicate

reductions in cerebral tissue pressure and brain shrinkage as brought about in a variety of clinical circumstances in the causation of intraventricular hemorrhage in premature infants. These experimental findings with sheep fetuses correlate well with a number of changes that have been demonstrated to occur in the human premature infant and provide significant new insights as to well-defined methods that may be instituted for the prevention of intraventricular hemorrhage in man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01388-14 ODIR												
PERIOD COVERED October 1, 1978 to September 30, 1979														
TITLE OF PROJECT (80 characters or less) Perinatal Asphyxia and Its CNS Consequences														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI: Ronald E. Myers</td> <td>Chief</td> <td>DBPS, ODIR, NINCDS</td> </tr> <tr> <td>Pauline Ting</td> <td>Staff Fellow</td> <td>DBPS, ODIR, NINCDS</td> </tr> <tr> <td>Gabrielle de Courten</td> <td>Visiting Associate</td> <td>DBPS, ODIR, NINCDS</td> </tr> <tr> <td>Michael Hirsch</td> <td>Visiting Associate</td> <td>DBPS, ODIR, NINCDS</td> </tr> </table>			PI: Ronald E. Myers	Chief	DBPS, ODIR, NINCDS	Pauline Ting	Staff Fellow	DBPS, ODIR, NINCDS	Gabrielle de Courten	Visiting Associate	DBPS, ODIR, NINCDS	Michael Hirsch	Visiting Associate	DBPS, ODIR, NINCDS
PI: Ronald E. Myers	Chief	DBPS, ODIR, NINCDS												
Pauline Ting	Staff Fellow	DBPS, ODIR, NINCDS												
Gabrielle de Courten	Visiting Associate	DBPS, ODIR, NINCDS												
Michael Hirsch	Visiting Associate	DBPS, ODIR, NINCDS												
COOPERATING UNITS (if any) Womens & Infants Hospital of Rhode Island, Providence, Rhode Island Umea University, Umea, Sweden														
LAB/BRANCH Office of the Director, IRP														
SECTION Developmental Brain Pathology Section (Rockville, Maryland 20852)														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS: .33	PROFESSIONAL: .33	OTHER: None												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) This project investigates the <u>clinical</u> and <u>neuropathologic</u> alterations produced in <u>early sheep fetuses</u> by exposure to <u>asphyxia</u> and to infusion of <u>hyperosmolar solutions</u> at different stages of pregnancy, at birth, and during the newborn period. It correlates <u>patterns of brain injury</u> produced with <u>physiologic changes</u> observed at the time of asphyxia or hyperosmolar treatment.														
73 - ODIR/IRP (DBPS)														

Project Description:

Objectives: To develop an animal experimental model of intraventricular hemorrhage (IVH) as a step in the analysis of its causation and to determine the neuropathologic effects of marked hypoxia of the midgestational fetus or newborn. To correlate the patterns of brain pathologic change produced with physiologic alterations observed during experimental treatment.

Methods Employed: Sixty to 80 day sheep fetuses are subjected to 2 hour episodes of marked hypoxia by respiring the mother with atmospheres low in oxygen. Other fetuses are infused with hyperosmolar solutions raising the osmotic pressure of serum to values in excess of 330 milliosmoles per liter. Respiratory, cardiovascular, acid-base, blood gas, and osmotic pressure changes produced are recorded and correlated with pathologic changes produced.

Major Findings: Exposure of midgestational fetuses to marked hypoxia failed to produce any germinal matrix hemorrhage (GMH) or IVH. Withdrawal of blood during exposure causing a shock-like state and rapid reinjection of the blood raising the intravascular pressure to high values failed to modify the basic response to hypoxia. Exposure to marked hypoxia did lead to widespread cortical necrosis and damage to white matter and basal ganglia. Injection of hyperosmolar solutions (sorbitol), on the other hand, regularly produced subdural hemorrhages but no involvement of the germinal matrix.

Significance: Animal exposure to hyperosmolar stimulation but not to hypoxia or raised intravascular pressures leads to intracranial hemorrhages. These experimental findings agree with a great deal of clinical data to suggest the critical changes leading to IVH are reduced cerebral tissue pressures caused by dehydration, hypovolemia, or hyperosmolar treatment during the first 72 hours following delivery in the newborn nursery in premature infants. The production of widespread hemispherical necrosis in the midterm fetuses from hypoxia provides the basis for understanding a wide variety of cerebral malformations including porencephaly and hydranencephaly.

Proposed Course of Project: The timing and characteristics of the infusion of hyperosmolar solutions and the possibility of causing intracranial hemorrhages in the midterm fetus by manipulating other variables will be investigated.

Publications:

Myers, R.E. and Myers, S.E.: Use of sedative, analgesic, and anesthetic drugs during labor and delivery: Bane or boon? Am. J. Obstet. Gynecol. 133: 83-104, 1979.

Myers, R.E., Joelsson, I., and Adamsons, K.: The effects of isoproterenol on fetal oxygenation. Acta. Obstet. Gynecol. Scand. 57: 317-322, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01464-13-ODIR
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemistry of Brain Damage due to Hypoxia or Anoxia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Ronald E. Myers Chief DBPS, ODIR, NINCDS
Kenneth R. Wagner Staff Fellow DBPS, ODIR, NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Office of the Director, IRP

SECTION
Developmental Brain Pathology Section (Rockville, Maryland 20852)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.33	PROFESSIONAL: .33	OTHER: 1.0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This work investigates alterations in activity or concentration of various brain enzymes and substrates in response to exposure to anoxia or hypoxia in various areas of cortex, basal ganglia, white matter, and nuclei in the brain stem. Tissue assays are carried out both during and following exposure to anoxia and hypoxia. Comparisons are made of the changes in enzyme activities and substrate concentrations in the brains of animals that develop brain edema and in the brains of those that recover without injury.

Project Description:

Objectives: To determine and compare the biochemical changes produced in different brain structures by hypoxia and anoxia and to relate these compositional changes to development of brain edema and distribution of brain pathology. A comparative study will define these changes in brain composition in response to anoxia and hypoxia in several animal species to attempt to account for differences in pathologic response.

Methods Employed: Monkeys and other animals under barbiturate anesthesia are exposed to controlled episodes of anoxia or hypoxia. Blood PO_2 , PCO_2 , and pH are analyzed and blood pressure, heart rate, respiration and EEG recorded. Brain tissue samples are taken and frozen after different durations of exposure and recovery and are analyzed for glucose, glycogen, ATP, ADP, phosphocreatine, lactate, pyruvate, and cyclic-AMP. Fresh tissues are also analyzed for ATPase activity, alkaline and acid phosphatase activities, etc.

Major Findings: Differences between anoxia and hypoxia: Anoxic brain tissue shows marked decreases of ATP and moderate increases of lactate. Hypoxic brain tissue shows much greater accumulations of lactate, an increased alkaline phosphatase activity, but only moderate decreases of ATP content.

Brain swelling after hypoxia: Brain tissue samples 5 hours after exposure to moderate hypoxia either show near-normal water, ATP and lactate contents and normal enzymatic activities or an increased water content, marked accumulation of lactate, decrease in ATP, and increased alkaline phosphatase activity. These two outcomes are seen in the brains of animals that either show complete recovery or develop brain edema.

Effects of glucose administration on response to anoxia: Administration of glucose or merely normally feeding rather than food-depriving animals prior to exposure to anoxia greatly diminishes brain tolerance to exposure to anoxia and alters the resulting brain pathology. Glucose-infused or normally fed animals show markedly greater increases of lactate in brain tissue following arrest of circulation than do saline-infused animals. A close correlation exists between magnitude of lactate accumulation in brain tissue and later development of brain edema and tissue necrosis. Serum glucose concentration prior to arrest correlates directly with magnitude of lactate accumulation and extent of brain pathology following exposure to circulatory arrest.

Topography of lactate accumulation in brain during circulatory arrest: Analysis of different brain structures following exposure to circulatory arrest shows high lactate accumulation in those structures vulnerable to injury while other structures accumulate lactate at lower concentrations. What explains the marked accumulation of lactic acid in vulnerable structures are their normally high glycogen contents. Prior feeding or infusion of glucose elevates the lactic acid values of all brain structures following exposure to circulatory arrest and greatly modifies the extent and distribution of pathologic change.

Significance: Post-hypoxic brain edema is closely correlated with accumulation of lactic acid in brain tissues at concentrations greater than 20 $\mu\text{mole/g}$. Infusion of glucose or normal feeding prior to exposure leads to excessive lactate accumulation, diminished tolerance to anoxia, and exaggerated brain pathologic response. Brain structures vulnerable to injury from anoxia show high glycogen contents in normally oxygenated animals and high lactic acid accumulation during circulatory arrest. Achievement of lactic acid accumulations greater than 18 to 20 $\mu\text{mole/g}$ results in tissue injury. Major differences exist in the carbohydrate content of different brain structures in different animal species and these lead to major differences in extent and distribution of lactate accumulation during circulatory arrest and account for major differences in pathologic response to challenge.

Proposed Course of Project: Further studies will define the temporal and quantitative relations between serum glucose concentration at exposure to anoxia and extent of lactic acid accumulation in brain tissues. In vitro mitochondrial models of anoxic and hypoxic exposure will be developed to further define the importance of concentrations of lactic acid on membrane structure and function and changes in mitochondrial activity in these various states.

Publications:

Myers, R.E.: Lactic Acid Accumulation as Cause of Brain Edema and Cerebral Necrosis Resulting From Oxygen Deprivation. In Korobkin, R. and Guilleminault, C. (Eds.): Advances in Perinatal Neurology. New York, Spectrum Publ., 1979, pp. 85-114.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE	PROJECT NUMBER
	NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02020-07 ODIR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Pathogenesis of Brain Injury Produced by Cardiovascular
and Pulmonary Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Ronald E. Myers Chief DBPS, ODIR, NINCDS
Shun-Ichi Yamaguchi Research Psychologist DBPS, ODIR, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Office of the Director, IRP

SECTION
Developmental Brain Pathology Section (Rockville, Maryland 20852)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .33	PROFESSIONAL: .33	OTHER: None
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER
 (s1) MINORS (s2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project investigates a variety of types of insults including circulatory arrest, arterial hypotension, hypoxia, asphyxia, cyanide intoxication, and exposure to carbon monoxide and its effects on the juvenile rhesus monkey brain. Alterations in cardiovascular performance and nervous system activity are examined during exposure and distribution of brain injury produced are determined by post-mortem brain examination after long-term survival. The patterns of pathology produced are correlated with alterations in cardiovascular and respiratory activity during exposure in an attempt to define the pathogenesis of specific patterns of brain injury.

Project Description:

Objectives: To develop animal experimental models of human brain pathology produced by cardiovascular and pulmonary disorders. These include studies of interference with oxygen supply to tissue due to cessation of circulation (cardiac arrest), hypotension, pulmonary disease, myocardial abnormalities, or intoxications with agents such as carbon monoxide or cyanide.

Methods Employed: Adult monkeys or other animals are anesthetized with pentobarbital and a femoral artery and vein is catheterized to record blood pressure and heart rate and to withdraw blood samples for analysis of acid-base and respiratory gas values. EKG, EEG and other physiologic parameters including cortical impedance, intracranial pressure, electrolyte composition of blood and CSF, glucose content of blood and CSF, etc. are monitored. The animals are subjected to one of the insults described and the physiologic and biochemical changes produced are measured. The animals are resuscitated and neurologic and pathologic abnormalities evaluated and correlated with the physiologic changes measured.

Major Findings: The brain pathology produced by circulatory arrest depends on nutritional state. Animals food-deprived for 24 hours are markedly tolerant to circulatory arrest - 12 to 14 minutes of circulatory arrest are required to produce first brain injury. Twenty four minutes may be well tolerated. Brain damage in food-deprived animals affects nuclear structures in brain stem leaving hemispherical structures intact. In contrast to this, exposure of normally-fed or glucose-infused animals to circulatory arrest of the same duration leads to brain edema, altered blood-brain-barrier function, and widespread cerebral necrosis. Brain pathologic response to anoxia depends on nutritional state.

The monkey can tolerate a mean blood pressure lowering to 35 mm Hg for prolonged periods without brain injury or damage to the heart. Animals exposed to a mean blood pressure between 25 to 35 mm Hg for longer than 45 minutes, though they also escape brain injury, die in the early hours following blood pressure restoration of cardiogenic shock. Animals exposed to a mean blood pressure below 25 mm Hg for 25 to 30 minutes all die during the first 6-48 hours after restoration of blood pressure as a result of brain edema and brain stem compression. Only a small proportion of animals survive to show static lesions in the brain. What static lesions can be demonstrated are related to large blood vessel compression secondary to brain edema.

Mode of death determines magnitude of in vitro brain swelling and extent of "autolytic" cell change. Rapid death leads to major in vitro water imbibition by brain and "autolytic" change while a prolonged terminus sharply limits such changes. Present evidence suggests that a preserved or impaired integrity of cell and organellar membranes in these two circumstances determines outcome: preserved membranes are associated with a major imbibition of water and marked cellular swelling with membrane disruption while leaky or impaired membranes are associated with minor changes in tissue water content and cell morphology.

Significance: These studies elucidating the pathogenesis of brain injury caused by circulatory or pulmonary diseases are critical to the development of techniques for the prevention or amelioration of associated brain pathology. Our present studies make clear the critical importance of history of food intake and serum glucose concentration on the nervous system response to oxygen lack. They also indicate that excessive lactic acid accumulation as a consequence of anoxia or hypoxia critically determines brain pathologic outcome by exerting critical effects on cell and organellar membrane integrity.

Publications:

Selkoe, D.J. and Myers, R.E.: Neurologic and cardiovascular effects of profound hypotension. Stroke. 10: 147-157, 1979.

Mirvis, D.M., Kopf, G.S., and Potalla, E.W.: Harmonic analysis of the left ventricular waveform of the primate. Cardiology 63: 79-93, 1978.

ANNUAL REPORT

October 1, 1978 through September 30, 1979

Medical Neurology Branch

National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report
October 1, 1978 through September 30, 1979
National Institute of Neurological and Communicative Disorders and Stroke
Medical Neurology Branch

John L. Sever, M.D., Acting Chief

Neuromuscular Diseases Section

Introduction: An inter-related multidimensional attack on the chosen target diseases is emphasized in our application of basic research techniques: tissue culture, histochemistry, electronmicroscopy, immunology, autoradiography, biochemistry, and clinical neurophysiology. In the human neurologic disorders studied, these techniques support thrusts to seek: (a) more precise morphologic, electrical, immunologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct and often new sub-forms; (c) specific or symptomatic treatment; and (d) induced animal models closely related to the human pathophysiologic states. Our emphasis is on the neuromuscular diseases -- they affect more than 1,000,000 persons in the country.

For the clinical investigations, 651 patients were admitted for a total of about 14,301 patient days, and there were 1,168 outpatient visits. About 416 patient muscle and nerve biopsies were processed histochemically and reported out -- about 52 of those were from outside hospitals. Many other outside biopsies are submitted for formal opinion. Clinical electrophysiologic studies were performed on 332 patients including 36 consult patients. In the past year 38 articles were published, 8 are in press, and there were more than 50 presentations to meetings.

Approximately 14 neurologists and other physicians and 9 technicians came this past year to learn clinical and laboratory research techniques in neuromuscular diseases. A number of medical students and residents from various other hospitals rotated through our service. Many of our former trainees are full professors, associate professors, and assistant professors in academic departments, and many are directors of Muscular Dystrophy Clinics and Myasthenia Gravis Clinics; many are Medical Advisory Board members of the National Muscular Dystrophy, Myasthenia Gravis, Amyotrophic Lateral Sclerosis, and Multiple Sclerosis associations.

We have a collaborative program on neuromuscular diseases in Paris with Hopital Salpetriere, CNRS, and Institut de Pathologie Moleculaire.

I. Amyotrophic Lateral Sclerosis: The disease usually causes death within 2-5 years, although some cases are more chronic. 90% of the cases are sporadic, 10% are dominantly inherited. The cause of ALS is unknown -- dysmetabolic vs. viral are the two main possibilities. We favor the former but are pursuing both.

A. Therapeutic Trials: Four drugs and one procedure have been studied over the past two years. Phthalazinol is an inhibitor of cAMP phosphodiesterase, and of cGMP phosphodiesterase to a lesser extent. In ALS patients it raised to normal the lowered endogenous cAMP of CSF but not the lowered

endogenous cGMP. In 25 ALS patients it did not alter the progression of the disease. In one patient with the bulbar form, progression has arrested but whether that was due to the drug or was spontaneous is not known. Phthalazinol has recently caused three side-effects of interest in 4 patients with both diabetes mellitus and ALS; increased glucosemia/glucosuria and diarrhea, and in one, the appearance of a new peptide band in isoelectric-focused CSF. Whether the findings can be related to the pathogenesis of the motor neuron degeneration in these patients is being explored. Human leucocyte interferon, an antiviral substance, did not benefit one ALS patient. Polyinosinic-polycytidylic-acid-poly-l-lysine (Poly-ICLC), an inducer of interferon did not benefit three ALS patients. Adenine arabinoside is an antiviral compound. Although the code has not been broken, there were no dramatic benefits from either 10-day course of the two coded agents (drug vs. placebo) given to 10 ALS patients. Plasmaphoresis for 8 weeks in 3 ALS patients was not beneficial.

B.. CSF biochemical studies: The CSF is a thesaural swamp of interesting central nervous system (CNS) transmitters and other metabolites. In ALS we have previously found HVA and cAMP to be low, but raising them to normal levels was not associated with clinical benefit. We found cGMP low and have not yet raised it to normal. (A chapter on cyclic nucleotide metabolism and the CSF is in press.) We are now quantitatively analyzing CSF total fatty acid content and composition by thin-layer and gas chromatography. In ALS patients vs. normals and disease controls, we have worked out the ionic-exchange and liquid chromatographic methods for quantitating bases and nucleosides in patients' CSF. Enolases, neuron-specific and non-neuronal, are now being studied in ALS CSF. Cultured motor neurons of 17-day fetal-rat ventral spinal cord serve as test-objects of possibly toxic fluids or agents related to ALS patients. To date, comparing CSF of ALS patients with controls, there was no detrimental effect visible morphologically. The level of neuron-specific enolase in such treated cultures is being determined.

C. Blood biochemical studies: (a) Fatty-acids of erythrocyte membranes are not abnormal in ALS. (b) ALS platelets have a reduced rate of initial uptake of ³H-serotonin and of initial release to 0.5 μ /ml thrombin, and normal cAMP and cGMP phosphodiesterase. (c) Leucocyte and erythrocyte insulin-receptors and glycogen content are being studied and correlated with plasma glucose and insulin levels. (d) Parameters of calcium homeostasis, which might or might not reflect parathyroid function, have shown: (i) greater reduction of retention of oral ⁴⁷CaCl₂ in ALS than in other neuromuscular diseases was found, and it was partly but not entirely attributable to inactivity; (ii) normal 25-hydroxy vitamin D levels; (iii) no resistance to dihydro-tachysterol (it mathematically corrected the decreased retention but did not cause clinical improvement); (iv) normal serum calcium, urinary 24-hour calcium excretion, and renal cAMP clearance; (v) mean serum parathyroid hormone levels higher than normal, but this was also found in other categories of neuromuscular diseases (myopathy, peripheral neuropathy). To be studied is the response to imposed serum fluctuations of calcium.

D. Motor Neuron Biology: Histochemical properties of lower motor neurons (LMNs) continue to be explored, seeking special properties of them and disease-characteristic defects thereof. To date 25 reactions -- enzymatic, non-enzymatic and lectin-binding -- have been applied, and the only ones seeming to be of possible relevance to pathogenesis are the high phosphorylase and low succinate dehydrogenase. Additional reactions will be explored. Bidirectional trophisms between motor neurons, muscle fibers and schwann cells are being studied by isolation of fractions containing putative factors and testing for influences on animal and human motor neurons, schwann cells and muscle fibers in culture.

E. Viral Studies: In ALS patients we continue to search for evidence of a viral cause. However, our negative evidence to date includes: (a) lack of sera or CSF antibodies to 13 viruses (and one protozoan), (b) lack of oligo-clonal bands in the CSF, (c) failure to respond to human leucocyte interferon and to the interferon-inducer poly-ICLC, and (d) no major benefit from the antiviral agent adenine arabinoside.

F. Diseases possibly relevant to ALS: Benign Focal Amyotrophy is important because of its excellent prognosis but usual misdiagnosis as fatal ALS. It was originally described by us 11 yrs. ago as a separate clinical syndrome, and in young-adult males possibly a distinct disease. Our updated experience indicates it is a limited form of lower-motor-neuron disease clinically confined to the upper extremities, unilateral or markedly asymmetric, gradual in onset, progressive for 1/2-4 years and then clinical stability, or only very minimal progression, for 2-11 yrs to present. We have recently found CSF oligoclonal Ig bands in several patients, raising a question of a viral/dysimmune pathogenesis. If related to ALS as a spontaneously arresting form, it may hold a clue to understanding and treating that disease. In central core disease, we have in press our newly formulated hypothetical pathogenesis. It is based on our histochemically showing marked paucity of type-II muscle fibers, normal appearance and distribution of the subtypes of type-I muscle fibers, and no densification of motor units by single-fiber-EMG (SFEMG). We propose the weakness to be based on a paucity of lower motor neurons (LMNs), especially of the hypothetical type-II LMNs, occurring during development, due to either impaired formation or increased normal loss as a part of "neurothanasos" (Hamburger's term for the normal loss of LMNs in embryonic chick cord). The muscle fibers themselves are proposed to be abnormally constructed, perhaps because of defective LMN trophic influence, because they contain cores and have a susceptibility to develop malignant hyperthermia. An aspect of neurothanasos may, on the basis of SFEMG and muscle histochemistry of II-fiber paucity without I-subtype grouping, also play a role in the pathokinesis of congenital rod disease and some forms of benign congenital hypotonia.

II. Polyneuropathy (peripheral neuropathy): The peripheral neuropathies comprise a group of disorders of various causes, but unknown in more than half the patients. They always cause serious physical handicap sooner or later, sometimes associated with intractable pain and ulceration and in extreme cases loss of feet and hands. Our studies seek to delineate the underlying causes and where possible develop a treatment. We also seek fuller understanding of the basic biology and pathologic responses of the lower motor and sensory neurons and their schwann cells. Dysschwannian neuropathies are ones in which

the neuronal-axon defect is considered secondary to schwann cell abnormality, whereas in dysneuronal neuropathies the lower motor and/or sensory neuron soma ± axon is the major site of abnormality.

A. Biology of the schwann cell: For basic biologic studies and for use as a test-object in human diseases, a technique was established for growing in tissue culture sciatic-nerve schwann cells from 3-day old rats. Ultrastructural and histochemical characteristics of the cultured rat schwann cells were identical to those of in vivo studied schwann cells in a very young animal, except for the presence of a basement membrane and myelin sheets in the latter.

To study human dysschwannian neuropathies we have developed techniques to grow reproducibly in tissue-culture human schwann cells obtained from diagnostic nerve biopsies. In primary or co-primary dysschwannian neuropathies (e.g., adrenomyeloneuropathy, metachromatic leucodystrophy, some forms of familial idiopathic "Charcot-Marie-Tooth" neuropathy) the schwann cells in culture should have the biochemical defect, which when identified can be treated in culture. Schwann cells might also express the defect in other neuropathies related to general metabolic defects such as diabetes mellitus. Our technique enables almost complete elimination of non-schwann cells. Histochemical, fluorescent, and ultrastructural characteristics of normal human schwann cells were determined. This will now serve as a basis to study various dysschwannian neuropathies. In one example of dysschwannian neuropathy, adrenomyeloneuropathy, we have detected in the cultured schwann cells ultrastructural and biochemical abnormalities -- the presence of very-long-chain fatty acids indicating a reincarnation and amplification of the defect in tissue culture.

B. Biochemical Studies: (a) Adrenomyeloneuropathy -- schwann cells and muscle cells were cultured from biopsies of two patients. Ultrastructurally increased amounts of lipid droplets and multilaminar inclusions were present in both cell types. Biochemically increased amounts of very-long-chain fatty-acids, C₂₂-C₂₆, were present in cultured schwann cells and even to a greater degree in cultured muscle cells. Cultured human skeletal muscle incorporated media-derived fatty-acids into several lipid classes including triglycerides, cholesterol esters, phospholipids and glycolipids. When cultured muscle of the patients was presented with C₂₂:0 or C₂₆:0 free fatty-acids in the medium, it accumulated 4-10 fold greater levels than control cultured muscle. The data demonstrated a generalized metabolic defect, and for the first time the defect was identified while the patient was alive.

(b) Substance P -- In CSF it was found by radioimmunoassay to be decreased in our neuropathy patients, but in no other neuromuscular diseases surveyed. All affected patients had sensory nerve involvement. Decrease of substance P was only approximately correlated with degree of loss of pain and temperature sensation.

C. Immunologic Studies: (1) In presumably-dysimmune relapsing neuropathy we have: (a) localized immunoglobulin complexes containing IgG, IgM and C₃ in sural nerves of 5 patients, and suggested the complexes to be a pathogenic mechanism; (b) found unfluctuating monoclonal IgG bands in CSF of chronic relapsing but not the acute "Guillain-Barre" form (which had polyclonal IgG), and suggested the monoclonal band might be a predictor of chronicity (c) found a serum IgM factor (? antibody) which bound specifically to

schwann cells in cultures of rat peripheral nerve in 4 of 5 patients and not in control sera, indicating a new tissue-culture test of pathogenic mechanisms; (d) found one case associated with a "non-secretory" osteosclerotic multiple myeloma with immunoglobulin deposition in peripheral nerve, indicating that to be a likely pathogenic mechanism.

(2) In presumably dysimmune progressive non-relapsing neuropathy: (a) eleven patients had a circulating monoclonal immunoglobulin spike without detectable myeloma or amyloid and with normal bone marrow -- immunoglobulin light chains were deposited in their nerve biopsies, and we suggested they represented circulating neurotoxic molecules; (b) one patient with IgM-k Waldenstrom's macroglobulinemia and neuropathy had deposits of IgM-k light chains in her sural nerve biopsy and lymphocytes bearing k-chains infiltrating that nerve -- treatment with chlorambucil and prednisone improved her neuropathy and lowered serum IgM levels.

(3) In the plasma cell dyscrasic form of amyloid neuropathy we have (a) found circulating immunoglobulin light-chains (IgG-kappa > IgG-lambda > IgM lambda) in all 10 pts., postulated non-amyloidogenic Ig light chain fragments as circulating neurotoxic molecules (c) found them deposited in peripheral nerves in 7 of 8 patients examined, and (d) in a unique case of polyneuropathy, amyloidosis and hypernephroma, crystal-violet-positive amyloid was found in muscle blood vessels and connective tissue but none in nerve; the excised tumor had amyloid of Ig- λ origin biochemically and immunochemically; antibodies against denatured λ -type amyloid protein bound to nerve, muscle and tumor (but antibodies against undenatured λ -protein did not); and by electron-microscopy deposits typically amyloid were present in nerve.

D. Therapy: (a) Polyinosinic-polycytidilic acid poly-l-lysine stabilized with carboxymethyl cellulose (Poly-ICLC), is a new treatment which we have found remarkably successful in a patient with chronic, presumably-dysimmune, relapsing polyneuropathy unresponsive to prednisone-plus-azathioprine. The patient went from electric-wheelchair dependency to walking 7 miles daily with 100 μ g/kg i.v. of weekly Poly-ICLC, now maintained for 18 months on 60-75 μ g/kg. These levels did not raise measurable interferon in the patient's serum. Three periods of several weeks off drug were associated with loss of strength, regained on resumption of drug. Although Poly-ICLC is an interferon-inducer, we postulate it is beneficial in this dysimmune disease by a new action we have found, marked lymphocytopenia (down to 10-20% of baseline) for 1-2 days after the drug with return to baseline by 4-5 days (granulocytes actually rose 3-fold at 6-24 hr. and fell only 30% below baseline at 2-3 days and returned to baseline by 3-5 days). A second patient with chronic dysimmune neuropathy resistant to prednisone-plus-cyclophosphamide now also appears to have benefited. We propose this to be a new antidysimmune treatment potentially beneficial to other dysimmune diseases. We are extending the trial to other dysimmune neuropathy patients, and to dermatomyositis/poly-myositis and myasthenia gravis patients. Preliminary data show no selective action of Poly-ICLC on any class or sub-class of lymphocytes, B, T, or natural-killer T. In preliminary studies, serially-sampled serum of the Poly-ICLC treated patient showed some ability to reduce mitogen responsiveness of his own lymphocytes, especially to PHA, within 6 hrs after injection; this is also the time point of maximum lymphocyte reduction. (b) Chlorambucil and

prednisone in Waldenstrom's macroglobulinemia, see II.c.(2) (c) Plasmaphoresis was clearly beneficial in one of two patients with chronic dysimmune neuropathy treated for 8 weeks. (d) High-single-dose alternate-day prednisone in chronic dysimmune, relapsing or progressive, polyneuropathy has a broader scope of responsivity than often believed. Favorable response is augered by the triad of (i) being dysschwannian in type (slow nerve-conduction times), (ii) relapsing, (iii) with elevated CSF protein; but even some non-relapsing patients (i.e., 2 with progressive course > 1 yr) without slowed nerve conduction times and with normal CSF have responded to long-term high-single-dose alternate-day prednisone. Long-term treatment is required -- too rapid reduction of dosage too soon results in exacerbation. Excellent results have been sustained for as long as 14 years in an adult and 11-1/2 years in a child (now age 22). Virtually all our corticosteroid-responsive patients are corticosteroid-dependent, requiring 5-20 mg single-dose q.o.d. to prevent exacerbation.

E. Other related conditions. (1) Unilateral calf hypertrophy was identified as a late sequel of discogenic sciatic radiculopathy.

III. Central Nervous System Disorders:

A. Spinocerebellar degenerations, which we have found virtually always to have a lower-motor-neuron component, comprise diseases of various causes, a few known, most not, which always result in serious physical handicap sooner or later in the course of the disease, and sometimes early death and/or mental deterioration. Our studies seek to delineate the underlying causes, where possible attempt to develop a treatment, and define basic cellular pathophysiologic mechanisms. Being reported is our new histochemical technique for adenylate cyclase, which synthesizes cAMP, considered to have an important role in cerebellar function. In the cerebellum the enzyme was found mainly in blood vessels, and of the neural-associated enzyme most was in basket-cell basket endings (not in Purkinje-cell somas). Treatment with a phosphodiesterase inhibitor to enhance cAMP is planned in spinocerebellar ataxia patients.

B. Progressive spastic paraplegia: This is a progressively crippling disorder to children and adults. The causes are not known. We have published a newly identified cause, adrenomyeloneuropathy, and have with tissue culture, v.s., demonstrated the biochemical defect for the first time while the patient is living.

Myopathies are non-neurogenic, primary or secondary diseases of muscle. Some, such as the dermatomyositis/polymyositis group, are often at least partially treatable, but their cause and details of their probably "dysimmune" pathogenesis are not known; others are not treatable but their cause is known, e.g., genetic deficiencies of phosphorylase, phosphofructokinase, acid maltase or carnitine-palmityl-transferase; while still others, such as Duchenne muscular dystrophy and other genetic disorders bearing the name "dystrophy", are of unknown pathogenesis and are untreatable. Some, such as malignant hyperthermia-rigidity, are preventable if identified.

Our tissue culture laboratory has been a major locus of studies. We

have qualitatively and quantitatively enhanced productivity in the culturing of human and animal muscle (and of human and animal schwann cells and animal neurons. Tissue culture of human muscle biopsies provides living muscle fibers growing free of all neural, vascular and humoral factors present in the patients. We obtain abundant, reproducible and mature growth of human fibers in culture, including spontaneous twitching, and can precisely select individual fibers for enzyme cytochemistry and immunocytochemistry at light-and electronmicroscopic levels and for various biochemical and microelectrode studies of them. This year we have grown 76 human muscle biopsies, as well as numerous rat muscle cultures.

I. Inherited Myopathies.

A. Biochemically distinct myopathies.

1. Lysosomal defects. The mechanism of muscle fiber damage is different from that of the afuelias. It probably involves leakage of the excess lysosomal hydrolytic enzymes to dissolve the fiber from within -- "an"endodissolution". Regeneration is minimal. (a) Acid maltase deficiency: Previously we demonstrated a reincarnation of the biochemical and morphologic abnormality in muscle cells cultured from acute-infantile, chronic-infantile and adult-onset forms of the disease, (i) establishing it as a true intrinsic defect of the muscle cell and (ii) providing a new test system for in vitro therapeutic trial, without risk to the patient. A recently late-onset proximal myopathy patient lacked any vacuoles or excess acid-phosphatase-positive areas in a biceps biopsy but had the classic findings in a quadriceps biopsy, with an equally complete lack of acid maltase in both original biopsies and in cultures of both, showing that (i) the enzyme defect is not necessarily accompanied by the morphologic defect and (ii) the erstwhile "classic" morphologic defect is not a pre-requisite to further biochemical analysis of our suspect patients. Muscle-fiber regeneration in acid maltase deficiency is minimal by histochemical criteria and the BB-isozyme of creatine kinase (v.i.) is correspondingly not elevated. (b) Cabbage-body myopathy is probably another lysosomal defect. The morphologic abnormality was reincarnated in muscle cultured from the patients. Assay of 12 lysosomal enzymes has not disclosed the defect.

2. Afuelias: We introduced the term "afuelias" to describe the defects, known and unknown, of (i) glycogen/glucose utilization and (ii) lipid, fatty-acid, ketone-body utilization. The former cause muscle-fiber breakdown during heavy exercise, especially ischemic exercise -- they include phosphorylase, phosphofructokinase and debrancher-enzyme deficiencies. The latter cause breakdown during fasting states -- they include failure to utilize long-chain fatty-acids, carnitine palmityl transferase deficiency and probably infantile fatal fasting rhabdomyolysis. (a) Glycogen/glucose utilization defects. We have reincarnated the phosphofructokinase and debranching enzyme defects in the patients' cultured muscle. However, the phosphorylase defect was "cured" in cultured fibers of 8 patients, as it is in regenerating fibers of the original biopsy. There has been a question whether enzyme rejuvenated in the cultured fibers is adult type phosphorylase -- we have established that it is by histochemical staining of isoelectric-focused gels and now by highly specific antibodies. Thus we have demonstrated a true rejuvenation of an

enzyme genetically programmed ultimately to be deficient in mature fibers. This opens a new therapeutic possibility, trying to provoke and maintain that phosphorylase in mature fibers of the patient. A summary of our other multidimensional correlated studies in the afuelias, including the induced model of iodoacetate-poisoned glyceraldehyde-3-phosphate dehydrogenase, has just been published. These include alternate-pathway therapy, our new diagnostic test of forearm ischemic exercise combined with localization of ^{99m}Tc -diphosphate, localization of excess calcium by histochemistry, autoradiography and electronmicroscopy in afuelically damaged fibers, and correlation of forearm ischemic exercise with single-fiber-EMG. (b) Lipid/fatty-acid/ketone-body utilization defects. Documented by us previously was the first defect in this category, impaired utilization of long-chain fatty-acids, some other cases of which were found by others to be due to carnitine palmityl transferase (CPT) deficiency. We are establishing this assay in our laboratory. Our original patients will be studied per their biopsied and cultured muscle. Our syndrome of fatal fasting infantile rhabdomyolysis is postulated to be in this category, but all studies to date have not shown an enzymatic deficiency (e.g., CPT was 2X normal). We will be studying cultured muscle of this. (c) Hypocyclasias: Reduced plasmalemmal adenylate cyclase but normal β -adrenergic receptors were found in three conditions, (i) muscle-fiber-hypotrophy-with-central-nuclei, (ii) myotonic atrophy, (iii) diazacholesterol-induced myotonia of intact rat muscle and of tissue-cultured rat muscle. In press is our study of muscle biopsies of affected infants from two families with X-linked recessive infantile-fatal muscle-fiber-hypotrophy-with central nuclei, flown to us from Amsterdam and Texas for tissue-culture. Both showed the same abnormalities: (1) cultured muscle cells had a marked, apparently uncontrolled proliferation, resembling that of neoplastic cells, which has persisted through many passages over 10 months, and was not controlled by CNS extract or CNS cocultures; (2) large multinucleated myotubes formed very early but never matured by light- or electronmicroscopic criteria and never contracted, only 60% of normal adenylate cyclase (basal, and NaF or isoprotorenol stimulated) in plasmalemma but normal β -adrenergic receptors (by ^{125}I -hydroxypindolol-binding). These findings demonstrated an intrinsic defect of the muscle cell, apparently an impaired control mechanism(s) related to cAMP. That could, among other effects, have resulted in impaired response to neurogenic maturational mechanisms, i.e., one kind of "myogenous dysinnervation". The identified defect now can be the basis of treating such patients with a phosphodiesterase inhibitor to increase cellular cAMP. In fact, one patient with somewhat similar biopsy findings was treated (with U. Rochester) -- there was no clinical benefit, but the patient's muscle fibers subsequently in culture did not show the abnormal growth, presumably indicating a different disease. (d) Other biochemical abnormalities. (i) Carnitine deficiency in myopathies has, by others, been found in several forms. It does not behave as a lysosomal defect or as an afuelia. To better screen our patients, we are setting up the assay. (ii) Adenylate deaminase (AD) deficiency of muscle, postulated by others to be pathogenetically significant, was studied by our improved histochemical method. Since the only abnormal muscle biopsy of > 100 was a patient with renal carcinoma, amyloidosis and peripheral neuropathy, we question a pathogenic role of AD deficiency.

II. Duchenne muscular dystrophy (DMD). This is the most prevalent of the old-terminology "muscular dystrophies". It is an X-linked hereditary progressive deterioration of muscle in boys, usually causing wheelchair or bed confinement by age 12 and death by age 20 years. Cause and treatment are not known. Current competing hypotheses of the pathogenesis of DMD are: (a) primary or secondary defect of blood supply to muscle, (b) primary defect of energy source within the muscle fiber, and (c) primary muscle-fiber plasmalemmal defect. Although nearly all others favor (c), we favor (a) or (b) or (b + a). Some of the findings in DMD muscle considered by others to be supportive of (c) we consider to be invalid results or not distinguishing between (c) and (b). In DMD the plasmalemma has long been known to be leaky, evidenced by elevated CPK and other "muscle enzymes" in the serum, but that certainly does not specify a primary plasmalemmal defect. Our studies have been concerned with: the nature of the muscle cell plasmalemma, the effects of its leakiness, plasmalemma of other cells in DMD patients, and intramuscular blood vessels and flow.

A. Plasmalemmal Composition. (a) The distribution of major erythrocyte phospholipids and the total fatty acid and fatty aldehyde composition of erythrocytes and plasma was not abnormal in DMD patients or definite DMD carriers, or myotonic atrophy patients, compared with a large number of normal and disease controls, contrary to several published reports. (b) Human and animal muscle cultured aneurally was studied in different developmental states with a battery of membrane probes histochemically, fluorescence-microscopically, electronmicroscopically and autoradiographically -- concanavalin A for α -d-mannoside and α -d-glucoside groups, ruthenium red for acid mucopolysaccharides, α -bungarotoxin for nicotinic acetylcholine receptors, and tannic acid. Tannic acid stained plasmalemma only of mature muscle fibers and thus can be used as a marker of muscle fiber maturity; it also stained t-tubules of rat and chicken muscle cultures and showed these structures absent in cultured human muscle. A base was established against which cultured Duchenne dystrophy muscle can now be compared. (c) The effect of specific plasmalemmal-reacting agents on living muscle cells is being studied.

B. Plasmalemmal porosity: outward leakage. (a) Radioimmunoassay of serum BB-isozyme of creatine kinase (CK) is being reported as a new method of demonstrating leaking immature or regenerative muscle fibers, while the MM-isozyme demonstrates leaking mature fibers. Although this approach gives an indication whether non-mature or mature fibers are leaking, it has not increased the detection of carriers of Duchenne dystrophy. (b) Serum myoglobin elevation was reported as a new method of detecting additional carriers of Duchenne dystrophy. (c) Hemopexin is an inducible, liver-produced, hemetransport protein. It was first discovered elevated in DMD patients and carriers a number of years ago by a current member of our group. We have now reported our confirmation of that. To support the hypothesis that the elevation is induced by the subtle myoglobin leakage from damaged muscle fibers, we have reported that: (i) with a sensitive radioimmunoassay, myoglobin leakage into the serum was found in nearly all DMD patients and half of the carriers; (ii) serum hemopexin elevation was induced in monkeys with experimentally crushed muscle or with repeated injections of small amounts

of myoglobin, which persisted long after CPK levels returned to normal (in the crush studies); (iii) a large amount of myoglobin released into the serum, clinically in rhabdomyolysis or experimentally injected in monkeys, induced an initial fall of serum hemopexin followed by a rise; (iv) of a large number of neuromuscular-disease patients surveyed with the immunodiffusion assay for hemopexin quantitation, only active myopathies, especially dermatomyositis/polymyositis and DMD patients and carriers, but also myasthenia gravis patients, had hemopexin elevations, all of those groups of patients having had elevated serum myoglobin; (v) molecular turnover studies using ¹²⁵I-hemopexin and ¹³¹I-albumin showed an increased turnover rate of hemopexin (increased synthesis > increased catabolism) in patients with Duchenne dystrophy, dermatomyositis/polymyositis and myasthenia gravis compared with normal and disease controls. Parallel turnover studies in monkeys showed that small amounts of heme (as could come from myoglobin or intravascular hemolysis) increased hemopexin synthesis while larger amounts of heme were required to increase the catabolism of hemopexin; those data demonstrated aspects of the hemopexin regulating mechanisms and explain what we observed in patients.

C. Plasmalemmal porosity: inward leakage. (a) Calcium ingress as part of our "calcium hypothesis" was published in more detail. That ingress was considered the trigger event for muscle responses to impaired plasmalemmal integrity, be it damage of unknown cause as in DMD or of known cause as in an endogenous afuelia or an exogenous ischemia, toxin, toxic-antibody, or toxic T-lymphocyte. If the calcium entry is slight and restricted, it is relatively benign, and probably is the "spark for repair/regeneration/hypertrophy" of muscle fibers. If the calcium entry is severe and unrestricted it begins a lethal cascade of events pushing muscle fibers past their point of no return, i.e., it is probably the "ultimate molecular assassin" or the "messenger of molecular doom". We have based our calcium hypothesis on our numerous interrelated studies of calcium in normal and damaged muscle fibers of patients with various neuromuscular diseases and various induced animal models thereof, utilizing light- and electronmicroscopic histochemistry, autoradiography, biochemistry and clinical scanning. The calcium mechanism can account for the large amount of obvious and subtle regeneration we see in DMD muscle by acridine orange and adenylate cyclase reactions. The calcium mechanism is not disease specific. We have previously described leakage of serum proteins into damaged muscle fibers that is not disease-specific.

D. Muscle blood vessels and blood flow. (a) Our ischemia hypothesis for DMD, which proposed a functional defect on the arterial side of the vascular tree, was based on our studies of the histochemopathology of DMD muscle, our experimental myopathy in animals, and our study of human ischemic limb muscles. An ischemia mechanism, although possible in DMD patients, has not yet been demonstrated in them directly. The next logical step we are now going to undertake by means of a laser-doppler technique for measuring blood flow in superficial capillaries of patients' muscle at time of biopsy and studying the vascular responses to perturbation by minor hypoxic stasis. In initial studies we have evaluated the technique as applied to human forearm skin blood vessels and to rat skeletal muscle vessels, and their responses. (b) Our new techniques for studying muscle blood vessels, being reported, include: (i) autoradiographic localization of β -adrenergic receptor with

125. I-hydroxybenzylpindolol, showing very high concentration in intramuscular vessels, much higher than in muscle fibers; (ii) histochemical localization of adenylate cyclase, showing it to be much higher in vessels than in normal muscle fibers (but high in regenerative fibers).

E. Blood cells. (a) An abnormal decrease of platelet dense bodies has been found in platelets of DMD patients (with CC and NIMH). (b) Platelet uptake of serotonin in DMD patients, now remeasured, again shows some reduction of initial uptake rates; and serotonin release rates were normal. (c) Platelet-cAMP-phosphodiesterase was increased in DMD patients and carriers and cGMP-phosphodiesterase increased only in carriers; both enzymes were normal in erythrocytes of DMD patients and carriers.

III. Polymyositis/Dermatomyositis Complex (PM/DM): PM/DM is an acquired disorder causing progressive deterioration of muscle in children and adults. The primary cause is not known but the pathogenic mechanism is considered dysimmune (autoimmune). Before the introduction of antidysimmune therapy, all patients were seriously incapacitated and many died.

A. Therapeutic efforts: Dysimmune component -- high-single-dose alternate-day prednisone (HSDAD-Pred) with or without azathioprine 3 mg/kg/day seems to be the best treatment. Cyclophosphamide is an alternative to azathioprine. In patients failing to respond to these drugs we will be trying Poly-ICLC. Calcinosis -- massive subcutaneous calcification, "calcinosis universalis", is a complication of dermatomyositis which is crippling and causes skin breakdown and infection. Calcium-solubilizing agents (EDTA, diphosphonate) in the past have failed. However, we have found in some severely affected patients the calcium has remarkably diminished as the muscle and skin were responding to our combined azathioprine-HSDAD-Pred program, and has remained diminished for several years, even as the drugs were gradually reduced.

B. Pathogenic mechanisms: The exact mechanism(s) of muscle damage in PM/DM is unknown. We previously found immunoglobulin complexes in muscle blood vessels in 83% of children and 29% of adults, supporting the hypothesis of a vascular mechanism of damage, especially in childhood DM. To directly study blood flow in the patients' muscle we will use a laser-doppler method at time of muscle biopsy. We will also study possible impairment of suppressor T-lymphocytes, a mechanism that might be more especially important in adult DM/PM (with NCI). DM/PM is considered to be a dysimmune response to either an exogenous antigen or a normal cell component "foreigned" by an exogenous agent. Specific treatment/elimination of an exogenous agent could be curative (as opposed to the merely suppressive action of all current treatments). We are continuing to seek evidence of an exogenous agent(s). We have not yet been able to rescue an agent nor to find reverse transcriptase. Collagen increase in DM/PM has been suggested by others as a possible pathogenic mechanism of muscle fiber damage. We have used antibodies against types I, II, III and IV collagens (which differ by virtue of the amino acids of their 3 polypeptide chains), against their procollagens and against fibronectin. We found types I and III collagen and procollagen and fibronectin in the normal endomysium and perimysium; they accumulate there in DM/PM, but in the same manner as in Duchenne dystrophy and thus the accumulation is not disease-specific. These are also increased in intramuscular blood vessels of

DM/PM, especially the childhood form, but not in Duchenne dystrophy -- this is more disease-specific but may be secondary to immunoglobulin complexes deposited in the vessels. Type IV collagen normally is only in cellular basement membranes and is not altered in DM/PM (or DMD). The cardiac involvement of DM/PM, demonstrable with non-invasive cardiologic techniques and occurring in the majority of 20 patients, was published; abnormalities consisted of conduction blocks, arrhythmias and systolic mitral prolapse.

C. Chronic vacuolar myopathy, seen in 18 of our patients, may or may not have a pathogenesis somewhat related to that of the DM/PM complex. We have, though, separated it off as a distinct disease (or syndrome). It is characterized by acid-phosphatase-positive vacuoles and contain multiform membranous whorls and masses, and collections of glycogen granules; there are also frequent collections of long parallel-arrayed double-helical tubule-like twists having 20 μ "diameter" and 100 μ periods. We will be publishing our findings that the muscle in culture reincarnates the typical vacuoles after 10 days of growth. In one case, the cultured muscle fibers had by electron-microscopy a number of unusual structures resembling virions situated near the nuclei or plasmalemma; re-scrutinizing the original biopsy revealed rare examples of identical structures.

IV. Other Myopathies and Neuromuscular Diseases of Uncertain Classification

A. Malignant hyperthermia-rigidity (MHR) is a syndrome, 70% fatal, of acute rise of body temperature accompanied by muscle rigidity during general anesthesia, usually provoked by halothane and/or succinylcholine. A number of the patients (if not all, by definition) have underlying not-well-defined neuromuscular disorders. One well-defined underlying condition is central core disease having a high incidence of MHR. In seeking a new test for patient-susceptibility to develop MHR, we have developed a focal model in a strain of pigs susceptible to MHR.

B. "Ragged-red" muscle fibers, which contain severe mitochondrial abnormalities, are the commonest histochemical manifestation in limb muscles of the heterogenous syndrome of oculocranosomatic neuromuscular diseases with ragged-red fibers (OCSNMD-RR). The patients usually having lacticacidosis and often ophthalmoplegia. Some patients have a syndrome of small stature, seizures, mental impairment, and lacticacidosis -- in limb muscle cultured from two such patients, we have reported that most of the mitochondrial changes, including increased number and greatly increased size of mitochondria, wide distorted "twisted-ribbon" cristae, and mushy-looking inclusion material, have been re-incarnated. We also reported that the same changes were produced in normal human muscle cultures after 2 days exposure to dinitrophenol, and the cultured ragged-red-fiber muscle was extremely susceptible to worsening of the in vitro changes by dinitrophenol. This demonstrates a mitochondrial defect which is reproducible in cultured muscle fibers and provides a test-system for seeking a possible genetic or occult-infectious basis. In the original biopsies and in the cultures the mushy-looking and crystalline inclusions lacked cytochrome oxidase staining by our EM-cytochemistry. Many of the OCSNMD-RR patients with ophthalmoplegia have cerebellar ataxia, mental impairment, some denervation evident in muscle biopsy, and spinal-

fluid protein increase. We have now reported that some such patients by CAT-scan have a decreased attenuation coefficient of cerebral white-matter and small brain-stem (shown by enlarged 4th ventricle and pre-pontine cisterns), changes correlated with the clinical state; the CAT-scan changes were also absolutely correlated with electrophysiologically determined delay of the bilateral late-response of the blink reflex.

C. Type-II muscle fiber atrophy. We continue to study the selective atrophy of the type-II (glycolytic-rich, oxidative poor) muscle fibers, especially the subtype-IIB fibers. This atrophy we have shown to be the basis of cachectic atrophy accompanying cancer and other chronic debilitating disorders. Evaluation of the cause of type-II fiber atrophy in cancer patients, theoretical mechanisms of which we published previously, is important because this "remote-effect" muscle weakness is often the most crippling aspect of cancer -- if the molecular mechanism(s) can be discovered it might be treatable independently of treatment and response of the cancer itself. An improvement of the muscle weakness and wasting could even make the patient better able to withstand the rigors of direct anti-cancer therapy. We have now reported 3 hypothetical mechanisms, which could even be summated in cancer patients: (a) insidiously decreased oral fuel (caloric) intake, which we have recently documented; (b) fuel wastage due to metabolic derangement within neoplastic or secondarily affected non-neoplastic cells; (c) possibly a circulating small-molecule remote-effect acting on type-II fibers. In exploring these mechanisms, we will be studying the role of insulin reception by and its action upon muscle fibers.

V. Basic biology of muscle. Several technical approaches have been pursued.

A. Tissue culture. (a) Human biopsies (76 patients) and rat muscle were cultured with improved techniques and used for a variety of cytochemical, immunocytochemical, ultrastructural, biochemical, autoradiographic and electrophysiologic studies. For example: (i) aneurally cultured adult human muscle developed muscle-type (adult-type) isozymes of phosphorylase and phosphofructokinase, but not of pyruvate kinase which appeared only in the brain-type (fetal-type); (ii) baseline electrical properties of aneurally cultured adult human were defined by microelectrode studies.

B. Electronmicroscopy. (i) To selectively stain RNA or DNA, a modification of platinum-thymine complex method was used. To be published are our results: The expected subcellular structures were stained in normal and regenerating muscle fibers; there was no staining of the crystal-like structures in mitochondria of ragged-red fibers, nor could DNA- or RNA-viral material be identified in them or in muscle fibers of chronic vacuolar myopathy; unexpected was the finding of uniform staining for nucleic acids of the plasma-lemma of cultured muscle cells (resembling that noted by others in tumorigenic cell cultures). (ii) (See other parts of this summary.)

C. Histochemistry. (i) Adenylate cyclase (AC) -- being prepared for detailed publication is: our new method, utilizing AMP-PNP as the substrate and Ca^{++} (which does not inhibit AC as Pb^{++} does) as the capture agent; specificity of the AC reaction by use of selective inhibitors vis-a-vis

guanylate cyclase reaction with GMP-PNP; and the localization and relative amounts of AC in blood vessels, nerve cells and muscle cells (normal, pathologic, regenerative) at light-microscopic and electronmicroscopic levels. (ii) Lectin probes, Con A, LC, RCA 120, WGA, Soy, for membrane polysaccharides were used for the first time in human muscle and spinal cord pathology and in human muscle cultures --the localizations are to be published. (iii) Phosphodiesterase-1. The histochemical distribution in normal and pathologic human and rat muscle was presented and will be reported. Differences were marked between human and rat muscle and between rat blood-vessels and muscle fibers. (iv) Superoxide dismutase -- a histochemical method is being developed.

D. Autoradiography. Three applications have been developed. (i) To animal models -- localization of β -adrenergic receptors in normal and denervated rat muscle with ¹²⁵I-hydroxybenzylpindolol; (ii) To human and animal tissue cryostat sections, ¹²⁵I- α -bungarotoxin in neuromuscular junctions, in plasmalemma of denervated fibers and in thymic epithelial cells; (iii) cultures of muscle, neurons and schwann cells -- β -adrenergic receptors and nAChRs, as above. Other receptors, e.g., α -adrenergic receptors and insulin receptors will now be sought. Study "i", in press, showing in normal muscle much greater amount of β -adrenergic receptors in arterial-tree vessels than in muscle fibers indicated (a) the fallacy of assuming β -adrenergic binding studied biochemically in whole-tissue muscle homogenates is only in muscle cells, and (b) the potential importance of arterial-tree, as well as muscle fiber, β -adrenergic receptors in human neuromuscular diseases. Study "ii" confirmed our earlier histochemical finding of increased nAChR in the plasmalemma of denervated muscle fibers and presence of nAChR in human thymic epithelial cells.

E. Biochemistry. Most of our attention has been directed to components of the plasmalemma, t-tubule and reticulum of human and animal muscle, with correlations with our other studies of the plasmalemma. Skeletal muscle plasmalemma (PL) sarcoplasmic reticulum (SR) and mitochondria (M) were prepared from homogenates of normal and denervated rat muscle, from human muscle from legs amputated for osteogenic sarcoma, from human diagnostic muscle biopsies, and from patient biopsy muscle grown in tissue culture. (1) β -adrenergic receptor (BAR) adenylate cyclase (AC) system. (a) Animal muscle ¹²⁵I. (i) Following denervation of rat muscle there are increased BAR's (per ¹²⁵I-hydroxybenzylpindolol binding), not blocked by cyclohexamide, and decreased AC in PL, SR and M. (ii) An aqueous sciatic nerve factor, as a putative trophic/regulatory factor, applied in vitro at higher concentrations decreased BAR of PL 30-40% in denervated muscle and 10-20% in normal muscle. (iii) Developmentally, in the rat BAR and AC are present from 18-day embryonic states (birth occurs at 21 days) but are not functionally coupled until 3 days after birth; this study was presented. (b) Human muscle. (i) The subcellular distribution of BAR in normal human muscle was defined and presented. The distribution, PL >> SR > M, correlated with our previous localization of AC in human muscle, supporting a functional coupling. (ii) Compared with control human muscle, in myotonic atrophy and x-linked recessive muscle-fiber hypotrophy with central nuclei, there was decrease of AC but no change of BAR; both BAR and AC were decreased in

various denervations, hypokalemic periodic paralysis and dermatomyositis. (2) Guanylate cyclase (GC). (a) Animal muscle. We previously reported increase of GC in all fractions (PL, SR, M and 10⁵ g supernatant) of denervated rat muscle. We now find the increase is not blocked in cyclophosphamide-treated animals, suggesting a mechanism other than new synthesis of GC must be responsible for the increase. (b) Human muscle. In normal human muscle the subcellular distribution and properties of GC were similar to those we observed in rat muscle. The specific enzyme activity among subcellular fractions was PL > SR > M > soluble fraction (10⁵g supernatant). Even though PL possessed the highest specific activity, the predominant portion of GC (60%) was in the soluble fraction. The enzyme required Mn²⁺, but Mg²⁺ could substitute to a considerable extent. Also demonstrated were elution profiles on sepharose columns, substrate dependency, ionic requirements and pH profiles. (3) Acetylcholinesterase (AChE). The subcellular distribution and properties of the 3 molecular forms of AChE in normal and denervated rat muscle were published.

F. Muscle Spindles. Published or in press were our histochemical and correlated electronmicroscopic studies of human and rat muscle spindles, detailing nuclear-chain and nuclear-bag-1 and nuclear-bag-2 fibers.

Myasthenia gravis (MG) is an acquired disorder affecting transmission at the neuromuscular junction, mainly in adults and older children. The primary cause is not known, but the pathogenic mechanism is considered to be dysimmune (or autoimmune). Untreated patients usually are seriously handicapped and many die. Palliative treatment with anticholinesterases and anti-pathogenic treatment, consisting of thymectomy, ACTH and, most recently, prednisone, have helped considerably, but much disability, some fatality, and drug side-effects do occur.

A. Therapy. 1. Thymectomy. The role of thymectomy in the treatment of MG and for which MG patients, has recently been questioned. To be published is a review of 55 consecutive thymectomies done over the past 10 years. It showed: thymic hyperplasia onset age 16-29, 84% improved; thymoma, 67% improved (all onset > age 29); onset > age 29, 71% improved (83% of patients with thymic hyperplasia, 70% of patients with "involved" thymus (v.i.)); zero operative mortality, low operative morbidity; severity of myasthenia not a contraindication, but an indication for surgery (84% improvement in patients "thymectomized" < 10 years of onset cf. 33% improvement rate if duration >10 years); transcervical surgical approach unsatisfactory, necessitating re-operation by sternal-splitting in 7/9 patients) and resulting in clinical improvement in all 7; our modified transverse sternal-splitting upper-sternotomy approach provides much greater surgical exposure than the transcervical route with minimal increase of post-operative morbidity, and presumably has less risk of uncontrollable bleeding (which has caused death with the transcervical approach by others); and less morbidity than vertical sternal-splitting, attributable to preserved lower sternal integrity allowing deeper, less painful respiration and earlier ambulation. Thus thymectomy is potentially beneficial in all patients with onset in teen-ager or later, and repeat thymectomy can be remarkably beneficial in patients previously improved who subsequently exacerbate and do not respond to medical management.

2. Prednisone. Long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred) was introduced to this disease by us 9 years ago, and one child was begun on treatment 13 years ago. Nearly all patients continue to have excellent benefit. However, virtually all responders are, even with very gradual tapering of the LT-HSDAD-Pred, dependent on it, requiring 5-20 mg q.o.d. Prednisone in higher doses reduces circulating lymphocytes ($T > B$), and lymphocyte-response to mitogens (T-mitogens $>$ B-mitogens), but those effects last no longer than 24-48 hours. We are now seeking the mechanism of benefit of the low maintenance dosage. Because prednisone has toxicity, we are seeking other drugs for MG. 3. New prednisone-responsive disorders. Three patients have been identified who, by detailed investigation, do not have one of the neuromuscular diseases (MG, dysschwannian polyneuropathy, dermatomyositis/polymyositis) known to respond to LT-HSDAD-Pred. Because their severe weakness was out of proportion to minimal or no histochemical and electrophysiologic abnormalities, LT-HSDAD-Pred was tried. Remarkable improvement was achieved in each, with return of weakness each time the dosage was lowered too much. They have a new disorder(s) and will be reported. We will explore whether their disorder is related to the new experimental dysimmune dysneuronal neuropathy having an aspect of impaired neuromuscular transmission we reported last year. 4. Poly-ICLC. We have found this to be a new anti-dysimmune drug. One MG patient showed apparent improvement on a very short course; more patients will be studied.

B. Pathogenesis. Questions regarding pathogenesis include possible altered host (patient) immunologic response, "foreigned" host cells, exogenous agent, role of thymus, role of lymphocytes, and role of nicotinic acetylcholine receptor (nAChR). 1. Thymus pathology. The rationale for the empirically-observed benefit of thymectomy is still being sought. In addition to the hyperplastic and thymomatous thymuses, we have now demonstrated that thymuses of older patients considered "atrophic" by pre-existing histopathologic criteria have evident in our fresh-frozen sections many small nests of lymphocytic and epithelial cells that look active -- we postulate they may have a pathogenic role in the older non-thymomatous MG patient. 2. Thymic nicotinic acetylcholine receptor (nAChR). We earlier demonstrated nAChR in thymic epithelial cells histochemically with peroxidase-labelled 125 I- α -bungarotoxin. Now we have demonstrated it autoradiographically with 125 I- α -bungarotoxin. The thymic epithelial-cell nAChR may be the molecule foreigned by an exogenous agent. 3. Thymic thymosin. Thymosin is a thymic hormone, discovered by A. Goldstein, capable of repairing and sustaining normal immune function of lymphocytes. With antibodies to thymosin we have achieved the first cytolocalization of thymosin -- it is in thymic epithelial cells of normal thymuses and of hyperplastic and thymomatous thymuses of MG patients. 4. Thymic cultures. We have cultured thymic cells from normal and MG human thymuses. We have never found muscle cells or cells with myofibrils. We find epithelial cells with typical desmosomes and tonofibrils. Epithelial cells form typical Hassall's corpuscles in culture. They (and not fibroblasts) contain thymosin by fluorescent antibody reaction. We will study the amount of their secretion of thymosin in vitro and its regulating mechanisms. We are now seeking evidence of nAChR by electronmicroscopic-cytochemical and autoradiographic means. 5. Suppressor cells. One hypothesis is that MG is caused by a defect of suppressor t-lymphocytes. We shall be studying the suppressor cells in MG. 6. Cerebrospinal fluid (CSF) immunoglobulin bands.

We reported that 7 of 23 MG patients had oligoclonal IgG bands and 5 of the others had monoclonal IgG bands. Since IgG "synthesis" (per Tourtellotte formula) in the CNS was normal, the CSF pathologic bands were probably from the serum. One band or more may reflect the anti-nAChR IgG, and therefore deserves consideration as a possible cause of the brisk reflexes of MG patients. 7. Nicotinic acetylcholine receptor (nAChR). IgG antibodies to nAChR are found in MG. We consider that there is a junctional (J) form of nAChR present only at the neuromuscular junction and an extrajunctional (E) form present both extrajunctionally in non-innervated fibers and to some extent at the junction of innervated fibers. Since we found that the anti-nAChR of MG patients reacts both at junctional and extrajunctional locations, we have proposed it to be against the E form. It is likely that the nAChR of thymic epithelial cells (v.s.) is the E form. (The antibodies in rabbits immunized with electric-fish nAChR are against the J form, per our report last year.) Previously we showed histochemically that the E-nAChR of denervated muscle fibers reacts with α -bungarotoxin; we have now shown that reaction autoradiographically with ¹²⁵I- α -bungarotoxin. Cultured muscle of humans, rats and chicks has diffuse plasmalemmal nAChR that reacts with the IgG nAChR antibodies of MG patients, suggesting it is E-type. Those nAChR receptors are mobile, as indicated by our current finding, utilizing rhodamine-labelled α -bungarotoxin, that diazacholesterol treatment in vitro increases their mobility. 8. Hemopexin. We have now reported that serum hemopexin is increased in MG patients; that was inexplicable until our recent finding of increased myoglobin in the serum of MG patients by use of a very sensitive complement-fixation technique. That small amount of myoglobin leakage may be a manifestation of a hitherto overlooked minimal subclinical plasmalemmal-leaking myopathy in many MG patients.

I. Periodic Paralysis (PP). These are hereditary or acquired disorders causing chronic weakness punctuated by attacks of paralysis. There are potassium-benefited and potassium-provoked forms. Associated metabolic abnormalities are known but the actual pathogenic mechanisms are not. Standard palliative/preventive therapy in the idiopathic hypokalemic form of PP is potassium, and more recently acetazolamide. 1. Treatment. In the hypokalemic form of PP the treatment we introduced, long-term acetazolamide, has continued to be the best prophylactic agent both for preventing attacks and improving inter-attack weakness. It is now in the textbooks as such. Two of our patients have been treated successfully for more than 13 years. Since muscle contains either no, or very little (per disparate studies of ourselves cf. others) carbonic anhydrase, the mechanism of acetazolamide benefit in hypokalemic PP remains unknown. 2. Pathogenesis. Human muscle was grown aneurally in tissue culture to obtain fibers free of all neural, circulating and other influences existing in the patient. We have successfully studied it with microelectrodes and obtained baseline values: resting membrane potential U (Vm) 52.4 ± 6.6 mV, input resistance 5.5 ± 3.4 M Ω , only 2/34 fibers electrically excitable at Vm, but when hyperpolarized to 80 mV an action potential could be elicited from all with threshold for excitation at 22.6 ± 8.7 mV and action-potential amplitude of 83.4 ± 28.9 mV. Against these we will now compare fibers cultured from patients with various periodic paralysis and myotonias. We can also study the effect on these parameters of motor neuron innervation in vitro of the cultured human fibers.

IIA. Myotonia Congenita and Paramyotonia Congenita. Myotonia is a crippling symptom in these inherited diseases of unknown causes. (1) Clinical Studies. Last year we reported acetazolamide as a new treatment providing excellent benefit in patients who had failed to respond to other anti-myotonia agents. It continues to have long-term effectiveness in those and several additional patients. (2) Pathogenesis. (see above, tissue culture).

IIIB. Myotonic Atrophy (Myotonic "Dystrophy"). This is an inherited multi-systemic disease, with progressive muscle weakness and wasting, of unknown pathogenesis. We have previously raised the possibility of at least a partially neurogenic aspect. With our new concept of "myogenous dys-innervation", we have now extended that hypothesis to include a possible myogenous muscle plasmalemmal non-receptivity to neural short- and long-term trophic influences. (1) Pathogenesis, patient studies. (a) Adenylate cyclase -- this we found decreased 30-60% in myotonic atrophy patients, with normal β -adrenergic receptors. We have now demonstrated those findings reincarnated in muscle fibers cultured from the patients. The muscle in culture can be used to further explore this defect. (b) Myotonic phenomena -- with our baseline values of microelectrode electrophysiologic parameters of cultured human muscle now established, we will study myotonic atrophy muscle in culture. In that preparation we will also study chloride and other ionic conductances and ionic dysequilibrium challenges. (c) Insulin receptors on leucocytes and erythrocytes are being studied. (2) Pathogenesis, animal models. Diazacholesterol-induced myotonia in the intact animal we discussed last year, including the lowered adenylate cyclase of muscle. Now we are preparing to report the effects of diazacholesterol on cultured rat muscle. The drug caused: change of contraction rhythm to more continuous and fibrillation-like movements; electronmicroscopically evident honey-comb appearance and dilation of sarcoplasmic reticulum and irregularity of tannic-acid stained plasmalemma; increased mobility of plasmalemmal nicotinic acetylcholine receptors; decreased binding of 125 I-Con A biochemically; and decreased adenylate cyclase (basal, and NaF and isoproterenol stimulated) but normal β -adrenergic receptors.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01039-17 MN
PERIOD COVERED <p style="text-align: center;">October 1, 1978 through September 30, 1979</p>		
TITLE OF PROJECT (80 characters or less) Amyotrophic Lateral Sclerosis (ALS), Other Lower Motor Neuron Diseases, and Peripheral Neuropathies		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: W. K. Engel, M.D., Chief, NMD Section, MNB, NINCDS OTHER: V. Askanas, M.D., Associate Neurologist, NINCDS M. Dalakas, M.D., Clinical Associate, MNB, NINCDS H. B. Levy, M.D., NIAID		
COOPERATING UNITS (if any) B. T. Adornato, M.D., Palo Alto Medical Clinic J. G. Nutt, M.D., U. Oregon T. E. Bertorini, M.D., U. Tennessee (Continued)		
LAB/BRANCH <p style="text-align: center;">Medical Neurology Branch</p>		
SECTION <p style="text-align: center;">Neuromuscular Diseases</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, MD 20205</p>		
TOTAL MANYEARS: <p style="text-align: center;">7</p>	PROFESSIONAL: <p style="text-align: center;">5</p>	OTHER: <p style="text-align: center;">2</p>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <p>In amyotrophic lateral sclerosis (ALS) and other diseases affecting the lower motor neurons, including peripheral neuropathies and some spinocerebellar degenerations, we are seeking (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, subforms; (c) most importantly, specific or symptomatic therapeutic response; (d) new methods of analyzing the abnormalities; and (e) animal models of the human pathophysiologic states.</p>		
Cooperating Units: B. R. Brooks, M.D., Johns Hopkins J. W. Griffin, M.D., Johns Hopkins H. R. Galnick, M.D., CC S. A. Houff, M.D., IDB, NINCDS J. L. Sever, M.D., IDB, NINCDS		

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Project Description:

Objectives: In amyotrophic lateral sclerosis (ALS) and other diseases affecting the lower motor neurons, including peripheral neuropathies and some spinocerebellar degenerations, we are seeking (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, subforms; (c) most importantly, specific or symptomatic therapeutic response; (d) new methods of analyzing the abnormalities; and (e) animal models of the human pathophysiological states.

Methods Employed: A variety of techniques, encompassing tissue-culture, histochemistry, biochemistry, autoradiography, radionuclide scanning, electrophysiology, electronmicroscopy, and immunology, are applied to patients with the various diseases covered in this category and to induced animal-models. Conducted were therapeutic trials, the efficacy of which was judged by clinical testing, functional evaluation, serial quantitative evaluation of muscle function using an apparatus designed by us for quantitating isometric muscle tension, clinical electrophysiology (including nerve conduction velocities); and biochemical data, especially as reflected in cerebrospinal fluid (CSF).

Major Findings:

I. Amyotrophic Lateral Sclerosis: The disease usually causes death within 2-5 years, although some cases are more chronic. 90% of the cases are sporadic, 10% are dominantly inherited. The cause of ALS is unknown -- dysmetabolic vs. viral are the two main possibilities. We favor the former but are pursuing both.

A. Therapeutic Trials: Four drugs and one procedure have been studied over the past two years, and the results are in the process of being formally reported or formally analyzed. Phthalazinol is an inhibitor of cAMP phosphodiesterase, and of cGMP phosphodiesterase to a lesser extent. In ALS patients it raised to normal the lowered endogenous cAMP of CSF but not the lowered endogenous cGMP. In 25 ALS patients it did not alter the progression of the disease. In one patient with the bulbar form, progression has arrested but whether that was due to the drug or was spontaneous is not known. Phthalazinol has recently caused three side-effects of interest in 4 patients with both diabetes mellitus and ALS; increased glucosemia/glycosuria and diarrhea, and, in one, the appearance of a new peptide band in isoelectric-focused CSF. Whether the findings can be related to the pathogenesis of the motor neuron degeneration in these patients is being explored. Human leucocyte interferon, an antiviral substance, did not benefit one ALS patient. Polyinosinic-polycytidylic-acid-poly-l-lysine (Poly-ICLC), an inducer of interferon, did not benefit three ALS patients (with NIAID). Adenine arabinoside is an antiviral compound. Although the code has not been broken, there were no dramatic benefits from either 10-day course of the two coded agents (drug vs. placebo) given to 10 ALS patients. Plasmaphoresis for 8 weeks in 3 ALS patients was not beneficial.

B. CSF biochemical studies: The CSF is a thesaural swamp of interesting central nervous system (CNS) transmitters and other metabolites. In ALS

we have previously found HVA and cAMP to be low, but raising them to normal levels was not associated with clinical benefit. We found cgMP low and have not yet raised it to normal. (A chapter on cyclic nucleotide metabolism and the CSF is in press.) We are now quantitatively analyzing CSF total fatty acid content and composition by thin-layer and gas chromatography. In ALS patients vs. normals and disease controls, we have worked out the ionic-exchange and liquid chromatographic methods for quantitating bases and nucleosides in patients' CSF. Enolases, neuron-specific and non-neuronal, are now being studied in ALS CSF (with NIMH). Cultured motor neurons of 17-day fetal-rat ventral spinal cord serve as test-objects of possibly toxic fluids or agents related to ALS patients. To date, comparing CSF of ALS patients with controls, there was no detrimental effect visible morphologically. The level of neuron-specific enolase in such treated cultures is being determined (with NIMH).

C. Blood biochemical studies: (a) Fatty-acids of erythrocyte membranes are not abnormal in ALS. (b) ALS platelets have a reduced rate of initial uptake of ^3H -serotonin and of initial release to 0.5 μml thrombin, and normal cAMP and cgMP phosphodiesterase. (c) Leucocyte and erythrocyte insulin-receptors and glycogen content are being studied and correlated with plasma glucose and insulin levels (with VAH, D.C.) (d) Parameters of calcium homeostasis, which might or might not reflect parathyroid function, have shown: (i) greater reduction of retention of oral $^{45}\text{CaCl}_2$ in ALS than in other neuromuscular diseases was found, and it was partly but not entirely attributable to inactivity; (ii) normal 25-hydroxy vitamin D levels; (iii) no resistance to dihydrotachysterol (it mathematically corrected the decreased retention but did not cause clinical improvement); (iv) normal serum calcium, urinary 24-hour calcium excretion, and renal cAMP clearance; (v) mean serum parathyroid hormone levels higher than normal, but this was also found in other categories of neuromuscular diseases (myopathy, peripheral neuropathy). All four sets of data are to be reported. To be studied is the response to imposed serum fluctuations of calcium.

D. Motor Neuron Biology: Histochemical properties of lower motor neurons (LMNs) continue to be explored, seeking special properties of them and disease-characteristic defects thereof. To date 25 reactions -- enzymatic, non-enzymatic and lectin-binding -- have been applied, and the only ones seeming to be of possible relevance to pathogenesis are the high phosphorylase and low succinate dehydrogenase. Additional reactions will be explored over the next year. Bidirectional trophisms between motor neurons, muscle fibers and schwann cells are being studied by isolation of fractions containing putative factors and testing for influences on animal and human motor neurons, schwann cells and muscle fibers in culture.

E. Viral Studies: In ALS patients we continue to search for evidence of a viral cause. However, our negative evidence to date includes: (a) lack of sera or CSF antibodies to 13 viruses (and one protozoan), (b) lack of oligo-clonal bands in the CSF, (c) failure to respond to human leucocyte interferon and to the interferon-inducer poly-ICLC, and (d) no major benefit from the antiviral agent adenine arabinoside (code not yet formally broken, v.s.).

F. Diseases possibly relevant to ALS: Benign Focal Amyotrophy is important because of its excellent prognosis but usual misdiagnosis as fatal ALS. It was originally described by us 11 yrs. ago as a separate clinical syndrome, and in young-adult males possibly a distinct disease. We are now preparing for publication our updated experience. It is a limited form of lower-motor-neuron disease clinically confined to the upper extremities, unilateral or markedly asymmetric, gradual in onset, progressive for 1/2-4 years and then clinical stability, or only very minimal progression, for 2-11 yrs to present. We have recently found CSF oligoclonal Ig bands in several patients, raising a question of a viral/dysimmune pathogenesis. If related to ALS as a spontaneously arresting form, it may hold a clue to understanding and treating that disease. In central core disease, we have in press our newly formulated hypothetical pathogenesis. It is based on our histochemically showing marked paucity of type-II muscle fibers, normal appearance and distribution of the subtypes of type-I muscle fibers, and no densification of motor units by single-fiber-EMG (SFEMG) (with Uppsala). We propose the weakness to be based on a paucity of lower motor neurons (LMNs), especially of the hypothetical type-II LMNs, occurring during development, due to either impaired formation or increased normal loss as a part of "neurothanasia" (Hamburger's term for the normal loss of LMNs in embryonic chick cord). The muscle fibers themselves are proposed to be abnormally constructed, perhaps because of defective LMN trophic influence, because they contain cores and have a susceptibility to develop malignant hyperthermia (see our Myopathy project). An aspect of neurothanasia may, on the basis of SFEMG and muscle histochemistry of II-fiber paucity without I-subtype grouping, also play a role in the pathokinesis of congenital rod disease and some forms of benign congenital hypotonia.

II. Polyneuropathy (peripheral neuropathy): The peripheral neuropathies comprise a group of disorders of various causes, but unknown in more than half the patients. They always cause serious physical handicap sooner or later, sometimes associated with intractable pain and ulceration and, in extreme cases, loss of feet and hands. Our studies seek to delineate the underlying causes and where possible develop a treatment. We also seek fuller understanding of the basic biology and pathologic responses of the lower motor and sensory neurons and their schwann cells. Dysschwannian neuropathies are ones in which the neuronal-axon defect is considered secondary to schwann cell abnormality, whereas in dysneuronal neuropathies the lower motor and/or sensory neuron soma ± axon is the major site of abnormality.

A. Biology of the schwann cell: For basic biologic studies and for use as a test-object in human diseases (see Immunologic Studies), a technique was established for growing in tissue culture sciatic-nerve schwann cells from 3-day old rats. Ultrastructural and histochemical characteristics of the cultured rat schwann cells were identical to those of in vivo studied schwann cells in a very young animal, except for the presence of a basement membrane and myelin sheets in the latter.

To study human dysschwannian neuropathies we have developed techniques to grow reproducibly in tissue-culture human schwann cells obtained from diagnostic nerve biopsies. In primary or co-primary dysschwannian neuropathies (e.g., adrenomyeloneuropathy, metachromatic leucodystrophy, some forms of familial idiopathic "Charcot-Marie-Tooth" neuropathy) the schwann cells in culture should have the biochemical defect, which when identified can be treated in culture. Schwann cells might also express the defect in other neuropathies related to general metabolic defects such as diabetes mellitus. Our technique enables almost complete elimination of non-schwann cells. Histochemical, fluorescent and ultrastructural characteristics of normal human schwann cells were determined and are being reported. This will now serve as a basis to study various dysschwannian neuropathies. In one example of dysschwannian neuropathy, adrenomyeloneuropathy, we have detected in the cultured schwann cells ultrastructural and biochemical abnormalities -- the presence of very-long-chain fatty acids indicating a reincarnation and amplification of the defect in tissue culture.

B. Biochemical Studies: (a) Adrenomyeloneuropathy -- schwann cells and muscle cells were cultured from biopsies of two patients. Ultrastructurally increased amounts of lipid droplets and multilaminar inclusions were present in both cell types. Biochemically increased amounts of very-long-chain fatty acids, C₂₂-C₂₆, were present in cultured schwann cells and even to a greater degree in cultured muscle cells. Cultured human skeletal muscle incorporated media-derived fatty-acids into several lipid classes including triglycerides, cholesterol esters, phospholipids and glycolipids. When cultured muscle of the patients was presented with C₂₂:0 or C₂₆:0 free fatty-acids in the medium, it accumulated 4-10 fold greater levels than control cultured muscle. The data demonstrated a generalized metabolic defect, and for the first time the defect was identified while the patient was alive. (b) Substance P -- In CSF it was found by radioimmunoassay to be decreased in our neuropathy patients, but in no other neuromuscular diseases surveyed (with U. Oregon and Harvard). All affected patients had sensory nerve involvement. Decrease of substance P was only approximately correlated with degree of loss of pain and temperature sensation.

C. Immunologic Studies: (1) In presumably-dysimmune relapsing neuropathy we have: (a) localized immunoglobulin complexes containing IgG, IgM and C₃ in sural nerves of 5 patients, and suggested the complexes to be a pathogenic mechanism; (b) found unfluctuating monoclonal IgG bands in CSF of chronic relapsing but not the acute "Guillain-Barre" form (which had polyclonal IgG), and suggested the monoclonal band might be a predictor of chronicity (with IDB); (c) found a serum IgM factor (? antibody) which bound specifically to schwann cells in cultures of rat peripheral nerve in 4 of 5 patients and not in control sera, indicating a new tissue-culture test of pathogenic mechanisms; (d) found one case associated with a "non-secretory" osteosclerotic multiple myeloma with immunoglobulin deposition in peripheral nerve, indicating that to be a likely pathogenic mechanism.

(2) In presumably dysimmune progressive non-relapsing neuropathy: (a) eleven patients had a circulating monoclonal immunoglobulin spike without detectable myeloma or amyloid and with normal bone marrow -- immunoglobulin light chains were deposited in their nerves biopsied, and we suggested they represented circulating neurotoxic molecules; (b) one patient with IgM-k Waldenstrom's macroglobulinemia and neuropathy had deposits of IgM-k light chains in her sural nerve biopsied and lymphocytes bearing k-chains infiltrating that nerve -- treatment with chlorambucil and prednisone improved her neuropathy and lowered serum IgM levels (with CC).

(3) In the plasma cell dyscrasic form of amyloid neuropathy we have (a) found circulating immunoglobulin light-chains (IgG-kappa > IgG-lambda > IgM lambda) in all 10 pts (b) postulated non-amyloidogenic Ig light chain fragments as circulating neurotoxic molecules (c) found them deposited in peripheral nerves in 7 of 8 patients examined, and (d) in a unique case of polyneuropathy, amyloidosis and hypernephroma, crystal-violet-positive amyloid was found in muscle blood vessels and connective tissue but none in nerve; the excised tumor had amyloid of Ig-λ origin biochemically and immunochemically; antibodies against denatured λ-type amyloid protein bound to nerve, muscle and tumor (but antibodies against undenatured λ-protein did not); and by electronmicroscopy deposits typically amyloid were present in nerve (with NIAMDD).

D. Therapy: (a) Polyinosinic-polycytidilic acid poly-l-lysine stabilized with carboxymethyl cellulose (Poly-ICLC), is a new treatment which we have found remarkably successful in a patient with chronic, presumably-dysimmune, relapsing polyneuropathy unresponsive to prednisone-plus-azathioprine (with NIAID). The patient went from electric-wheelchair dependency to walking 7 miles daily with 100 μg/kg i.v. of weekly Poly-ICLC, now maintained for 18 months on 60-75 μg/kg. These levels did not raise measurable interferon in the patient's serum. Three periods of several weeks off drug were associated with loss of strength, regained on resumption of drug. Although Poly-ICLC is an interferon-inducer, we postulate it is beneficial in this dysimmune disease by a new action we have found, marked lymphocytopenia (down to 10-20% of baseline) for 1-2 days after the drug with return to baseline by 4-5 days (granulocytes actually rose 3-fold at 6-24 hr. and fell only 30% below baseline at 2-3 days and returned to baseline by 3-5 days). A second patient with chronic dysimmune neuropathy resistant to prednisone-plus-cyclophosphamide now also appears to have benefited. We propose this to be a new antidysimmune treatment potentially beneficial to other dysimmune diseases. We are extending the trial to other dysimmune neuropathy patients, and to dermatomyositis/polymyositis and myasthenia gravis patients. Preliminary data show no selective action of Poly-ICLC on any class or sub-class of lymphocytes, B, T, or natural-killer T (with IDB and NCI). In preliminary studies, serially-sampled serum of the Poly-ICLC treated patient showed some ability to reduce mitogen responsiveness of his own lymphocytes, especially to PHA, within 6 hrs after injection; this is also the time point of maximum lymphocyte reduction. (b) Chlorambucil and prednisone in Waldenstrom's macroglobulinemia, see above. (c) Plasmaphoresis was clearly beneficial in one of two patients with chronic dysimmune neuropathy treated for 8 weeks.

(d) High-single-dose alternate-day prednisone in chronic dysimmune, relapsing or progressive, polyneuropathy has a broader scope of responsivity than often believed. Favorable response is augered by the triad of (i) being dys-schwannian in type (slow nerve-conduction times), (ii) relapsing, (iii) with elevated CSF protein; but even some non-relapsing patients (i.e., 2 with progressive course > 1 yr) without slowed nerve conduction times and with normal CSF have responded to long-term high-single-dose alternate-day prednisone. Long-term treatment is required -- too rapid reduction of dosage too soon results in exacerbation. Excellent results have been sus-tained for as long as 14 years in an adult and 11-1/2 years in a child (now age 22). Virtually all our corticosteroid-responsive patients are cortico-steroid-dependent, requiring 5-20 mg single-dose q.o.d. to prevent exacer-bation.

E. Other related conditions. (1) Unilateral calf hypertrophy was identi-fied as a late sequel of discogenic sciatic radiculopathy.

III. Central Nervous System Disorders:

A. Spinocerebellar degenerations, which we have found virtually always to have a lower-motor-neuron component, comprise diseases of various causes, a few known, most not, which always result in serious physical handicap sooner or later in the course of the disease, and sometimes early death and/or mental deterioration. Our studies seek to delineate the underlying causes, where possible attempt to develop a treatment, and define basic cellular pathophysiologic mechanisms. Being reported is our new histo-chemical technique for adenylate cyclase, which synthesizes cAMP, considered to have an important role in cerebellar function. In the cerebellum the enzyme was found mainly in blood vessels, and of the neural-associated enzyme most was in basket-cell basket endings (not in Purkinje-cell somas). Treatment with a phosphodiesterase inhibitor to enhance cAMP is planned in spinocerebellar ataxia patients.

B. Progressive spastic paraplegia: This is a progressively crippling dis-order to children and adults. The causes are not known. We have pub-lished a newly identified cause, adrenomyeloneuropathy, and have with tissue culture, v.s., demonstrated the biochemical defect for the first time while the patient is living.

Significance to Bio-Medical Research and the Program of the Institute: These findings provide new information (a) on the pathologic and pathogenic aspects of the various lower motor neuron disorders, peripheral neuropathies, spinocerebellar degenerations, and progressive spastic paraplegias, (b) on the treatment of some, and (c) on animal-models of some of these disorders.

Proposed Course of Project: To more fully develop the interlinked basic and clinical studies, underway, directed toward clarification of the pathogenesis and identification of the etiology, and, most importantly, toward elaboration of means of treatment and prevention of these disorders.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01034-17 MN
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Myopathies		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PIs: W. King Engel, M.D., Chief, NMD, MNB, NINCDS Valerie Askanas, MD., Ph.D., Associate Neurologist, NMD, MNB, NINCDS OTHERS: B. Lavenstein, M.D., NINCDS B. Reddy, Ph.D., NINCDS A. Tahmoush, M.D., NINCDS M. Foidart, M.D., NINCDS J. McLaughlin, Ph.D., NINCDS M. Dalakas, M.D., NINCDS B. Joshi, M.D., NINCDS H. Sadowski, M.D., NINCDS D. Gettelfinger, M.D., NINCDS		
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TOTAL MANYEARS: 6.5	PROFESSIONAL: 4.5	OTHER: 2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) To more fully elaborate the clinical, tissue-cultural, histochemical, biochemical, ultrastructural, radioisotopic electrophysiologic and immunologic abnormalities of patients with the various <u>myopathies</u> and certain other neuromuscular disorders. To further sub-classify patients in each category using those parameters. To seek pathogenic mechanisms, using a variety of different techniques including ones listed above, applied to the patient's body fluids and tissues, particularly to the <u>muscle biopsy</u> specimens. To tissue-culture human abnormal muscle in order to <u>reincarnate</u> the disease in culture and then to treat it <u>in vitro</u> . To induce, by chemicals and by immunologic means, models of human <u>myopathies</u> in animals and in tissue-cultured human and animal muscle.		
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Objectives: To more fully elaborate the clinical, tissue-cultural, histochemical, biochemical, ultrastructural, radioisotopic electrophysiologic and immunologic abnormalities of patients with the various myopathies and certain other neuromuscular disorders. To further subclassify patients in each category using those parameters. To seek pathogenic mechanisms, using a variety of different techniques including ones listed above, applied to patient's body fluids and tissues, especially to the muscle biopsy specimens. To tissue-culture human abnormal muscle in order to reincarnate the disease in culture and then to treat it in vitro. To induce, with chemicals, or by immunologic means, models of human myopathies in animals, and in tissue-cultured human and animal muscle. Especially, to treat myopathic disorders by different methods in order to learn which is most effective within each disease category.

Methods Employed: A variety of techniques encompassing tissue-culture, histochemistry, biochemistry, autoradiography, radionuclide scanning, electrophysiology, electronmicroscopy, and immunology are applied to patients with the various myopathies their tissue-cultured muscle, and induced animal-models thereof.

Patient Material: Patients and diagnostic material from Medical Neurology Branch patients and from outside patients from whom diagnostic muscle biopsies were obtained and sent here for study.

Major Findings:

Myopathies are non-neurogenic, primary or secondary diseases of muscle. Some, such as the dermatomyositis/polymyositis group, are often at least partially treatable, but their cause and details of their probably "dysimmune" pathogenesis are not known; others are not treatable but their cause is known, e.g., genetic deficiencies of phosphorylase, phosphofructokinase, acid maltase or carnitine-palmityl-transferase; while still others, such as Duchenne muscular dystrophy and other genetic disorders bearing the name "dystrophy", are of unknown pathogenesis and are untreatable. Some, such as malignant hyperthermia-rigidity, are preventable if identified.

Our tissue culture laboratory has been a major locus of studies. We have qualitatively and quantitatively enhanced productivity in the culturing of human and animal muscle (and of human and animal schwann cells and animal neurons, per our Amyotrophic Lateral Sclerosis/Neuropathy Project). Tissue culture of human muscle biopsies provides living muscle fibers growing free of all neural, vascular and humoral factors present in the patients. We obtain abundant, reproducible and mature growth of human fibers in culture, including spontaneous twitching, and can precisely select individual fibers for enzyme-cytochemistry and immunocytochemistry at light- and electronmicroscopic levels and for various biochemical and microelectrode studies of them. This year we have grown 76 human muscle biopsies, as well as numerous rat muscle cultures. Specific studies are noted below.

I. Inherited Myopathies.A. Biochemically distinct myopathies.

1. Lysosomal defects. The mechanism of muscle fiber damage is different from that of the afuelias. It probably involves leakage of the excess lysosomal hydrolytic enzymes to dissolve the fiber from within -- an "endo-dissolution". Regeneration is minimal. (a) Acid maltase deficiency: Previously we have demonstrated a reincarnation of the biochemical and morphologic abnormality in muscle cells cultured from acute-infantile, chronic-infantile and adult-onset forms of the disease, (i) establishing it as a true intrinsic defect of the muscle cell and (ii) providing a new test system for in vitro therapeutic trial, without risk to the patient. This year we have set up the acid-maltase assay in our own group. A recently late-onset proximal myopathy patient lacked any vacuoles or excess acid-phosphatase-positive areas in a biceps biopsy but had the classic findings in a quadriceps biopsy, with an equally complete lack of acid maltase in both original biopsies (with Inst. de Pathologie Moleculaire, Paris) and in cultures of both, showing that (i) the enzyme defect is not necessarily accompanied by the morphologic defect and (ii) the erstwhile "classic" morphologic defect is not a pre-requisite to further biochemical analysis of our suspect patients. Muscle-fiber regeneration in acid maltase deficiency is minimal by histochemical criteria and the BB-isozyme of creatine kinase (v.i.) is correspondingly not elevated. (b) Cabbage-body myopathy is probably another lysosomal defect. The morphologic abnormality was reincarnated in muscle cultured from the patients. Assay of 12 lysosomal enzymes has not disclosed the defect (with Inst. de Pathologie Moleculaire, Paris).

2. Afuelias: We have introduced the term "afuelias" to describe the defects, known and unknown, of (i) glycogen/glucose utilization and (ii) lipid, fatty-acid, ketone-body utilization. The former cause muscle-fiber breakdown during heavy exercise, especially ischemic exercise -- they include phosphorylase, phosphofructokinase and debrancher-enzyme deficiencies. The latter cause breakdown during fasting states -- they include failure to utilize long-chain fatty-acids, carnitine palmityl transferase deficiency and probably infantile fatal fasting rhabdomyolysis. (a) Glycogen/glucose utilization defects. We have reincarnated the phosphofructokinase and debranching enzyme defects in the patients' cultured muscle. However, the phosphorylase defect was "cured" in cultured fibers of 8 patients, as it is in regenerating fibers of the original biopsy. There has been a question whether enzyme rejuvenated in the cultured fibers is adulttype phosphorylase -- we have established that it is by histochemical staining of isoelectric-focused gels and now by highly specific antibodies (with Inst. de Pathologie Moleculaire Paris and U. Miami).

Thus we have demonstrated a true rejuvenation of an enzyme genetically programmed ultimately to be deficient in mature fibers. This opens a new therapeutic possibility, trying to provoke and maintain that phosphorylase in mature fibers of the patient. A summary of our other multidimensional

correlated studies in the afuelias, including the induced model of iodoacetate-poisoned glyceraldehyde-3-phosphate dehydrogenase, has just been published. These include alternate-pathway therapy, our new diagnostic test of forearm ischemic exercise combined with localization of ^{99m}Tc-diphosphate, localization of excess calcium by histochemistry, autoradiography and electronmicroscopy in afuelically damaged fibers, and correlation of forearm ischemic exercise with single-fiber-EMG (with U. Tennessee, U. South Dakota, Uppsala). (b) Lipid/fatty-acid/ketone-body utilization defects. Documented by us previously was the first defect in this category, impaired utilization of long-chain fatty-acids, some other cases of which were found by others to be due to carnitine palmityl transferase (CPT) deficiency. We are establishing this assay in our laboratory. Our original patients will be studied per their biopsied and cultured muscle. Our syndrome of fatal fasting infantile rhabdomyolysis is postulated to be in this category, but all studies to date have not shown an enzymatic deficiency (e.g., CPT was 2X normal). We will be studying cultured muscle of this. (c) Hypocyclasias: Reduced plasma-lemmal adenylate cyclase but normal β -adrenergic receptors were found in three conditions, (i) muscle-fiber-hypotrophy-with-central-nuclei, (ii) myotonic atrophy, (iii) diazacholesterol-induced myotonia of intact rat muscle and of tissue-cultured rat muscle (last two discussed in Episodic Weakness/Myotonia Project). In press is our study of muscle biopsies of affected infants from two families with X-linked recessive infantile-fatal muscle-fiber-hypotrophy-with central nuclei, flown to us from Amsterdam and Texas for tissue-culture. Both showed the same abnormalities: (1) cultured muscle cells had a marked, apparently uncontrolled proliferation, resembling that of neoplastic cells, which has persisted through many passages over 10 months, and was not controlled by CNS extract or CNS co-cultures; (2) large multinucleated myotubes formed very early but never matured by light- or electronmicroscopic criteria and never contracted, only 60% of normal adenylate cyclase (basal, and Naf or isoproterenol stimulated) in plasmalemma but normal β -adrenergic receptors (by ¹²⁵I-hydroxypindolol-binding). These findings demonstrated an intrinsic defect of the muscle cell, apparently an impaired control mechanism(s) related to cAMP. That could, among other effects, have resulted in impaired response to neurogenic maturational mechanisms, i.e., one kind of "myogenous dysinnervation". The identified defect now can be the basis of treating such patients with a phosphodiesterase inhibitor to increase cellular cAMP. In fact, one patient with somewhat similar biopsy findings was treated (with U. Rochester) -- there was no clinical benefit, but the patient's muscle fibers subsequently in culture did not show the abnormal growth, presumably indicating a different disease. (d) Other biochemical abnormalities. (i) Carnitine deficiency in myopathies has, by others, been found in several forms. It does not behave as a lysosomal defect or as an afuelia. To better screen our patients, we are setting up the assay. (ii) Adenylate deaminase (AD) deficiency of muscle, postulated by others to be pathogenically significant, was studied by our improved histochemical method. Since the only abnormal muscle biopsy of > 100 was a patient with renal carcinoma, amyloidosis and peripheral neuropathy, we question a pathogenic role of AD deficiency.

II. Duchenne muscular dystrophy (DMD). This is the most prevalent of the old-terminology "muscular dystrophies". It is an X-linked hereditary progressive deterioration of muscle in boys, usually causing wheelchair or bed confinement by age 12 and death by age 20 years. Cause and treatment are not known. Current competing hypotheses of the pathogenesis of DMD are: (a) primary or secondary defect of blood supply to muscle, (b) primary defect of energy source within the muscle fiber, and (c) primary muscle-fiber plasmalemmal defect. Although nearly all others favor (c), we favor (a) or (b) or (b + a). Some of the findings in DMD muscle considered by others to be supportive of (c) we consider to be invalid results or not distinguishing between (c) and (b). In DMD the plasmalemma has long been known to be leaky, evidenced by elevated CPK and other "muscle enzymes" in the serum, but that certainly does not specify a primary plasmalemmal defect. Our studies have been concerned with: the nature of the muscle cell plasmalemma, the effects of its leakiness, plasmalemma of other cells in DMD patients, and intramuscular blood vessels and flow.

A. Plasmalemmal Composition. (a) The distribution of major erythrocyte phospholipids and the total fatty acid and fatty aldehyde composition of erythrocytes and plasma was not abnormal in DMD patients or definite DMD carriers, or myotonic atrophy patients, compared with a large number of normal and disease controls, contrary to several published reports. (b) Human and animal muscle cultured aenurally was studied in different developmental states with a battery of membrane probes histochemically, fluorescence-microscopically, electronmicroscopically and autoradiographically -- concanavalin A for α -d-mannoside and α -d-glucoside groups, ruthenium red for acid mucopolysaccharides, α -bungarotoxin for nicotinic acetylcholine receptors, and tannic acid. Tannic acid stained plasmalemma only of mature muscle fibers and thus can be used as a marker of muscle fiber maturity; it also stained t-tubules of rat and chicken muscle cultures and showed these structures absent in cultured human muscle. A base was established against which cultured Duchenne dystrophy muscle can now be compared. (c) The effect of specific plasmalemmal-reacting agents on living muscle cells is being studied.

B. Plasmalemmal porosity: outward leakage. (a) Radioimmunoassay of serum BB-isozyme of creatine kinase (CK) is being reported as a new method of demonstrating leaking immature or regenerative muscle fibers, while the MM-isozyme demonstrates leaking mature fibers. Although this approach gives an indication whether non-mature or mature fibers are leaking, it has not increased the detection of carriers of Duchenne dystrophy (with CC). (b) Serum myoglobin elevation was reported as a new method of detecting additional carriers of Duchenne dystrophy (with Columbia). (c) Hemopexin is an inducible, liver-produced, heme-transport protein. It was first discovered elevated in DMD patients and carriers a number of years ago by a current member of our group. We have now reported (with UCSD) our confirmation of that. To support the hypothesis that the elevation is induced by the subtle myoglobin leakage from damaged muscle fibers, we have reported that: (i) with a sensitive radioimmunoassay, myoglobin leakage into the serum was found in nearly all

DMD patients and half of the carriers (with Columbia); (ii) serum hemopexin elevation was induced in monkeys with experimentally crushed muscle or with repeated injections of small amounts of myoglobin, which persisted long after CPK levels returned to normal (in the crush studies); (iii) a large amount of myoglobin released into the serum, clinically in rhabdomyolysis or experimentally injected in monkeys, induced an initial fall of serum hemopexin followed by a rise; (iv) of a large number of neuromuscular-disease patients surveyed with the immunodiffusion assay for hemopexin quantitation, only active myopathies, especially dermatomyositis/polymyositis and DMD patients and carriers, but also myasthenia gravis patients, had hemopexin elevations, all of those groups of patients having had elevated serum myoglobin; (v) molecular turnover studies using ¹²⁵I-hemopexin and ¹³¹I-albumin showed an increased turnover rate of hemopexin (increased synthesis > increased catabolism) in patients with Duchenne dystrophy, dermatomyositis/polymyositis and myasthenia gravis compared with normal and disease controls. Parallel turnover studies in monkeys showed that small amounts of heme (as could come from myoglobin or intravascular hemolysis) increased hemopexin synthesis while larger amounts of heme were required to increase the catabolism of hemopexin; those data demonstrated aspects of the hemopexin regulating mechanisms and explain what we observed in patients.

C. Plasmalemmal porosity: inward leakage. (a) Calcium ingress as part of our "calcium hypothesis" was published in more detail, that ingress was considered the trigger event for muscle responses to impaired plasmalemmal integrity, be it damage of unknown cause as in DMD or of known cause as in an endogenous aetiology or an exogenous ischemia, toxin, toxic-antibody, or toxic T-lymphocyte. If the calcium entry is slight and restricted, it is relatively benign, and probably is the "spark for repair/regeneration/hypertrophy" of muscle fibers. If the calcium entry is severe and unrestricted it begins a lethal cascade of events pushing muscle fibers past their point of no return, i.e., it is probably the "ultimate molecular assassin" or the "messenger of molecular doom". We have based our calcium hypothesis on our numerous interrelated studies of calcium in normal and damaged muscle fibers of patients with various neuromuscular diseases and various induced animal models thereof, utilizing light- and electronmicroscopic histochemistry, autoradiography, biochemistry and clinical scanning. The calcium mechanism can account for the large amount of obvious and subtle regeneration we see in DMD muscle by acridine orange and adenylate cyclase reactions. The calcium mechanism is not disease specific. We have previously described leakage of serum proteins into damaged muscle fibers that is not disease-specific.

D. Muscle blood vessels and blood flow. (a) Our ischemia hypothesis for DMD, which proposed a functional defect on the arterial side of the vascular tree, was based on our studies of the histochemopathology of DMD muscle, our experimental ischemic myopathy in animals, and our study of human ischemic limb muscles. An ischemia mechanism, although possible in DMD patients, has not yet been demonstrated in them directly. The next logical step we are now going to undertake by means of a laser-doppler technique for measuring blood

flow in superficial capillaries of patients' muscle at time of biopsy and studying the vascular responses to perturbation by minor hypoxic stasis (with NHLBI). In initial studies we have evaluated the technique as applied to human forearm skin blood vessels and to rat skeletal muscle vessels, and their responses. (b) Our new techniques for studying muscle blood vessels, being reported, include: (i) autoradiographic localization of β -adrenergic receptor with 125 I-hydroxybenzylpindolol, showing very high concentration in intramuscular vessels, much higher than in muscle fibers; (ii) histochemical localization adenylate cyclase, showing it to be much higher in vessels than in normal muscle fibers (but high in regenerative fibers).

E. Blood cells. (a) An abnormal decrease of platelet dense bodies has been found in platelets of DMD patients (with CC and NIMH). (b) Platelet uptake of serotonin in DMD patients, now remeasured, again shows some reduction of initial uptake rates; and serotonin release rates were normal. (c) Platelet-cAMP-phosphodiesterase was increased in DMD patients and carriers and cGMP-phosphodiesterase increased only in carriers; both enzymes were normal in erythrocytes of DMD patients and carriers.

III. Polymyositis/Dermatomyositis Complex (PM/DM):

PM/DM is an acquired disorder causing progressive deterioration of muscle in children and adults. The primary cause is not known but the pathogenic mechanism is considered dysimmune (autoimmune). Before the introduction of anti-dysimmune therapy, all patients were seriously incapacitated and many died.

A. Therapeutic efforts: Dysimmune component -- high-single-dose alternate-day prednisone (HSDAD-Pred) with or without azathioprine 3 mg/kg/day seems to be the best treatment. Cyclophosphamide is an alternative to azathioprine. In patients failing to respond to these drugs we will be trying Poly-ICLC (see our ALS Project). Calcinosis -- massive subcutaneous calcification, "calcinosis universalis", is a complication of dermatomyositis which is crippling and causes skin breakdown and infection. Calcium-solubilizing agents (EDTA, diphosphonate) in the past have failed. However, we have found in some severely affected patients the calcium has remarkably diminished as the muscle and skin were responding to our combined azathioprine-HSDAD-Pred program, and has remained diminished for several years, even as the drugs were gradually reduced.

B. Pathogenic mechanisms: The exact mechanism(s) of muscle damage in PM/DM is unknown. We previously found immunoglobulin complexes in muscle blood vessels in 83% of children and 29% of adults, supporting the hypothesis of a vascular mechanism of damage, especially in childhood DM. To directly study blood flow in the patients' muscle we will use a laser-doppler method at time of muscle biopsy (see Duchenne dystrophy, above) (with NHLBI). We will also study possible impairment of suppressor T-lymphocytes, a mechanism that might be more especially important in adult DM/PM (with NCI). DM/PM is considered to be a dysimmune response to either an exogenous antigen or a normal cell component "foreigned" by an exogenous agent. Specific treatment/elimination

of an exogenous agent could be curative (as opposed to the merely suppressive action of all current treatments). We are continuing to seek evidence of an exogenous agent(s). We have not yet been able to rescue an agent (with IDB) nor to find reverse transcriptase. Collagen increase in DM/PM has been suggested by others as a possible pathogenic mechanism of muscle fiber damage. We have used antibodies against types I, II, III and IV collagens (which differ by virtue of the amino acids of their 3 polypeptide chains), against their procollagens and against fibronectin. We found types I and III collagen and procollagen and fibronectin in the normal endomysium and perimysium; they accumulate there in DM/PM, but in the same manner as in Duchenne dystrophy and thus the accumulation is not disease-specific. These are also increased in intramuscular blood vessels of DM/PM, especially the childhood form, but not in Duchenne dystrophy -- this is more disease-specific but may be secondary to immunoglobulin complexes deposited in the vessels. Type IV collagen normally is only in cellular basement membranes and is not altered in DM/PM (or DMD).

The cardiac involvement of DM/PM, demonstrable with non-invasive cardiologic techniques and occurring in the majority of 20 patients, was published; abnormalities consisted of conduction blocks, arrhythmias and systolic mitral prolapse (with NHLBI, VAH DC, Georgetown U.).

C. Chronic vacuolar myopathy, seen in 18 of our patients, may or may not have a pathogenesis somewhat related to that of the DM/PM complex. We have, though, separated it off as a distinct disease (or syndrome). It is characterized by acid-phosphatase-positive vacuoles and contain multiform membranous whorls and masses, and collections of glycogen granules; there are also frequent collections of long parallel-arranged double-helical tubule-like twists having 20 μ "diameter" and 100 μ periods. We will be publishing our findings that the muscle in culture reincarnates the typical vacuoles after 10 days of growth. In one case, the cultured muscle fibers had by electron-microscopy a number of unusual structures resembling virons situated near the nuclei or plasmalemma; re-scrutinizing the original biopsy revealed rare examples of identical structures.

IV. Other Myopathies and Neuromuscular Diseases of Uncertain Classification:

A. Malignant hyperthermia-rigidity (MHR) is a syndrome, 70% fatal, of acute rise of body temperature accompanied by muscle rigidity during general anesthesia, usually provoked by halothane and/or succinylcholine. A number of the patients (if not all, by definition) have underlying not-well-defined neuromuscular disorders. One well-defined underlying condition is central core disease, having a high incidence of MHR. In seeking a new test for patient-susceptibility to develop MHR, we have developed a focal model in a strain of pigs susceptible to MHR (with U. of Berne).

B. "Ragged-red" muscle fibers, which contain severe mitochondrial abnormalities, are the commonest histochemical manifestation in limb muscles of the heterogenous syndrome of oculocranosomatic neuromuscular diseases with ragged-red fibers (OCSNMD-RR). The patients usually having lacticacidosis and often ophthalmoplegia. Some patients have a syndrome of small stature,

seizures, mental impairment, and lacticacidosis -- in limb muscle cultured from two such patients, we have reported (with DNB) that most of the mitochondrial changes, including increased number and greatly increased size of mitochondria, wide distorted "twisted-ribbon" cristae, and mushy-looking inclusion material, have been re-incarnated. We also reported that the same changes were produced in normal human muscle cultures after 2 days exposure to dinitrophenol, and the cultured ragged-red-fiber muscle was extremely susceptible to worsening of the in vitro changes by dinitrophenol. This demonstrates a mitochondrial defect which is reproducible in cultured muscle fibers and provides a test-system for seeking a possible genetic or occult-infectious basis. In the original biopsies and in the cultures the mushy-looking and crystalline inclusions lacked cytochrome oxidase staining by our EM-cytochemistry.

Many of the OCSNMD-RR patients with ophthalmoplegia have cerebellar ataxia, mental impairment, some denervation evident in muscle biopsy, and spinal-fluid protein increase. We have now reported (with SNB, U. Tennessee) that some such patients by CAT-scan have a decreased attenuation coefficient of cerebral white-matter and small brain-stem (shown by enlarged 4th ventricle and pre-pontine cisterns), changes correlated with the clinical state; the CAT-scan changes were also absolutely correlated with electrophysiologically determined delay of the bilateral late-response of the blink reflex (with EEG).

C. Type-II muscle fiber atrophy. We continue to study the selective atrophy of the type-II (glycolytic-rich, oxidative poor) muscle fibers, especially the subtype-IIB fibers. This atrophy we have shown to be the basis of cachectic atrophy accompanying cancer and other chronic debilitating disorders. Evaluation of the cause of type-II fiber atrophy in cancer patients, theoretical mechanisms of which we published previously, is important because this "remote-effect" muscle weakness is often the most crippling aspect of cancer -- if the molecular mechanism(s) can be discovered it might be treatable independently of treatment and response of the cancer itself. An improvement of the muscle weakness and wasting could even make the patient better able to withstand the rigors of direct anti-cancer therapy. We have now reported 3 hypothetical mechanisms, which could even be summated in cancer patients: (a) insidiously decreased oral fuel (caloric) intake, which we have recently documented; (b) fuel wastage due to metabolic derangement within neoplastic or secondarily affected non-neoplastic cells; (c) possibly a circulating small-molecule remote-effect acting on type-II fibers. In exploring these mechanisms, we will be studying the role of insulin reception by and its action upon muscle fibers.

V. Basic biology of muscle. Several technical approaches have been pursued.

A. Tissue culture. (a) Human biopsies (76 patients) and rat muscle were cultured with improved techniques and used for a variety of cytochemical, immunocytochemical, ultrastructural, biochemical, autoradiographic and electrophysiological studies (see other parts of this Project). For example: (i) aneurally

cultured adult human muscle developed muscle-type (adult-type) isozymes of phosphorylase and phosphofructokinase, but not of pyruvate kinase which appeared only in the brain-type (fetal-type) (with Inst. de Pathologie Moleculaire, Paris); (ii) baseline electrical properties of aneurally cultured adult human were defined by microelectrode studies (see Myotonia Project).

B. Electronmicroscopy. (i) To selectively stain RNA or DNA, a modification of platinum-thymine complex method was used. To be published are our results: The expected subcellular structures were stained in normal and regenerating muscle fibers; there was no staining of the crystal-like structures in mitochondria of ragged-red fibers, nor could DNA- or RNA-viral material be identified in them or in muscle fibers of chronic vacuolar myopathy; unexpected was the finding of uniform staining for nucleic acids of the plasmalemma of cultured muscle cells (resembling that noted by others in tumorigenic cell cultures). (ii) (See other parts of this Project.)

C. Histochemistry. (i) Adenylate cyclase (AC) -- being prepared for detailed publication is: our new method, utilizing AMP-PNP as the substrate and Ca^{++} (which does not inhibit AC as Pb^{++} does) as the capture agent; specificity of the AC reaction by use of selective inhibitors vis-a-vis than guanylate cyclase reaction with GMP-PNP; and the localization and relative amounts of AC in blood vessels, nerve cells and muscle cells (normal, pathologic, regenerative) at light-microscopic and electronmicroscopic levels. (ii) Lectin probes, Con A, LC, RCA 120, WGA, Soy, for membrane polysaccharides were used for the first time in human muscle and spinal cord pathology and in human muscle cultures -- the localizations are to be published. (iii) Phosphodiesterase-I. The histochemical distribution in normal and pathologic human and rat muscle was presented and will be reported. Differences were marked between human and rat muscle and between rat blood-vessels and muscle fibers. (iv) Superoxide dismutase -- a histochemical method is being developed.

D. Autoradiography. Three applications have been developed. (i) To animal models -- localization of β -adrenergic receptors in normal and denervated rat muscle with ^{125}I -hydroxybenzylpindolol; (ii) To human and animal tissue cryostat sections ^{125}I localization of nicotinic acetylcholine receptors (nAChRs) with ^{125}I - α -bungarotoxin in neuromuscular junctions, in plasmalemma of denervated fibers and in thymic epithelial cells; (iii) cultures of muscle, neurons and schwann cells -- β -adrenergic receptors and nAChRs, as above. Other receptors, e.g., α -adrenergic receptors and insulin receptors will now be sought. Study "i", in press, showing in normal muscle much greater amount of β -adrenergic receptors in arterial-tree vessels than in muscle fibers indicated (a) the fallacy of assuming β -adrenergic binding studied biochemically in whole-tissue muscle homogenates is only in muscle cells, and (b) the potential importance of arterial-tree, as well as muscle fiber, β -adrenergic receptors in human neuromuscular diseases. Study "ii" confirmed our earlier histochemical finding of increased nAChR in the plasmalemma of denervated muscle fibers and presence of nAChR in human thymic epithelial cells (see Myasthenia Gravis Project).

E. Biochemistry. Most of our attention has been directed to components of the plasmalemma, t-tubule and reticulum of human and animal muscle, with correlations with our other studies of the plasmalemma. Skeletal muscle plasmalemma (PL) sarcoplasmic reticulum (SR) and mitochondria (M) were prepared from homogenates of normal and denervated rat muscle, from human muscle from legs amputated for osteogenic sarcoma, from human diagnostic muscle biopsies, and from patient biopsy muscle grown in tissue culture. (1) β -adrenergic receptor (β AR) adenylate cyclase (AC) system. (a) Animal muscle. (i) Following denervation of rat muscle there are increased β AR's (per 125^I -hydroxybenzylpindolol binding), not blocked by cyclohexamide, and decreased AC in PL, SR and M. (ii) An aqueous sciatic nerve factor, as a putative trophic/regulatory factor, applied in vitro at higher concentrations decreased β AR of PL 30-40% in denervated muscle and 10-20% in normal muscle. (iii) Developmentally, in the rat β AR and AC are present from 18-day embryonic states (birth occurs at 21 days) but are not functionally coupled until 3 days after birth; this study was presented. (b) Human muscle. (i) The subcellular distribution of β AR in normal human muscle was defined and presented. The distribution, PL >> SR > M, correlated with our previous localization of AC in human muscle, supporting a functional coupling. (ii) Compared with control human muscle, in myotonic atrophy and x-linked recessive muscle-fiber hypotrophy with central nuclei, there was decrease of AC but no change of β AR; both β AR and AC were decreased in various denervations, hypokalemic periodic paralysis and dermatomyositis. (2) Guanylate cyclase (GC). (a) Animal muscle. We previously reported increase of GC in all fractions (PL, SR, M and 10⁵g supernatant) of denervated rat muscle. We now find the increase is not blocked in cyclophosphamide-treated animals, suggesting a mechanism other than new synthesis of GC must be responsible for the increase. (b) Human muscle. In normal human muscle the subcellular distribution and properties of GC were similar to those we observed in rat muscle. The specific enzyme activity among subcellular fractions was PL > SR > M > soluble fraction (10⁵g supernatant). Even though PL possessed the highest specific activity, the predominant portion of GC (60%) was in the soluble fraction. The enzyme required Mn²⁺, but Mg²⁺ could substitute to a considerable extent. Also demonstrated were elution profiles on sepharose columns, substrate dependency, ionic requirements and pH profiles. (3) Acetylcholinesterase (AChE). The subcellular distribution and properties of the 3 molecular forms of AChE in normal and denervated rat muscle were published.

F. Muscle Spindles. Published or in press were our histochemical and correlated electronmicroscopic studies of human and rat muscle spindles, detailing nuclear-chain and nuclear-bag-1 and nuclear-bag-2 fibers.

Significance to Bio-Medical Research and the Program of the Institute: These findings provide new information on the pathologic and pathogenic aspects of the various myopathies, on the treatment of some, and on animal models of some of the myopathies.

Proposed Course of Project: The studies underway are part of a long-term project consisting of interrelated investigations which will continue for several years.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01190-15 MN
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Myasthenia Gravis (MG)		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: W. King Engel, M.D., Chief, Neuromuscular Diseases Section, NINCDS OTHER: Valerie Askanas, M.D., Ph.D., Associate Neurologist, NINCDS Marinos C. Dalakas, M.D., NINCDS Charles McIntosh, M.D., Surgery Branch, NHLBI Dale McFarlin, M.D., Chief, Neuro-Immunology Branch, NINCDS Bennett Lavenstein, M.D., NINCDS Lawrence Kagen, M.D., Columbia University, NY		
COOPERATING UNITS (if any) Bruce T. Adornato, M.D., Palo Alto Med. Clinic, CA Alan Goldstein, Ph.D., George Washington U. Moyhee Eldefrawi, M.D., U. MD School of Medicine (Continued)		
LAB/BRANCH Medical Neurology Branch		
SECTION Neuromuscular Diseases Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 4.0	PROFESSIONAL: 3	OTHER: 1.9
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) To apply clinical, immunologic, tissue-culture, histochemical, pharmacologic, electrophysiologic, autoradiographic, radionuclide-scanning, and electronmicroscopic techniques to investigate the etiology and pathogenesis of <u>myasthenia gravis</u> . Especially, to seek new or improved methods of treatment. <hr/> Cooperating Units: D. L. Madden, M.D., NINCDS Sidney A. Houff, M.D., Clinical Associate, IDB, NINCDS John L. Sever, M.D., Chief, IDB, NINCDS Benjamin Castleman, M.D., Harvard Medical School		

Project Description:

Objectives: To apply clinical, immunologic, tissue-culture, histochemical, electromicroscopic, pharmacologic, electrophysiologic, autoradiographic and radionuclide-scanning techniques to investigate the etiology and pathogenesis of myasthenia gravis. Especially, to seek new or improved methods of treatment and diagnosis.

Methods Employed: A variety of basic and clinical investigative techniques, v.i., were applied to patients with myasthenia gravis and other disorders of neuromuscular transmission, and to induced animal-models thereof.

Patient Material: Myasthenia gravis patients, and patients with other disorders of neuromuscular transmission, participated in the investigative studies and therapeutic trials. Sera, muscle and thymus were obtained during diagnostic or therapeutic procedures.

Major Findings:

Myasthenia gravis (MG) is an acquired disorder affecting transmission at the neuromuscular junction, mainly in adults and older children. The primary cause is not known, but the pathogenic mechanism is considered to be dysimmune (or autoimmune). Untreated patients usually are seriously handicapped and many die. Palliative treatment with anticholinesterases and anti-pathogenic treatment, consisting of thymectomy, ACTH and, most recently, prednisone, have helped considerably, but much disability, some fatality, and drug side-effects do occur.

A. Therapy.

1. Thymectomy. The role of thymectomy in the treatment of MG, for which MG patients, has recently been questioned. To be published is a review of 55 consecutive thymectomies done over the past 10 years. It showed: thymic hyperplasia, onset age 16-29, 84% improved; thymoma, 67% improved (all onset > age 29); onset > age 29, 71% improved (83% of patients with thymic hyperplasia, 70% of patients with "involved" thymus (v.i.); zero operative mortality, low operative morbidity; severity of myasthenia not a contra-indication, but an indication for surgery (84% improvement in patients "thymectomized" < 10 years of onset cf. 33% improvement rate if duration > 10 years); transcervical surgical approach unsatisfactory, necessitating re-operation by sternal-splitting in 7/9 patients) and resulting in clinical improvement in all 7; our modified transverse sternal-splitting upper-sternalotomy approach provides much greater surgical exposure than the transcervical route with minimal increase of post-operative morbidity, and presumably has less risk of uncontrollable bleeding (which has caused death with the transcervical approach by others); and less morbidity than vertical sternal-splitting, attributable to preserved lower sternal integrity allowing deeper, less painful respiration and earlier ambulation (with NHLBI and Harvard). Thus thymectomy is potentially beneficial in all patients with onset in teen-ager or later, and repeat thymectomy can be remarkably beneficial in patients previously improved who subsequently exacerbate and do not respond to medical management.

2. Prednisone. Long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred) was introduced to this disease by us 9 years ago, and one child was begun on treatment 13 years ago. Nearly all patients continue to have excellent benefit. However, virtually all responders are, even with very gradual tapering of the LT-HSDAD-Pred, dependent on it, requiring 5-20 mg q.o.d. Prednisone in higher doses reduces circulating lymphocytes (T > B), and lymphocyte-response to mitogens (T-mitogens > B-mitogens), but those effects last no longer than 24-48 hours. We are now seeking the mechanism of benefit of the low maintenance dosage. Because prednisone has toxicity, we are seeking other drugs for MG.

3. New prednisone-responsive disorders. Three patients have been identified who, by detailed investigation, do not have one of the neuromuscular diseases (MG, dysschwannian polyneuropathy, dermatomyositis/polymyositis) known to respond to LT-HSDAD-Pred. Because their severe weakness was out of proportion to minimal or no histochemical and electrophysiologic abnormalities, LT-HSDAD-Pred was tried. Remarkable improvement was achieved in each, with return of weakness each time the dosage was lowered too much. They have a new disorder(s) and will be reported. We will explore whether their disorder is related to the new experimental dysimmune dysneuronal neuropathy having an aspect of impaired neuromuscular transmission we reported last year.

4. Poly-ICLC. We have found this to be a new anti-dysimmune drug (see ALS and Peripheral Neuropathy project). One MG patient showed apparent improvement on a very short course; more patients will be studied.

B. Pathogenesis. Questions regarding pathogenesis include possible altered host (patient) immunologic response, "foreigned" host cells, exogenous agent, role of thymus, role of lymphocytes, and role of nicotinic acetylcholine receptor (nAChR).

1. Thymus pathology. The rationale for the empirically-observed benefit of thymectomy (v.s.) is still being sought. In addition to the hyperplastic and thymomatous thymuses, we have now demonstrated that thymuses of older patients considered "atrophic" by pre-existing histopathologic criteria have evident in our fresh-frozen sections many small nests of lymphocytic and epithelial cells that look active -- we postulate they may have a pathogenic role in the older non-thymomatous MG patient.

2. Thymic nicotinic acetylcholine receptor (nAChR). We earlier demonstrated nAChR in thymic epithelial cells histochemically with peroxidase-labelled α -bungarotoxin. Now we have demonstrated it autoradiographically with 125 I- α -bungarotoxin (with Georgetown U.). The thymic epithelial-cell nAChR may be the molecule foreigned by an exogenous agent.

3. Thymic Thymosin. Thymosin is a thymic hormone, discovered by A. Goldstein, capable of repairing and sustaining normal immune function of lymphocytes. With antibodies to thymosin we have achieved the first cytolocalization of thymosin -- it is in thymic epithelial cells of normal thymuses and of hyperplastic and thymomatous thymuses of MG patients (with George Washington U.).

4. Thymic cultures. We have cultured thymic cells from normal and MG human thymuses. We have never found muscle cells or cells with myofibrils. We find epithelial cells with typical desmosomes and tonofibrils. Epithelial cells form typical Hassall's corpuscles in culture. They (and not fibroblasts) contain thymosin by fluorescent antibody reaction (with George Washington U.). We will study the amount of their secretion of thymosin in vitro and its regulating mechanisms. We are now seeking evidence of nAChR by electron-microscopic-cytochemical and autoradiographic means.

5. Suppressor cells. One hypothesis is that MG is caused by a defect of suppressor t-lymphocytes. We shall be studying the suppressor cells in MG.

6. Cerebrospinal fluid (CSF) immunoglobulin bands. We reported that 7 of 23 MG patients had oligoclonal IgG bands and 5 of the others had monoclonal IgG bands. Since IgG "synthesis" (per Tourtellotte formula) in the CNS was normal, the CSF pathologic bands were probably from the serum (with IDB). One band or more may reflect the anti-nAChR IgG, and therefore deserves consideration as a possible cause of the brisk reflexes of MG patients.

7. Nicotinic acetylcholine receptor (nAChR). IgG antibodies to nAChR are found in MG. We consider that there is a junctional (J) form of nAChR present only at the neuromuscular junction and an extrajunctional (E) form present both extrajunctionally in non-innervated fibers and to some extent at the junction of innervated fibers. Since we found that the anti-nAChR of MG patients reacts both at junctional and extrajunctional locations, we have proposed it to be against the E form. It is likely that the nAChR of thymic epithelial cells (v.s.) is the E form. (The antibodies in rabbits immunized with electric-fish nAChR are against the J form, per our report last year.) Previously we showed histochemically that the E-nAChR of denervated muscle fibers reacts with α -bungarotoxin; we have now shown that reaction autoradiographically with ¹²⁵I- α -bungarotoxin. Cultured muscle of humans, rats and chicks has diffuse plasmalemmal nAChR that reacts with the IgG nAChR antibodies of MG patients, suggesting it is E-type. Those nAChR receptors are mobile, as indicated by our current finding, utilizing rhodamine-labelled α -bungarotoxin, that diazacholesterol treatment in vitro increases their mobility.

8. Hemopexin. We have now reported that serum hemopexin is increased in MG patients (see Myopathy project); that was inexplicable until our recent finding of increased myoglobin in the serum of MG patients by use of a very sensitive complement-fixation technique (with Columbia). That small amount of myoglobin leakage may be a manifestation of a hitherto overlooked minimal subclinical plasmalemmal-leaking myopathy in many MG patients.

Significance to Bio-Medical Research and the Program of the Institute: These findings present new information on the pathologic and pathogenic aspects of myasthenia gravis, and other defects of neuromuscular transmission, on treatment, and on corresponding animal-models.

Proposed Course of Project: To develop more fully the interlinked basic and clinical studies underway directed toward clarification of the pathogenesis and identification of the etiology, and toward elaboration of better means of treatment and prevention.

Publications:

Adornato, B.T., Houff, S. A., Engel, W. K., Dalakas, M.C., Madden, D.L. and Sever, J. L.: Abnormal immunoglobulin bands in cerebrospinal fluid in myasthenia gravis. Lancet ii:367-368, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01189-11 MN
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Episodic Weakness and Myotonic Disorders		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: W. King Engel, M.D., Chief, Neuromuscular Diseases Section, NINCDS OTHER: N. Bojji Reddy, Ph.D., Guest Worker, MN, NINCDS Valerie Askanas, M.D., Ph.D., Associate Neurologist, Medical Neurology Branch, NINCDS Albert J. Tahmouh, M.D., Clinical Associate, NINCDS G. K. Bergey, NICHD P. G. Nelson, NICHD		
COOPERATING UNITS (if any) Laboratory of Developmental Neurobiology, NICHD Roger A. Brumback, M.D., VA Hospital, Fargo, ND		
LAB/BRANCH Medical Neurology Branch		
SECTION Neuromuscular Diseases Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.5	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) To define more clearly and to treat those disorders affecting the neuromuscular apparatus which present primarily with <u>episodic weakness</u> or paralysis or are characterized by a significant amount of <u>myotonia</u> , i.e., conditions in which the main site of intermittent dysfunction is somewhere within the muscle fiber plasma-lemma, T-system, sarcoplasmic reticulum, or myofibrillar complex --i.e., the total excitation-contraction coupling mechanism. With respect to <u>periodic paralysis syndromes</u> , studies are done with agents which are transiently either therapeutic or provocative, with a view to obtaining more information regarding abnormalities of pertinent metabolic pathways and methods of treatment. The various <u>myotonia disorders</u> are studied with respect to more clearly defining the molecular abnormalities, seeking the underlying pathogeneses and treatment thereof, and finding better ways of symptomatically treating their myotonia. Induced animal-models of myotonia are also used for these purposes. Tissue culture of the human abnormal muscle is used to reincarnate the disease in culture and thence its		
51 - MNB/IRP		

analysis, e.g., with microelectrodes, and its treatment; cultured human or animal muscle is also used for induction of models of diseases in vitro by chemical agents.

Project Description:

Objectives: To define more clearly and to treat those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis, or are characterized by a significant amount of myotonia, conditions in which the main site of intermittent dysfunction is somewhere within the muscle fiber plasmalemma, T-system, sarcoplasmic reticulum, or myofibrillar complex -- i.e., the total excitation-contraction coupling mechanism. With respect to periodic paralysis syndromes, studies are done with agents which are transiently either therapeutic or provocative, with a view to obtaining more information regarding abnormalities of pertinent metabolic pathways and methods of treatment. The various myotonia disorders are studied with respect to more clearly defining the molecular abnormalities, seeking the underlying pathogeneses and treatment thereof, and finding better ways of symptomatically treating their myotonia. Induced animal-models of myotonia are also used for these purposes. Tissue culture of the human abnormal muscle is used to reincarnate the disease in culture and thence its analysis, e.g., with microelectrodes, and its treatment in vitro; cultured human or animal muscle is also used for induction of models of disease in vitro by chemical agents.

Methods Employed: Various techniques of clinical investigation, including electromyography, clinical biochemistry, and muscle biopsy with samples for histochemical analysis, electronmicroscopy, tissue culture and biochemical assays were utilized. Cultured muscle was studied with various techniques, including intracellular microelectrode electrophysiology. Therapeutic trials to raise or lower potassium or sodium and provocative loading tests were used. Acetazolamide was administered as a prophylactic agent for hypokalemic periodic paralysis, and as a treatment of myotonia. Diazachol-esterol, a myotonogenic agent, was administered to animals and to human and animal muscle growing in culture.

Patient Material: Patients of all ages are admitted to the Medical Neurology Branch for this project if they have: intermittent muscular weakness associated with familial periodic paralysis, hypo- or hyperkalemic; isolated examples of periodic paralysis with potassium disturbances; thyrotoxic periodic paralysis; paramyotonia congenita; myotonia congenita; or myotonic atrophy. (Patients with myasthenia gravis are part of another Medical Neurology Branch project.)

Major Findings:

I. Periodic Paralysis (PP). These are hereditary or acquired disorders causing chronic weakness punctuated by attacks of paralysis. There are potassium-benefited and potassium-provoked forms. Associated metabolic abnormalities are known but the actual pathogenic mechanisms are not. Standard palliative/preventive therapy in the idiopathic hypokalemic form of PP

is potassium, and more recently acetazolamide. 1. Treatment. In the hypokalemic form of PP, the treatment we introduced, long-term acetazolamide, has continued to be the best prophylactic agent both for preventing attacks and improving inter-attack weakness. It is now in the textbooks as such. Two of our patients have been treated successfully for more than 13 years. Since muscle contains either no, or very little (per disparate studies of ourselves cf. others) carbonic anhydrase, the mechanism of acetazolamide benefit in hypokalemic PP remains unknown. 2. Pathogenesis. Human muscle was grown aneurally in tissue culture to obtain fibers free of all neural, circulating and other influences existing in the patient. We have successfully studied it with microelectrodes (with NICHD) and obtained baseline values: resting membrane potential $U(V_m)$ 52.4 ± 6.6 mV, input resistance 5.5 ± 3.4 M Ω , only 2/34 fibers electrically excitable at V_m , but when hyperpolarized to 80 mV an action potential could be elicited from all with threshold for excitation at 22.6 ± 8.7 mV and action-potential amplitude of 83.4 ± 28.9 mV. Against these we will now compare fibers cultured from patients with various periodic paralyses and myotonias. We can also study the effect on these parameters of motor neuron innervation in vitro of the cultured human fibers.

IIA. Myotonia Congenita and Paramyotonia Congenita. Myotonia is a crippling symptom in these inherited diseases of unknown causes. (1) Clinical Studies. Last year we reported acetazolamide as a new treatment providing excellent benefit in patients who had failed to respond to other anti-myotonia agents. It continue to have long-term effectiveness in those and several additional patients. (2) Pathogenesis. (see above, tissue culture).

IIIB. Myotonic Atrophy (Myotonic "Dystrophy"). This is an inherited multi-systemic disease, with progressive muscle weakness and wasting, of unknown pathogenesis. We have previously raised the possibility of at least a partially neurogenic aspect. With our new concept of "myogenous dys-innervation", we have now extended that hypothesis to include a possible myogenous muscle plasmalemmal non-receptivity to neural short- and long-term trophic influences. (1) Pathogenesis, patient studies. (a) Adenylate cyclase -- this we found decreased 30-60% in myotonic atrophy patients, with normal β -adrenergic receptors. We have now demonstrated those findings reincarnated in muscle fibers cultured from the patients. The muscle in culture can be used to further explore this defect. (b) Myotonic phenomena -- with our baseline values of microelectrode electrophysiologic parameters of cultured human muscle now established, we will study myotonic atrophy muscle in culture (with NICHD). In that preparation we will also study chloride and other ionic conductances and ionic dysequilibrium challenges. (c) Insulin receptors on leucocytes and erythrocytes are being studied. (2) Pathogenesis, animal models. Diazacholesterol-induced myotonia in the intact animal we discussed last year, including the lowered adenylate cyclase of muscle. Now we are preparing to report the effects of diazacholesterol on cultured rat muscle. The drug caused: change of contraction rhythm to more continuous and fibrillation-like movements; electronmicroscopically evident honey-comb appearance and dilation of sarcoplasmic reticulum and irregularity of tannic-acid stained

plasmalemma; increased mobility of plasmalemmal nicotinic acetylcholine receptors; decreased binding of ¹²⁵I-Con A biochemically; and decreased adenylate cyclase (basal, and NaF and isoproterenol stimulated) but normal β -adrenergic receptors.

Significance: These findings present new information on the pathologic and pathogenic aspects of the periodic paralyses and the disorders with myotonia, on their treatment, and on corresponding animal-models.

Proposed Course of Project: To explore in more detail, with patients and animals, the mechanism of action of acetazolamide prophylaxis in hypokalemic periodic paralysis and the pathogenesis of the disease itself. To seek even better therapeutic agents. To explore the underlying nature of myotonia and the method of its benefit from acetazolamide and to seek improved methods of treating myotonia and the underlying disorders.

Publications:

Tahmoush, A., Bergey, G. K., Askanas, V., Nelson, P. G. and Engel, W. K.: Electrical properties of aneurally cultured adult human muscle. Trans. Soc. Neuroscience, in press, 1979.

ANNUAL REPORT

October 1, 1978 through September 30, 1979

Surgical Neurology Branch

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1978 through September 30, 1979
Surgical Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders
and Stroke

Paul L. Kornblith, M. D., Chief

Summary of Studies in the Surgical Neurology Branch

This annual report is the first of the newly reorganized Surgical Neurology Branch beginning October 1, 1978. This period of time has been occupied with reorganization, refurbishing of the research and clinical facilities and preparation of a research and clinical program in the neurosurgical sciences. The primary areas of our research activities have included:

1. Biological, immunological and chemotherapeutic studies in human brain tumors.
2. Biological and immunological factors in peripheral nerve injury, neoplasia and regeneration.
3. Diagnostic studies.
4. Neurophysiological studies.

In addition to these major programs preliminary organization of studies of pituitary function in neoplastic and normal states have been initiated.

1. BIOLOGICAL, IMMUNOLOGICAL AND CHEMOTHERAPEUTIC STUDIES IN HUMAN BRAIN TUMORS

A. Biological Studies

In order to determine the ways in which human brain tumors will behave in a given patient it is necessary to have cellular models of their biological activity. Such cellular models are provided by the tissue culture system. In this system it is possible to grow approximately 90% of human brain tumors in an environment outside of the human body. Utilizing this approach we have been able to show that certain characteristics of cultured human brain tumor cells not only parallel those of the cells in a patient but also offer us 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. Secondly, 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, therefore, the question is being able 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 and differences of this tumor growth to the growth of normal cells. In order to achieve successful

evaluation of all of these primary and secondary goals we have established a tissue culture laboratory at the NIH for the study of human brain tumors.

One of the major accomplishments of our group in the past six months has been the development of an appropriately trained scientific team capable of carrying out the necessary studies. This group includes an electron microscopist, Dr. Barry Smith; a neuropathologist, Dr. Paul McKeever; and appropriate technical support. In addition to these personnel, facilities for tissue culture explantation, including hoods, incubators, and appropriate monitoring devices have been built. We have initiated cultures of approximately 20 human brain tumors in this short interval of time. In addition, we have brought from the Massachusetts General Hospital many of our cultured tumors selected from the over 2,000 tumors which we have studied at that Institution. A bank of human brain tumor cultures has thus been established for our study. The observations which we have made on these initial tumors from the NIH have been supplemented, therefore, by observations which have continued on tumors brought from outside of the NIH.

The types of observations which we are able to make include the rate of growth of individual as well as populations of tumor cells; the ability of tumor cells to grow under stressful conditions such as in soft agar or in low serum concentrations; their ability to invade neighboring tissue; their chromosomal pattern and abnormalities; and their growth and malignancy in animal hosts. This latter technique is perhaps the most crucial in determining whether an individual human tumor can reproduce via tissue culture a tumor in the in vivo situation.

These studies of tumor malignancy are supplemented by studies of tumor characterization in which tumor cell populations are characterized as to their cell or cells of origin. It is extremely important in deciding whether or not a given tumor will behave in a malignant or in a benign fashion, relatively speaking, to determine what type of cell predominates. Frequently, human brain tumors are of mixed cell origin or at least mixed cell origin as regards the degree of malignancy of the cells comprising the tumor mass. Our tumor characterization includes detailed studies of cellular ultrastructure membrane and cytoplasmic biophysical properties and biochemical properties, such as S100 protein, the enzyme cortisol acetyltransferase and glial fibrillary acidic protein. In addition, studies of myelin basic protein and cerebral gangliosides may be of importance. Such studies have been initiated on the tumor cell lines under evaluation.

From these biological studies it appears possible (as we have reported previously) to determine the degree of malignancy and to characterize the cell of origin of a given tumor. This data can be useful in determining the prognosis of the individual patient and also in the planning of individualized, optimal therapy. For example, it is possible to assess the likely responsiveness to radiation therapy from the type of kinetic growth pattern of a particular tumor. The most rapidly growing tumors in tissue culture are the most sensitive to radiation. Likelihood and rapidity of recurrence may also be determined.

B. Immunological Studies

It is highly likely that immunotherapy will be an important part of brain tumor therapy in the near future. With this in mind development of a systematic approach to the study of the immunological aspects of brain tumors both with respect to the tumor and the host has been undertaken. This includes the cellular as well as the humoral facets of immunological interaction. We have been extremely fortunate to have Dr. Eugene Quindlen, a skilled humoral immunologist, as well as Dr. Maurice Gately, an excellent cellular immunologist, join our group. We thus have special expertise in the two crucial areas of immunological interaction in human tumors. We have established a laboratory for the study of immunology which includes extensive technology for the separation of immunoglobulins, for the characterization of immune globulin and antigen activity and for the characterization of cellular subpopulations of lymphocytes as well as the study of their interaction with brain tumor target cells.

The approach which we are using is to study the immune response in tissue culture utilizing individual patients' tumor cells as targets. The cultured tumor lines enable us to study the interaction in a very direct way with humoral factors, and cellular factors studied either separately or in combination.

We have studied the cytotoxic antibody responses in the serum of all human patients with gliomas that have been operated on here at the NIH in this interval. Additionally, immune adherence techniques have been used to characterize the antibody response. The fractions responsible for the immune adherence response have been found to be largely IgM while both IgG and IgM are involved in the cytotoxic responses. These studies have indicated the feasibility of the determination of the system of antitumor immune responses in which the role of specific antibody fractions can be delineated. It may well be that the immune response is not purely one of blocking lymphocyte interaction with target cells but that the humoral response may in certain instances be helpful in destroying tumor cells. Such a conclusion is supported by the observation that the presence of cytotoxic antibody in the serum of patients does indeed correlate with longer survival. The fact that there is a difference between the cytotoxic and immune adherence antibodies is supported by the fact that there is a marked difference between patients who have immune adherence responses and those who have cytotoxic responses and that the responses do not necessarily correlate directly.

The humoral immunological techniques have a potential direct diagnostic value. The detection of antibody activity in the serum of patients harboring brain tumors can be made in over 80% of such patients. A simple diagnostic test of immune response in individual patients for telling whether they may or may not have a brain tumor is thus a practical reality. More needs to be determined about the specificity of the response. To date we know that it is present in "normals" at the level of only 9%. Patients with metastatic tumors show a 30-40% positive response.

The major goal of our next phase of work will be to determine the characteristics of the cellular and humoral immunological patients who have primary astrocytic tumors. Based on these responses we hope to be able to plan in a

rational way a program of immunotherapy to enhance cellular and/or humoral immune responses and to help the patients' own body defenses to deal with their own tumors.

C. Chemotherapy Studies

At the present time the major therapeutic modality being explored to alter the prognosis in human brain tumors is that of chemotherapy. The most effective forms of chemotherapy - the nitrosourea compounds - are effective in only 50% of patients. Therefore it is important to determine which patients will respond to which agent. There are several new agents such as dianhydrogalactitol and procarbazine which show promise as additional therapeutic modalities. We have therefore developed a system, again using our tissue culture approaches, whereby the individual patient response can be determined. Using this response we have been able to show that there is a direct correlation between individual patient response to a given agent in their tissue cultures and their response clinically. This has necessitated the use of CAT scan follow-up as well as clinical evaluation on a serial basis. The observation that there is a correlation enables us to do more extensive studies in determining why there are or are not specific responders to chemotherapeutic agents. These studies of specific responders and nonresponders have included uptake and release of labelled drug as well as amino acid uptake and preliminary DNA strand break repair, membrane fluidity and surface charge. From these more extensive and sophisticated studies we may be able to learn what makes a cell or a given tumor respond or not respond to chemotherapy. Our preliminary correlations suggest that cellular uptake of labelled drug as well as release occurs in a pattern more favorable to chemotherapeutic effectiveness in the responders than in the nonresponders.

We are presently developing our model system for a more detailed and thorough study of how the drug interaction with the more commonly used agents (e.g. nitrosoureas, vincristine, procarbazine, methotrexate) as well as some of the newer agents can be evaluated on a patient by patient basis with the plan of developing a rational prospective chemotherapeutic plan and with the hope that agents or factors enhancing the response may be developed. Both cytotoxic and cell differentiation agents (e.g. cyclic adenosine monophosphate, dimethylformamide, DMSO, and hexamethylene bis acetamide) are being explored for their potential in controlling neoplastic cell growth as well as for possible interactions leading to enhanced killing of neoplastic cells.

As part of an exploration of the effects of the various components of standard glioma therapy, we have examined the effects of phenytoin (used as a prophylactic anticonvulsant after glioma surgery) and found an inhibition of the growth of at least 50% of glial brain tumors. We have previously published human brain tumor data on this inhibition and have now documented these findings in two rat glioma tumor models (C6 and RT9). At the NIH we have done a series of tests on RT9 using a subcutaneous tumor model in acute and chronic survival experiments. Work to elucidate the basis of this tumor growth inhibitory effect of phenytoin is proceeding.

A list of past publications related to this study are as follows:

PUBLICATIONS:

Liszczyk, T.M., Phillips, J. P., and Kornblith, P.L.: Procedure for the embedment and ultrastructural visualization of cells cultured on plastic microtest plates. J. Immunol. 15: 131-134, 1977.

Quindlen, E.A., Wood, W.C., and Kornblith, P.L.: Mechanisms of humoral cytotoxicity testing: the role of rabbit serum, xenoantibody and human IgM. J. Immunol. 118: 1836-1842, 1977.

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Gerweck, L.E., Kornblith, P.L., Burlett, P., Wang, J., and Sweigert, S.: Radiation sensitivity of cultured human glioblastoma cells. Radiology 125: 231-234, 1977.

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Dohan, F.C., Jr., Kornblith, P.L., Wellum, G.R., Pfeiffer, S.E., and Levine, L.: S-100 protein and 2', 3' -cyclic nucleotide 3'phosphohydrolase in human brain tumors. Acta neuropathol. (Berl) 40: 123-128, 1977.

2. BIOLOGICAL AND IMMUNOLOGICAL FACTORS IN PERIPHERAL NERVE REGENERATION

A vein grafting technique for rat sciatic nerve has been developed and perfected during the year to serve as a model system for the study of molecular and cellular factors in neuronal regeneration after injury. The vein graft serves to provide a chamber into which various molecular factors such as collagen or growth factors (epidermal growth factor, nerve growth factor, fibronectin, etc.) or cells such as cultured fibroblasts, Schwann cells, central glia or glioma cells can be introduced to study their effects on axonal regrowth through the graft. Vein grafts alone, cut nerves alone, sutured reapproximated nerves and nerve grafts have been utilized as controls with the studies covering the post-injury period from immediately post-injury to six months.

The studies to date have shown enhanced regeneration in the vein grafted nerves compared to injured but untreated nerves, which is in accord with previous studies of nerve regeneration. Comparison with nerve autografts and allografts is in process. In cases where the vein graft has been from a strain of rat other than the recipient an immunological reaction has taken

place with loss of myelinization but some axonal regeneration. The presence of human glioma cells in the graft has, however, despite the heterograft, promoted excellent regeneration, suggesting either or both immunosuppression and release of a "trophic" factor promoting regeneration. Microcrystalline collagen placed in the graft, on the other hand, does not provoke any immunological reaction but blocks regeneration, probably on a mechanical basis. Studies with nerve growth factor as well as other growth and differentiation factors and glial conditioned media are under way.

The goal of these studies is to define the optimal molecular factors for axonal outgrowth and to provide for optimal micro- and macro-environments for neuronal regeneration. It may thus be possible in the not too distant future to construct immunologically "safe", biologically optimal grafts for peripheral nerve regeneration. The principles developed will likely be applicable as well to the much more complex problems of regeneration in the spinal cord and brain.

3. DIAGNOSTIC STUDIES

RESEARCH IN THE "NEURORADIOLOGY AND COMPUTED TOMOGRAPHY SECTION"

The largest effort of the Neuroradiology and Computed Tomography Section research activity has been concentrated on computed tomography (CT) both in its transmission and emission (Positron Emission Tomography) modalities.

A. Transmission CT

1. A study of the CT attenuation profiles in the periventricular regions in patients affected by a wide variety of pathological conditions in which periventricular areas of hypodensity are recognized (hydrocephalus, multiple sclerosis, the leukodystrophies, leukoencephalopathies related to dysmetabolic processes or to mitochondrial abnormalities, leukomalacia in the area of the terminal matrix, progressive multifocal leukoencephalopathy, disseminated necrotizing leukoencephalopathy, subacute sclerosing panencephalitis, viral inflammatory processes, postradiation necrosis of the brain, and periventricular spreading of primary or secondary CNS neoplasm) is being carried out. Categorization and differentiation of the various CT profiles have been accomplished. Profile characteristics for particular conditions have been demonstrated. For instance, on the basis of the CT attenuation data, it is now possible to discriminate between the periventricular hypodensity related to hydrocephalus (especially in its acute form) and the hypodensity related to the various types of leukoencephalopathy. The concept of the "ventricular wall barrier", as identified by CT, has been introduced. Computed tomography permits the distinction between the intact and the disrupted (impaired) barrier. In parallel with the clinical activity experimental studies in primates with an obstructive hydrocephalus model to evaluate timing of appearance and evolution of the periventricular hypodensity (thought to be related to the transependymal passage of CSF) have been performed.

2. Dual-energy CT (Tomochemistry) - A significant amount of dual-energy CT data on patients has been accumulated. The goal is to establish dual-energy CT "signature" of the various tissues. With this technique, the

CT recognition of even minimal amounts of certain tissues (particularly fat) as well as electrolytic fluids (CSF) is greatly improved as compared to conventional CT. The split detector developed in this section for the purpose of dual-energy CT scanning has been helpful in these studies.

3. CT research on the spine and spinal cord has continued with comparison of CT and radionuclide scanning of the spine in metastatic processes. Efforts at improving the CT resolution of the spinal cord and the spinal CSF have continued.

4. A CT study on the edema in primates has been completed. Cryogenically induced cerebral edema in the rhesus monkey was analyzed by serial CT scans in both axial and coronal planes. The onset, progression (peak at the fourth and fifth day) and resolution of the vasogenic cerebral edema have been assessed. An attempt was made to correlate the low CT attenuation values of the involved areas with the specific gravity of corresponding fresh edematous brain specimens.

5. A new and advanced computer program has been developed for measuring CSF volume (ventricular and subarachnoid cavities).

6. Algorithm improvements. Significant activity has been put into eliminating algorithmic artifacts from CT scans. This work has been highly productive, and in fact has been adopted by the leading CT manufacturer with a definite benefit to image quality.

B. Positron Emission Tomography (PET)

1. In preparation for operational PET capabilities a number of research projects related to a variety of clinical conditions (cerebrovascular disease, cerebral tumors - particularly the gliomas, cerebral edema of various etiologies, leukoencephalopathies, epilepsy, CSF circulation and turnover, determination of blood volume and flow, as well as metabolic rate - e.g., glucose - of the brain and spinal cord) have been designed and outlined in various reports to the NINCDS-NIH directorates.

2. Neuro-PET scanner - A large effort is being generated in the design and development of an advanced high resolution positron emission tomograph for human head and small animal studies. The design is now complete and procurement and software developments are underway.

3. Experimental positron studies in support of the above experiments have been conducted to determine the limitations and capabilities of PET.

4. Cyclotron - A study has been conducted of cyclotron characteristics and needs for PET and suitable recommendations have been made along with an acceptable solution to the thorny problem of location and installation.

5. PET algorithm development - Major progress has been made in ways of handling the non-uniform sampling associated with PET, as well as corrections for certain artifacts peculiar to PET (attenuation, random coincidence, scattered coincidences).

C. Experimental Spinal Cord Angiography

D. Radiographic and Radioisotopic Angiography of the Spinal Cord

E. Isotope Ventriculography and Isotope Cisternography

These last three research projects (C, D, E) remain ongoing, although only a modest amount of work has been dedicated to them in the past year.

4. NEUROPHYSIOLOGICAL STUDIES

Under the direction of Dr. C. L. Li investigations of the neurophysiological mechanisms of epilepsy and pain have been carried out.

Recording with intracellular electrodes from an epileptic focus in the cerebral cortex under the effect of strychnine hydrosulphate revealed that epileptiform discharges were, in most instances, associated with neuronal depolarization which could last for as long as 2,020 msec. During this period the input resistance of the neuron decreased. The experiment also provided evidence which strongly suggests that cortical epileptiform discharges are set off by impulses from the centrum medianum parafascicular complex of the thalamus.

For the study of the mechanisms of pain, the experiments were designed in two stages. In the first stage investigation of various fiber components in a peripheral nerve was made. In the second stage pain impulses generated in a peripheral nerve and their interaction with other sensory impulses in the central nervous system were investigated. The first stage of this study has been completed (see Acta. Neurol. Scand. 59:31-45, 1979) and the second stage is now in process. The preliminary result of these experiments show that many cells in the nucleus reticularis gigantocellularis respond with characteristic patterns to stimulation of the A-delta and C-fibers in the radial nerve. In future experiments, these responses will be tested by conditioning pulses applied to the nerve and by administration of B-endorphin to the animal.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02367-01 SN

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biological, Immunological and Chemotherapeutic Studies of Human Brain Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul L. Kornblith	Chief	SNB	NINCDS
Other:	Barry H. Smith	Medical Officer	SNB	NINCDS
	Eugene A. Quindlen	Senior Staff Fellow	SNB	NINCDS
	Maurice K. Gately	Senior Staff Fellow	SNB	NINCDS
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COOPERATING UNITS (if any)

Division of Radiation Therapy, NCI

LAB/BRANCH

Surgical Neurology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study 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 of this work is to improve the therapy of patients by understanding the basic cellular biology of malignant human brain tumors.

PROJECT DESCRIPTION:

I. OBJECTIVES: There are three primary objectives of this research program. The first objective is to determine how increased understanding of the cellular biology of human brain tumors can lead to a better development of therapeutic and prognostic approaches. The second objective is to determine how a detailed study of immunological parameters in human brain tumors can be used to develop diagnostic and immunotherapeutic modalities for these patients. The third objective is to develop a program for rational planning of chemotherapeutic usage based on individual patient response.

II. MATERIALS AND METHODS:

A. The primary method to be used for this project is that of the tissue culture of human brain tumor cells. In this technique cells removed at operation are placed in a medium consisting of F10 with 10-12% fetal calf serum. The tissue removed at surgery is minced to 1 mm chunks and then explanted into plastic Falcon bottles with appropriate amounts of the standard medium. After cellular outgrowth begins the medium is changed regularly and then cells are subcultured when needed with .25% trypsin. The cells are passaged and harvested to provide the basic cellular material for all of our other techniques.

B. Biological techniques: The detailed characterization of the tissue culture cells requires performance of PPLO or mycoplasma testing to determine that the cells are free of contamination. It also requires light and electron microscopies, biophysical and biochemical studies. The electron microscopic studies involve scanning, transmission and surface replica studies to evaluate the surface and intracellular characteristics of the tumor cells. Direct correlations can thus be made of surface characteristics with malignancy. Biophysical studies include microelectrode recordings with single cell determination of cell brain resting potential, time constant and cellular resistivity. The biochemical studies including analysis of S-100 glial fibrillary acidic protein, myelin basic protein and gangliosides can be performed on cultures at varying time periods from initial explantation. Characterization of the degree of functional malignancy can be accomplished by means of using the cell's ability to grow in low serum .5%, ability to grow in soft agar and to penetrate nucleopore filters. The cells may also be studied in an animal model system such as the immunosuppressed hamster to determine whether they are able to produce a tumor similar to that seen in the patient.

C. Immunological studies: Two basic types of humoral immunology studies have been carried out. The first involves a microcytotoxicity assay. In this microcytotoxicity assay cells are transferred from Falcon plastic flasks (in a suspension of approximately 30-50 thousand cells per cc) to the individual wells of a Falcon microtiter plate using a Terasaki syringe at an approximate 100 density of cells per well.

These cells are allowed to establish themselves for 12-18 hours and then immunological testing with antibody and complement is carried out. The antibody can be either whole prepared from serum or serum which has been fractionated into its globulin components. The complement used is a combination of human pooled serum or human cord serum with rabbit serum as a primary complement source. It is important that the rabbit serum be obtained from rabbits approximately 4 to 6 weeks of age. The complement preparation is added approximately 1 hour after the addition of the serum and the plates are then incubated for 18 hours at 37°. Finally, the plates are stained with hematoxylin-Giemsa and the cells counted. By careful arrangement of the cells in the plate it is possible to analyze the effects of 4 to 6 individual patient sera on a line simultaneously. This approach allows for excellent statistical quantitation and determination of what is known as the cytotoxic index (C.I.). This index is essentially the ratio of cells that have been eliminated by the immunological interaction to those in the untreated control wells. A cytotoxic index of .2 or above is generally statistically significant. Precise statistical determinations are made on each set of observations.

Immune adherence testing is carried out with the use of antibody from specific patients, target cells and red blood cells. The antibody-coated red blood cells attach to the surface of a target cell and when one sees adherence of numbers of red cells to a given target cell it is considered to be a positive response. This technique has the advantage of allowing careful serial titer dilution and also permits absorption with various cellular or tissue components.

The chemotherapeutic studies are carried out by means of a similar microtiter system in which the diluted chemotherapeutic agents are under study (primarily the nitrosoureas DCNU and CCNU). The target cells from individual patients are exposed for varying periods of time to these therapeutic agents. Direct observations can be made of cell killing by means of phase microscopy and time-lapse microphotography as well as by cell counting similar to that done in the immunological techniques. A cytotoxic index can be established for each agent at each concentration. The cell killing at concentrations closest to those achievable in patients can then be compared to actual clinical responses seen in patients who are receiving such therapy.

MAJOR FINDINGS:

The biological analyses of tissue culture tumors have provided two major findings. First, in those tumors which are generally malignant, i.e. the astrocytic tumor group, it has been possible to demonstrate that the precise degree of malignancy (i.e. how rapidly a tumor will grow) can be determined with our standard battery of characterization tests. The most valuable tests in this group include the ability of the tumor to grow in immunosuppressed animals as well as the ability of the cells to grow under stressful conditions such as in low serum or in soft agar. The presence of microvilli on the surface of cells as well as the kinetic

growth characteristics are also useful characterization parameters.

In the area of immunological study significant progress has been made and we now feel it is possible to use an allogeneic cytotoxicity as a diagnostic tool. Eighty percent of patients with malignant brain tumors of varying grades will have a positive response against the common tumor cell line whereas only 9% of normals show such a response. The highest percentage of responders (approximately 90%) occurs in the lowest grade of malignant tumors, which are the most difficult to detect by current diagnostic techniques. This offers the possibility of detecting a tumor at an early enough time to effect more helpful therapy for individual patients. The immune adherence and microcytotoxicity immunological studies together with careful fractionation of the molecular components of the immune response indicate that there are different classes of antibody and by manipulation of these different classes of antibody once can understand with much greater detail the way in which cellular lysis occurs.

Chemotherapeutic testing has demonstrated that there is a direct correlation of the in vitro and clinical responses. The correlation is sufficiently suggestive that we feel that, with more experience, it will be possible prior to commencement of the therapeutic program to determine whether or not a given patient will respond to a particular agent and thereby later the therapeutic program to meet the needs of each individual.

PROPOSED COURSE:

The project described above represents an establishment at the NIH of a program which has been developed and carried out at Massachusetts General Hospital over the past 12 years. The major concerns in our immediate future will be to integrate our new group of scientific experts into this program. It is essential at this point to intensify our efforts both in depth as well as extent to make these approaches of significant and lasting value for the care of neurosurgical brain tumor patients. In regard to biological studies, emphasis will be placed upon more sophisticated techniques for evaluating cell-cell interaction and determining the ways in which modification of cell growth such as cyclic AMP, concanavalin A and other cellular growth or cell surface modifying factors can be used to more precisely determine cellular biological activity. Cell surface dynamics (i.e. alteration of intercellular and intracellular-surface relationships) will need to be evaluated in careful detail by electron microscopy and biochemical study. It will only be by the use of such techniques that we can determine how the biological data which appears to be so relevant can be put to clinical use.

It is in the immunological area that the greatest degree of effort will need to be expended so that immunotherapy can be made a reality. It has been apparent from the preliminary studies that the field is so extremely complex that both the cellular and the humoral aspects of the host response, as well as the specificity of the antigenic properties

responsible for the eliciting of the host response will need to be determined. A major portion of the effort of our entire group will therefore be directed to this area. The humoral response will be dissected in detail as to the classes of antibody responsible as well as the types of antigens which are the basis for the various types of humoral responses. It is already apparent that immunoadherence and cytotoxicity are two significantly different aspects of humoral responsivity and need to be studied in greater detail. Detailed absorption studies are necessary to determine the specificity of the reactivity. Antigen purification will be crucial to the elucidation of basic immune response and also a key to potential programs of active immunotherapy. Cellular immunological studies will be required to define the nature and rates of suppressor cells and helper cells as well as the ways in which antibody, complement and lymphocytes interact in patients. The questions of the role of lymphocyte maturity in these interactions will also be of concern.

In the area of chemotherapeutic studies, it will be necessary to determine whether the initial observations of selective responsivity and individualized, customized therapy prove useful in larger populations of patients. The mechanisms for these responses need to be determined. As our experience increases in this area, we hope to plan a prospective trial in which we devise or alter therapeutic plans based on in vitro responsiveness to given chemotherapeutic agents.

PUBLICATIONS:

Abelson, H.T., Fosburg, M., Gorka, C., and Kornblith, P.: Identification of dihydrofolate reductase in human central-nervous-system tumours. Lancet 1: 184-185, 1978.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02368-01 SN
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PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biological and Immunological Factors in Peripheral Nerve Regeneration

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. H. Smith	Medical Officer	SNB NINCDS
	P. L. Kornblith	Chief	SNB NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Surgical Neurology Branch

SECTION

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NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

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CHECK APPROPRIATE BOX(ES)

- (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER
- (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The cellular, biological and immunological factors in peripheral nerve regeneration are being studied in a rat vein-graft model. The vein graft serves as a chamber into which various biologic agents such as collagen or "trophic" factor such as nerve growth factor as well as specific cell types grown in tissue culture can be added to study their effects on axonal regeneration. Quantitative light and electron microscopic measures are being utilized to analyze the effects. To date, human tumor cells in the vein-graft have enhanced regeneration whereas microcrystalline collagen has inhibited the process. The influence of fetal dorsal root ganglion cells, cerebellar cells, cortical cells, and fibroblasts are currently under study.

[Prior work done on this study was published as follows: Smith, B.H.: Changing concepts of neuroglia functions. Neurosurgery 2: 175-180, 1978.]

PROJECT DESCRIPTION:

I. OBJECTIVES: A study of the cellular and macromolecular factors influencing the success or failure of regeneration of axons after peripheral nerve injury.

II. MATERIALS AND METHODS:

A. Nerve injury and graft: The left or right sciatic nerve in either an Osborne-Weber or caesarian-delivered Fisher rat is transected sharply. Thereafter it is either 1) left alone for control; 2) reapproximated directly via a nerve graft with 8-0 nylon; 3) repaired with a 5 mm segment of vena cava obtained from a second animal of either the same or the other rat strain.

B. Vein graft placement: Under surgical microscopic control, the 5 mm segment of donor vein is sewn to the perineurium of the proximal and distal nerve ends with a total of four 8-0 nylon sutures. Care is taken to assure minimum tension.

C. Cell preparation for placement in graft: Rat or human cells are obtained from either the tissue culture stock lines in our laboratory or grown from new fetal cell cultures of the central nervous system as well as fibroblastic elements. Cultures from the different tissues as well as regions of the brain are quite distinct morphologically in tissue culture and maintain these characteristics over time. At the time for injection into the nerve graft, they are removed from the flask surface, centrifuged to provide a concentrated pellet and injected via a #27 needle into the graft.

D. Macromolecular factors: Microcrystalline collagen (nonantigenic) is placed within the vein graft prior to suturing under microscopic control to fill the vein graft cavity.

E. Follow-up periods: Animals so treated (see A-D above) are then followed for periods ranging from 0 time to 6 months (1 wk, 2 wk, 3 wk, 4 wk, 6 wk, 8 wk, 12 wk, 16 wk, and 24 wks) prior to histologic study.

F. Histologic and ultrastructural examinations: All nerves and/or grafts are removed for study and cut into 1 mm segments to allow for precise reconstruction of the nerve. Fixation is accomplished with glutaraldehyde with standard Epon embedding. Thick sections are then cut for light microscope evaluation and thin sections are prepared for electron microscopy (uranyl acetate staining). Quantitation of numbers regenerating myelinated and unmyelinated axons is done by both light and EM methods.

III. MAJOR FINDINGS: The major findings to date include:

A. Poor regeneration and lack of myelination have been prominent findings in cases where Osborne-Mandel grafts have been placed in C.D. Fisher rats (or vice versa). These findings are in accord with those of other investigators. The importance of immune factors for any nerve grafting technique are thus clearly indicated. The possible influence on regeneration of autologous immune reactions to previously "unseen" antigens in patients with nerve injury is also of interest in this respect.

B. Human glioma cells (line L.M.) injected into the graft do not appear to provoke a significant immunological reaction. Instead they seem to promote regeneration of myelinated axons. Some tumor cells survive in the graft but there is no significant growth of the tumor over the periods tested (see methods). It is of particular interest that, despite the presence of foreign tumor cells, nerve regeneration is enhanced. Perhaps these cells produce a factor that enhances neuronal process formation in accord with previous evidence that such factors can be produced by glioma cells.

C. Microcrystalline collagen, placed in the graft, inhibits the regeneration process over the periods tested to date of up to 8 weeks. Some of this inhibition may be due to purely mechanical effects, but there is also provocation of a cellular reaction with resultant walling off of some collections of collagen. No axons grew through these areas. These findings provide a model for further studies of the influence of scar formation with collagen deposition on nerve regeneration. This also suggests that great care in the utilization of microcrystalline collagen.

D. The various lines of fetal rat cells (cerebellar, cortical, fibroblastic, dorsal root ganglion) from culture are being tested in the rat vein-graft model. Only the shortest time periods have been examined histologically and the data is thus far inconclusive.

IV. PROPOSED COURSE:

Much remains to be done with the quantitation of the present data and with the histological examination of the longer-term (greater than 8 wks) vein graft-cellular injection animals. New proposed experiments include the introduction of three growth factors - nerve growth factor (NGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF) as well as fibronectin to alter the endogenous cell population. Cultured neurons will also be introduced into the graft. Functional evaluation of recovery will also be undertaken.

The goal of these studies is to provide insight into the molecular and cellular factors that influence axonal regrowth and ultimately to manipulate them to enhance the recovery of neuronal function after injury.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01047-17 SN												
PERIOD COVERED October 1, 1978 to September 30, 1979														
TITLE OF PROJECT (80 characters or less) Isotope Ventriculography and Isotope Cisternography														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">G. Di Chiro</td> <td style="width: 50%;">Chief, Neuroradiology and Computed Tomography Section</td> <td style="width: 10%;">SN NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>G.S. Johnston</td> <td>Chief, Nuclear Medicine Dept.</td> <td>NM CC</td> </tr> <tr> <td></td> <td>A.E. Jones</td> <td>Assistant Chief</td> <td>NM CC</td> </tr> </table>			PI:	G. Di Chiro	Chief, Neuroradiology and Computed Tomography Section	SN NINCDS	OTHER:	G.S. Johnston	Chief, Nuclear Medicine Dept.	NM CC		A.E. Jones	Assistant Chief	NM CC
PI:	G. Di Chiro	Chief, Neuroradiology and Computed Tomography Section	SN NINCDS											
OTHER:	G.S. Johnston	Chief, Nuclear Medicine Dept.	NM CC											
	A.E. Jones	Assistant Chief	NM CC											
COOPERATING UNITS (if any) Nuclear Medicine, Clinical Center, NIH														
LAB/BRANCH Surgical Neurology Branch														
SECTION Neuroradiology and Computed Tomography Section														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS: .0	PROFESSIONAL: .0	OTHER: .0												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) <u>Isotope ventriculography</u> and <u>isotope cisternography</u> are diagnostic tools permitting the morphologic and dynamic study of the <u>cerebrospinal fluid</u> pathways more accurately than has ever been possible with any other diagnostic test. The adjunction of <u>emission computed tomography</u> to our diagnostic armamentarium should improve significantly the information content of our isotope ventriculograms and cisternograms.														

Project Description:

Objectives: A gamma emitting isotope injected within the cerebrospinal fluid pathways will permit in subsequent head scans the pictorial outline of the ventricular system (isotope or radionuclide ventriculography) and of the subarachnoid intracranial spaces (isotope or radionuclide cisternography). Information about the anatomical status of the cerebrospinal fluid cavities, and, by multiple serial scans, of the normal and abnormal dynamics of the cerebrospinal fluid itself will be obtained. The spinal CSF spaces may also be evaluated.

Methods Employed: The radionuclide cisternography and ventriculography procedures are now well established.

Recently we have devoted particular attention to one aspect of the CSF flow, i.e., its descent to the spinal subarachnoid space.

Major Findings: None

Significance to Bio-Medical Research and the Program of the Institute: Legions of authors are studying this remarkable fluid (CSF) which still remains uncomprehended since Cotugno first described it in 1764. In particular we now have a diagnostic tool to gather information about the "terra incognita" which is represented by the basal and convexity subarachnoid pathways, as well as the spinal CSF compartment. In this area, the CSF spinal descent studies should enable us to determine what is the importance of the spinal CSF route of flow as an alternative pathway of resorption. The observations of the spinal descent pattern of the CSF have also heuristic significance in regard to a possible analysis of metabolites and drugs distribution through the CSF from the endocranial cavity to the spinal theca.

Proposed Course of Project: Further information about the normal and abnormal cerebrospinal fluid cavities, and the normal and pathologic flow of CSF will be gathered by the techniques of radionuclide cisternography and ventriculography. The adjunction of the capabilities for emission computed tomography (our purchase of the ORTEC-ECAT device which will soon be installed) will permit significant refinements in the techniques of radionuclide cisternography and ventriculography. In particular, emission computed tomography, using radiopharmaceuticals tagged with positron emitters (e.g., chelating substances labeled with ^{68}Ga) will allow for a better demonstration of the tagged CSF in the deep CSF cavities. This improved demonstration will be possible through the tomographic display with images representing axial transverse slices. The problem of the superimposition of the radioactivity in the superficial tissues, so disturbing in the interpretation of conventional radionuclide CSF scintiphotographic studies, will be practically eliminated.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01195-15 SN
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Radiographic and Radioisotopic Angiography of the Spinal Cord

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Di Chiro	Chief, Neuroradiology and Computed Tomography Section	SN NINCDS
OTHER:	J.L. Doppman	Chief	DR CC
	J.R. Herdt	Deputy Chief	DR CC
	P.L. Kornblith	Chief	SN NINCDS
	E. Quindlen	Senior Staff Fellow	SN NINCDS
	G.S. Johnston	Chief	NM CC
	A.E. Jones	Assistant Chief	NM CC

COOPERATING UNITS (if any) Greater S. E. Community Hosp., Wash., DC; Hosp. of the Univ. of PA, Phila., PA; Medical Examiner's Office, Phila., Dept. of Public Health, Phila., PA; Diagnostic Radiology and Nuclear Medicine Depts., Clinical Center, NIH.

LAB/BRANCH
Surgical Neurology Branch

SECTION
Neuroradiology and Computed Tomography Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .0	PROFESSIONAL: .0	OTHER: .0
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CHECK APPROPRIATE BOX(ES)
 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER
 (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
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 intraspinal pathology may give information not available by any other diagnostic test.

Preliminary experience with computed tomography of the spine after injection of contrast medium indicates that this new methodology may be useful in the evaluation of certain vascular lesions of the spinal cord.

Project Description:

Objectives: The introduction of cerebral angiography (1927) has markedly increased our knowledge of the vascular pathology of the brain. The vascular pathology of the spinal cord, on the other hand, remains a largely unexplored area.

Since 1964 we have been carrying out angiographic studies of the spinal cord and developed this technique into a reliable diagnostic tool. Selective injection of the contrast medium has made the difference between an occasional demonstration, and the consistent visualization of the spinal cord vasculature.

The usefulness of selective arteriography in cases of spinal cord arteriovenous malformations is now well established. We are continuing to use this technique to: 1) Learn more about the pathophysiology of the spinal cord arteriovenous malformations so that a better treatment of these important and frequent lesions may be developed. 2) Evaluate how useful spinal cord angiography is in cases of spinal cord tumors. 3) Establish whether or not this technique can be of diagnostic value in the study of obstructive spinal cord vascular disease. 4) Assess the usefulness of this technique in intervertebral disc pathology. 5) Evaluate the diagnostic possibilities of this procedure in post-traumatic spinal cord injury with or without vertebral fractures. 6) Establish the value and limits of newly introduced radioisotopic angiography of the spinal cord. 7) Explore the possible emergency therapeutic means which could be employed to treat and cure, or at least minimize the effects of the dreadful postangiographic cord complications. 8) Acquire new information regarding the fine vasculature of the human spinal cord, with particular emphasis on the intrinsic vessels (sulcal and central arteries and other perforating or penetrating branches). This goal is accomplished by post-mortem microangiographic techniques in cadavers of all age groups. We are paying particular attention to cords of aged adults.

Methods Employed: Selective arteriograms with modern catheter techniques are carried out in patients in whom spinal cord vascular or tumoral lesions are suspected. The subtraction technique is used to better visualize the injected vessels. In addition, in the last fiscal year we have gained considerable experience with the direct radiographic magnification angiograms.

For the technique of radioisotope angiography of the spinal cord a bolus of 15 mCi of ^{99m}Tc human serum albumin (1 to 2 ml) is injected in a left antecubital vein. Immediately afterwards, cine-scintiphotographic or rapid flow Polaroid views of the various segments of the spine are obtained with an Anger scintillation camera. In the last fiscal year our scintiphotographic data have been significantly ameliorated by a computer assisted analysis and reconstruction of images, as well as by isometric contour computer display of the data.

For the technique of computed tomographic angiography of the spinal cord we use a computed tomography (CT) body scanner and we carry out timed serial tomograms of the area of interest of the spine after the intravenous intro-

duction of a bolus of angiographic contrast medium.

For the post-mortem studies of the vessels of the human spinal cords, (aged adults) we have used our previously developed microangiographic techniques.

Based on the observation made elsewhere, that in two patients who died soon after aortography with spinal cord complications, the iodine content in the CSF was enormously increased, we are attempting an emergency therapeutic method consisting of flushing out the "iodine contaminated" CSF.

Major Findings: We have continued to accumulate experience in the areas of:

- 1) Selective arteriography in cases of herniation of thoracic discs.
- 2) CSF lavage in patients who develop symptoms and signs of cord involvement after abdominal aortography or other types of arteriographic studies.
- 3) Post-mortem microangiographic evaluation of the aged human cord.

The newest development has been the introduction of computed tomography (CT) in the analysis of vascular diseases of the spinal cord. We have gathered preliminary experience in eight patients with arteriovenous malformation of the spinal cord using a CT scanner. We have been able to recognize the pathological vessels in four of the arteriovenous malformations. In one case, in which occlusion of the pathological vessels was obtained through percutaneous embolization, we were able to demonstrate the positive result of this therapeutic procedure.

Significance to Bio-Medical Research and the Program of the Institute: Radiographic and radioisotopic angiography of the spinal cord are increasing our understanding of the large group of conditions in which vascular lesions of the cord represent the basic pathologic element.

Proposed Course of Project: Post-mortem microangiography of the aged adults' cords should offer new insights on such conditions as obstructive vascular disease of the cord due to arteriosclerosis and cervical spondylosis, and possibly on degenerative and demyelinating cord diseases.

We are "watching" for possible further technical developments of the technique of selective arteriography of the spinal cord. We have recently established the value of direct radiographic magnification, and we are considering initiating the use of angiotomography for a better visualization of the smaller vessels, possibly the intrinsic arteries and veins of the cord.

Improved x-ray vascular contrast media will also enhance the diagnostic possibilities of spinal cord angiography. We are following very closely the recent developments in the area of polymeric, ion-balanced and non-ionic iodinated x-ray contrast media.

Radioisotope angiography of the spinal cord is a method which we have been using extensively as a screening and followup procedure. By increasing our resolution through a computer-assisted reconstruction and enhancement of the images, we should be able to extract more definitive diagnostic information from this simple and innocuous technique.

Computed tomographic (transmission) angiography of the spine and spinal cord represents one of the areas in which we will concentrate a great deal of interest.

We have some expectation that positron emission tomography particularly with the use of the high resolution Neuro-PET (designed in our Section), will allow us to study blood flow and metabolism (glucose metabolic rate?) of the cord in a non invasive fashion.

Publications: None

Project Description:

Objectives: The clinical value of the NIH developed technique of selective arteriography in the management of arteriovenous malformations and tumors (in particular hemangioblastomas) of the spinal cord is now well established.

In order to expand the clinical applications of arteriography of the spinal cord we are working with experimental angiographic and microangiographic models in primates.

Previously, we have concentrated our attention on the area of experimental obstructive vascular disease of the spinal cord in the rhesus monkey. More recently much of our experimental investigation has dealt with a catastrophic iatrogenic pathological condition, postradiation myelomalacia (myelitis), which occurs more frequently than is generally realized.

In the area of postradiation myelitis we are particularly interested in establishing whether the basic pathological lesion of this dreadful complication is primarily neurogenic or vascular.

Methods Employed: Preradiation angiographic studies (selective technique) of the thoracolumbar segment of the spinal cord are carried out in young, healthy rhesus monkeys. Soon after, selective irradiation of the thoracolumbar cord using a LINAC accelerator is initiated. Total dosage and modalities of delivery are chosen to approximate the radiation protocol which most often seems to cause myelomalacia in human patients.

At the end of the radiation, the monkeys are kept under careful observation for periods of many months. Neurological testing of the lower limbs is performed twice a week. If and when the monkeys show signs of developing or established paraplegia, repeat selective arteriography of the irradiated segment is carried out. Following this, the animals are perfused for microangiography of the spinal cord and then sacrificed. The cord is studied by gross observation, microangiography, routine histology and special myelin stains. Careful gross and histological analysis of the neighboring aortic segment, its branches and the pertinent radiculomedullary arteries is also carried out.

Major Findings: We are on the course of evaluating the pathological changes of the spinal cord from monkeys in which we successfully induced postradiation paraplegia (myelopathy).

Significance to Bio-Medical Research and the Program of the Institute: We should be able to shed some light on the pathogenesis of the postradiation myelitis. This is not a rare complication in human patients (over 500 cases have been reported in literature).

Proposed Course of Project: Appraisal of the postradiation data which we have already collected as well as new data in other irradiated animals now under observation. We will attempt to study (by angiography and micro-

angiography) human patients (or human specimens) with postradiation spinal cord damage.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02073-06 SN																																																
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TITLE OF PROJECT (80 characters or less) New Title: Computed Tomography (Transmission) Previous Title: Computer Assisted Tomography (Transmission Computed Tomography)																																																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td data-bbox="90 308 135 334">PI:</td> <td data-bbox="199 308 337 334">G. Di Chiro</td> <td data-bbox="456 308 804 353">Chief, Neuroradiology and Computed Tomography Section</td> <td data-bbox="885 308 997 329">SN NINCDS</td> </tr> <tr> <td></td> <td data-bbox="199 359 337 381">R.A. Brooks</td> <td data-bbox="456 359 692 381">Senior Staff Fellow</td> <td data-bbox="885 359 997 381">SN NINCDS</td> </tr> <tr> <td></td> <td data-bbox="199 387 325 408">K.G. Rieth</td> <td data-bbox="456 387 692 408">Senior Staff Fellow</td> <td data-bbox="885 387 997 408">SN NINCDS</td> </tr> <tr> <td></td> <td data-bbox="199 414 311 435">V.J. Sank</td> <td data-bbox="456 414 680 435">Research Assistant</td> <td data-bbox="885 414 997 435">SN NINCDS</td> </tr> <tr> <td data-bbox="90 435 176 456">OTHER:</td> <td data-bbox="199 435 374 456">P.L. Kornblith</td> <td data-bbox="456 435 519 456">Chief</td> <td data-bbox="885 435 997 456">SN NINCDS</td> </tr> <tr> <td></td> <td data-bbox="199 462 325 483">B.H. Smith</td> <td data-bbox="456 462 729 483">Senior Staff Physician</td> <td data-bbox="885 462 997 483">SN NINCDS</td> </tr> <tr> <td></td> <td data-bbox="199 489 337 511">E. Quindlen</td> <td data-bbox="456 489 692 511">Senior Staff Fellow</td> <td data-bbox="885 489 997 511">SN NINCDS</td> </tr> <tr> <td></td> <td data-bbox="199 517 325 538">J.R. Herdt</td> <td data-bbox="456 517 609 538">Deputy Chief</td> <td data-bbox="885 517 948 538">DR CC</td> </tr> <tr> <td></td> <td data-bbox="199 544 325 565">M. Vermess</td> <td data-bbox="456 544 729 565">Assistant Deputy Chief</td> <td data-bbox="885 544 948 565">DR CC</td> </tr> <tr> <td></td> <td data-bbox="199 571 362 592">G.S. Johnston</td> <td data-bbox="456 571 519 592">Chief</td> <td data-bbox="885 571 948 592">NM CC</td> </tr> <tr> <td></td> <td data-bbox="199 598 325 619">A.E. Jones</td> <td data-bbox="456 598 642 619">Assistant Chief</td> <td data-bbox="885 598 948 619">NM CC</td> </tr> <tr> <td></td> <td data-bbox="199 625 348 647">R.M. Kessler</td> <td data-bbox="456 625 642 647">Staff Physician</td> <td data-bbox="885 625 948 647">NM CC</td> </tr> </table>			PI:	G. Di Chiro	Chief, Neuroradiology and Computed Tomography Section	SN NINCDS		R.A. Brooks	Senior Staff Fellow	SN NINCDS		K.G. Rieth	Senior Staff Fellow	SN NINCDS		V.J. Sank	Research Assistant	SN NINCDS	OTHER:	P.L. Kornblith	Chief	SN NINCDS		B.H. Smith	Senior Staff Physician	SN NINCDS		E. Quindlen	Senior Staff Fellow	SN NINCDS		J.R. Herdt	Deputy Chief	DR CC		M. Vermess	Assistant Deputy Chief	DR CC		G.S. Johnston	Chief	NM CC		A.E. Jones	Assistant Chief	NM CC		R.M. Kessler	Staff Physician	NM CC
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COOPERATING UNITS (if any)Neurosurg. Service, Dept. of Psychiatry and Neurology, WRAMC, Wash., DC; Physics Dept., Tufts Univ., Medford, MA; Cyclotron Lab., Harvard Univ., Cambridge, MA; Diagnostic Radiology, Nuclear Medicine Depts., CC, NIH; Pediatric Oncology Branch, DCT, NCI, NIH.																																																		
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SUMMARY OF WORK (200 words or less - underline keywords) <u>Computed Tomography (CT)</u> in its transmission and emission modalities, represents the main research area of the Neuroradiology and Computed Tomography Section. Ongoing clinical research projects in transmission CT include the following pathological processes: degenerative, demyelinating and atrophic processes of the brain; hydrocephalus, brain edema; postradiation cerebral necrosis; surgically correctable lesions in young patients affected by chronic epilepsy; diseases of the spine and the spinal cord; attempts at tissue characterization of normal and abnormal (e.g., tumoral) cerebral tissue. Physics projects: improved dual-energy CT scanning using both a split-detector and a dual kVp method; analysis of aliasing effects and developments of methods for their elimination; phantom studies for the evaluation of artifacts and calibration of CT machines. Feasibility tests to build a new type of CT device which will use <u>protons</u> are under way.																																																		

Names, Laboratory and Institute Affiliations, etc. (Cont'd)

OTHER: D.M. Conca	Senior Staff Fellow	NM CC
D.G. Poplack	Senior Investigator	POB DCT NCI

Project Description:

Objectives: To advance the clinical applications of CT. Attention is being devoted to trying to improve the resolution of the CT devices. Advanced quantitative assessment of the attenuation values (profiles, regions of interest) are used in an attempt to improve the diagnostic specificity of CT. The goal is to enhance our capability of distinguishing between different types of lesions which present with similar qualitative findings. Differentiation of the various tissues' chemical components (tomochemistry) through dual-energy scanning or, possibly in the future, by means of proton CT, is a promising line of our research.

Methods Employed: Clinical CT scanning is now a standard diagnostic procedure. Groups of patients with various disease conditions are studied by CT of the brain and/or body.

A split detector of original design has been built to carry out simultaneous dual-energy scanning.

Basic experiments being carried out as a preliminary step to build the PROTO-Scanner, involve determination in various phantoms of the absorption coefficient of the proton beam produced by a cyclotron. The phantoms include organic materials (particularly organic solutions of various concentrations).

Major Findings: In the clinical area we have:

1) Carried out a study of the CT attenuation profiles in the periventricular regions in patients affected by a wide variety of pathological conditions in which periventricular areas of hypodensity are recognized (hydrocephalus, multiple sclerosis, the leukodystrophies, leukoencephalopathies related to dismetabolic processes or to mitochondrial abnormalities, leukomalacia in the area of the germinal matrix, progressive multifocal leukoencephalopathy, disseminated necrotizing leukoencephalopathy, subacute sclerosing panencephalitis, viral inflammatory processes, post-radiation necrosis of the brain, and periventricular spreading of primary or secondary CNS neoplasm). Categorization and differentiation of the various CT profiles have been accomplished. Profile characteristics for particular conditions have been demonstrated. For instance, on the basis of the CT attenuation data, we are now able to discriminate between the periventricular hypodensity related to hydrocephalus (especially in its acute form) and the hypodensity connected with the various types of leukoencephalopathy. The concept of the "ventricular wall barrier," as identified by CT, has been introduced. Computed tomography permits the distinction between the intact and the disrupted (impaired) barrier.

2) Dual-energy CT (Tomochemistry) - A significant amount of dual-energy CT data on patients has been accumulated. Our goal is to establish dual-energy CT "signature" of the various tissues. With this technique, the CT recognition of even minimal amounts of certain tissues (particularly fat) as well as electrolytic fluids (CSF) is greatly improved as compared to conventional CT. The split detector developed in our Section for the purpose of dual-energy scanning has been helpful in these studies.

3) A new and advanced computer program has been developed for measuring CSF volume (ventricular and subarachnoid cavities).

4) Our CT research on the spine and spinal cord has continued. In the last year emphasis has been put on the comparison between CT and radionuclide scanning of the spine in metastatic processes. Efforts at improving the CT resolution of the spinal cord and the spinal CSF have continued and further experience with our technique of computer assisted myelography (CAM) has been accumulated. (See also computed tomographic angiography of the spinal cord - Project No. Z01 NS 01195-14 SN.)

5) Observed interesting findings concerning postradiation necrosis of the brain. These findings may mimic brain tumors (recurrence or spread). Their recognition, therefore, is of capital importance.

6) Analyzed the brain CT findings (in particular hypodensity due to demyelination) after therapeutic intrathecal methotrexate (administered for controlling meningeal leukemia and metastatic lesions).

7) Analyzed a large group of patients affected by chronic epilepsy to determine how frequently surgically correctable epileptogenic lesions can be detected solely by CT.

In the animal experimental area we have:

1) Completed a CT study on the edema in primates. Cryogenically induced cerebral edema in the rhesus monkey was analyzed by serial CT scans in both axial and coronal planes. The onset, progression (peak at the fourth - fifth day) and resolution of the vasogenic cerebral edema have been assessed. An attempt was made to correlate the low CT attenuation values of the involved areas with the specific gravity of corresponding fresh edematous brain specimens.

2) In tandem with our clinical activity connected with the differentiation of the various types and stages of hydrocephalus, we have been performing experimental studies in primates with an obstructive hydrocephalus model to evaluate timing of appearance and evolution of the periventricular hypodensity (thought to be related to the transependymal passage of CSF).

3) In tandem with our clinical activity on the spinal cord and spinal CSF we have carried out experiments in primates trying to develop better visualization of the cord and the spinal CSF through intravenous enhancement

(intravenous CT myelography). From preliminary observations, it would appear that possibly there is an early relative enhancement of the cord followed by late relative enhancement of the CSF. By exploiting these features one could extract valuable additional information from CT of the spine after simple intravenous injection of contrast medium and, thus avoid the intrathecal administration.

In the physics area the most important findings are:

1) Algorithm improvements. Significant activity has been put into eliminating algorithmic artifacts from CT scans. This work has been highly productive, and in fact has been adopted by the leading CT manufacturer with a definite benefit to image quality.

2) Development of a dual-energy Hounsfield tissue signature, and demonstration of its usefulness in patient diagnosis.

3) Development of an offset detector method for the elimination of aliasing artifacts in fan-beam third-generation scanners.

Significance to Bio-Medical Research and the Program of the Institute: The diagnostic abilities in the area of neuroradiological disease are fundamentally altered by the introduction of CT. The progress in this area is fast. Statements regarding the future significance of this methodology could be surpassed and rendered obsolete in a short time.

Proposed Course of Project: In the Neuroradiology and Computed Tomography Section, CT will be the main area of research for years to come. We will proceed with a multipronged approach: 1) clinical work on the brain, sella turcica and pituitary gland (microadenomas!), spinal cord and eye; 2) experimental research on primates; 3) tomochemistry of the CNS; 4) theory (mathematics, physics); 5) planning and building a new type of CT device.

A new journal "JOURNAL OF COMPUTER ASSISTED TOMOGRAPHY" originates from this Section (Eds. Di Chiro and Brooks).

Publications: Di Chiro, G., Arimitsu, T., Pellock, J.S. and Landes, R.D.: Periventricular leukomalacia related to neonatal anoxia: recognition by computed tomography. J. Comput. Assist. Tomogr. 2:352-355, 1978

Wendling, L.R., Bleyer, W.A., Di Chiro, G. and McIlvanie, S.K.: Transient, severe periventricular hypodensity after leukemic prophylaxis with cranial irradiation and intrathecal methotrexate. J. Comput. Assist. Tomogr. 2:503-505, 1978

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- Di Chiro, G. and Brooks, R.A.: Technical Aspects of Computed Tomography. New York, Elsevier North-Holland, Inc. (In press)

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

New Title: Positron Emission Tomography

Previous Title: Computer Assisted Tomography (Emission Computed Tomography).

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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R.A. Brooks	Senior Staff Fellow	SN NINCDS
V.J. Sank	Research Assistant	SN NINCDS
T.N. Chase	Director	IRP NINCDS
P.L. Kornblith	Chief	SN NINCDS
B.H. Smith	Senior Staff Physician	SN NINCDS
E. Quindlen	Senior Staff Fellow	SN NINCDS
G.S. Johnston	Chief	NM CC
A.E. Jones	Assistant Chief	NM CC
R.M. Kessler	Staff Physician	NM CC
OTHER: L. Sokoloff	Chief	LCM NIMH

COOPERATING UNITS (if any) BEIB, DRS, NIH; Naval Res. Lab., Wash., DC; Brookhaven Natl. Lab., Upton, NY; Wash. Univ., St. Louis, MO; Div. of Nucl. Med., Dept. of Rad. Sciences. UCLA, Los Angeles, CA; Lab. of Cerebral Metabolism, NIMH, NIH. Nuclear Med. Dept., CC, NIH; ODIR, NINCDS

LAB/BRANCH

Surgical Neurology Branch

SECTION

Neuroradiology and Computed Tomography Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

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CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Positron Emission Tomography (PET) allows us to obtain pictorial data (e.g., axial transverse images of the brain) as well as dynamic functional data (such as regional cerebral glucose consumption rate (mg/min/100 gm) of brain substance), measurements of the storage, degradation and turnover of tagged metabolites, follow-through of the movement of the CSF in the deep CSF intracranial cavities). The unique property of PET is that it provides physiologic information not available with any other imaging procedures.

During the last year significant progress has been made in our Section on the design of a high-resolution high-sensitivity scanner for head and animal studies - the Neuro-PET.

Project Description:

Objectives: Recent new developments have made a significant difference in the practical and clinical application of PET. The two most important of these developments are: 1) efficient PET devices (scanners) have been developed and some are commercially available, and 2) the original Sokoloff's autoradiographic technique in experimental animals has been converted into a PET method for living human subjects (see below).

Methods Employed: The radioactivity originating from a positron emitting radionuclide, generally introduced by the intravenous route, is detected in a single plane (tomography) with an axial-transverse or horizontal incidence, and the image of this distribution in a slice or cut through the body area of interest is produced, displayed and recorded in a variety of fashions. The most interesting radionuclide is at present ^{18}F 2-deoxyglucose (^{18}FDG). The application of this tracer is a direct derivation of the original, NIH developed, Sokoloff's autoradiographic technique in experimental animals. In living human patients, it is now possible with ^{18}F (110 minute half-life) tagged 2-deoxyglucose to obtain pictorial data (axial transverse images) as well as quantitation (mg/min/100 gm of brain substance) of the cerebral glucose metabolism. Other positron emitting radiopharmaceuticals of interest are ^{68}Ga chelated tracers (^{68}Ga is generator produced and has a 68 minute half-life). The ^{68}Ga tracers would be particularly interesting for the analysis of the CSF circulation (PET cisternography). The use of tracers tagged with the short-lived ^{11}C , ^{13}N and ^{15}O will require a cyclotron on the NIH premises.

Major Findings:

1) In preparation for our operational PET capabilities a number of research projects related to a variety of clinical conditions (cerebrovascular diseases, cerebral tumors - particularly the gliomas, cerebral edema of various etiologies, leukoencephalopathies, epilepsy, CSF circulation and turnover, determination of blood volume and flow, as well as metabolic rate - e.g., glucose - of the brain and spinal cord) have been designed and outlined in various reports to the NINCDS-NIH directorates.

2) Neuro-PET scanner - A large effort is being generated in the design and development of an advanced high resolution positron emission tomograph for human head and small animal studies. The design is now complete and procurement and software development is underway. The accomplished advances include:

A) The experimental demonstration of the feasibility of using small (8 mm) bismuth germanate crystals for obtaining high-resolution PET images.

B) The development of a crystal-photomultiplier attachment and packing method so that the necessary number of detectors can be placed close together.

C) The development of software corrections for scattering and other artifacts in positron emission tomography.

D) The development of an improved mechanical motion for the PET gantry to produce optimum sampling of data.

3) Major progress has been made developing techniques to handle the non-uniform sampling associated with PET, as well as corrections for certain artifacts peculiar to PET (attenuation, random coincidence, scattered coincidences).

4) Cyclotron - A study has been conducted of Cyclotron characteristics and needs for PET and suitable recommendations have been made along with an acceptable solution to the thorny problem of location and installation.

Significance to Bio-Medical Research and the Program of the Institute:
Following are the research projects which are considered significant to the program of the NINCDS and which we subdivide into two groups, (1) those using relatively long-lived radionuclides and (2) those employing short-lived radionuclides.

Group 1

A) Regional cerebral glucose consumption using ^{18}F FDG in the various stages of stroke, as well as TIA. There are reasons to believe that ^{18}F FDG PET should be more sensitive than conventional CT, radionuclide brain scans (RNS) and cerebral angiography (CA_n) in the evaluation of the stroke patient. There is already some evidence of mismatches (non-coupling) between flow and glucose utilization. In cerebrovascular lesions, a differentiation between ischemic (reversible) and infarcted (irreversible) lesions can perhaps be made.

B) Regional cerebral glucose consumption using ^{18}F FDG in epilepsy. Of particular interest are studies of epileptic patients presenting with EEG and ECoG foci and negative neuroradiological tests (CT, RNS, CA_n, PEG). Also, patients with deeply located lesions and normal or nonlocalizing EEG findings represent an important group to evaluate by this method.

C) Regional cerebral glucose consumption using ^{18}F FDG in the various astrocytoma types (I-IV). The question to be considered here is whether or not the glucose metabolic rate in the various glioma types is different depending upon the differentiation of the neoplasm. Also the assessment of borderline cases, i.e., patients in whom the clinical suspicion of brain tumor is not convincingly validated by neuroradiological findings, could be facilitated using this technique.

D) Regional cerebral glucose consumption using ^{18}F FDG in edematous regions of the brain. The critical aspect of this study will be an analysis of the functionality of the edematous cerebral tissue. The areas of edema (surrounding tumors, inflammatory processes, MS plaques, bleedings or other edema-generating foci) will be recognized by conventional CT. Comparative analysis of the glucose metabolic rate in the edema areas vs. control regions could be carried out.

E) Regional cerebral glucose consumption using ^{18}F FDG in the brain of patients affected by Parkinson's or Huntington's diseases to establish the presence and extent of disturbed glucose metabolism in the various overt stages of these two pathological conditions and, for Huntington's disease, in patients at risk.

F) Regional cerebral glucose consumption using ^{18}F FDG in degenerative diseases of the brain, particularly in leukoencephalopathies. At present we are involved in a comprehensive study of a large variety of leukoencephalopathic processes. It would be most interesting to assess the glucose metabolic rate of the leukodystrophic regions. Also, in some cases, the differentiation between periventricular leukomalacia and transependymal CSF migration (hydrocephalus) may be difficult and of critical diagnostic importance. Perhaps ^{18}F FDG PET may prove useful here.

G) PET would permit a detailed analysis of flow, distribution and destiny of intrathecally injected CSF tracers. In conventional cisternography this detailed analysis is difficult for the deep regions which, on the other hand, are well suited for PET assessment. The anticipated improvement of CSF dynamics appraisal by PET has far-reaching implications for the study of such common pathological entities as hydrocephalus, dementia and other psychiatric disorders. As a tracer for CSF PET, ^{68}Ga DTPA appears to be an optimal choice (see FDA-approved-for-cisternography INDTPA).

H) We have some expectation that with high-resolution Neuro-PET we may possibly be able to evaluate the flow and metabolic rate (glucose) of the cervical spinal cord.

I) ^{18}F -DOPA could be used for direct external measurement of storage, degradation and turnover of intracerebral dopamine. The implications of the possible usage of this or other (^{18}F haloperidol, ^{18}F serotonin) radiopharmaceuticals for the external evaluation of the regional catecholamine metabolism will be far reaching.

J) Very preliminary reports indicate that the protein metabolic rate could be explored using tagged amino acids (such as valine) very much in the same fashion as the tagged deoxyglucose is used for evaluation of the original cerebral glucose consumption rate.

Group 2

A) Measurement and validation of regional cerebral blood flow (rCBF) using one (or several) method(s) possible with cyclotron produced radionuclides. The compounds suitable for this purpose include C^{15}O_2 , $^{13}\text{NH}_3$, $^{13}\text{N}_2\text{O}$, H_2^{15}O , ^{11}C -iodoantipyrine. The cyclotron produced $^{77}\text{K}_2$ has also been used for rCBF detection. Actually the validation of a PET method to study rCBF represents an indispensable prerequisite for many other physiologic studies. A satisfactory blood flow agent is still to be agreed upon: for instance, the distribution of $^{13}\text{NH}_3$ is believed by one team of investigators to be related and informative about rCBF, whereas members of another team dismiss $^{13}\text{NH}_3$ as an

agent to study rCBF on the basis that this compound perfusion is affected by metabolic variations and pH changes.

- B) Measurement of cerebral blood volume (CBV) using ^{11}C O or C^{15}O .
- C) Use and validation of ^{11}C -deoxyglucose (^{11}C -2DG) with its advantages over ^{18}F -fluorodeoxyglucose (^{18}F -2FDG) for determination of local cerebral glucose utilization in man in normal and pathological conditions.
- D) Measurement of regional cerebral O_2 metabolic rate using $^{15}\text{O}_2$.
- E) Measurement and validation of regional oxygen extraction rate using the $^{15}\text{O}_2 + \text{C}^{15}\text{O}_2$ method (ratio of the two compounds equilibrium values).
- F) Measurement and validation of method using ^{11}C labeled essential precursor aminoacids to study protein synthesis and turnover in the brain. From the nuclear medicine literature, promising aminoacids for this type of study are methionine, phenylalanine, tryptophane, leucine, and particularly valine.
- G) Measurement and validation of functional localization of precursors of neurotransmitters, neuroreceptor agonists and antagonists, and distribution-turnover of neuroleptic drugs.
- H) CSF. Studies on the CSF circulation based on radionuclide cisternography and, more recently, CT cisternography (with metrizamide) have reached an impasse. Discordant observations and opinions are ubiquitous in the pertinent literatures. The fact is that the CSF flow (third circulation) as well as the diffusion-transport of the various substances (proteins, aminoacids, metabolites, catecholamines) brain \rightleftharpoons CSF are still scantily understood. The blood-CSF barrier and the related appealing CSF "sink" theory need to be elucidated. The implications of studies in this area for a better comprehension of the various types of dementias (and not only the dementia related to normal pressure hydrocephalus) are far-reaching. ^{11}C , ^{13}N , ^{15}O labeled physiological CSF components may be followed by PET as they move from and to the brain and its surrounding vascular structures. Proteins, aminoacids, metabolites such as homovanilic acid, 5-hydroxy-indole-acetic-acid, catecholamines as well as certain compounds which have a great capability of passing through the blood-brain-CSF barrier (e.g., aminonitriles which have already been ^{11}C labeled) represent some of the substances which may be used. They may be introduced intrathecally or systemically. Labeled drugs, already used or potentially usable for intrathecal therapy, are another group of compounds which would be worthwhile to follow in their passage through the CSF pathways.

Proposed Course of Project: A commercial PET device (ORTEC-ECAT) is being delivered to the NIH. Our high-resolution Neuro-PET scanner is being built. Installation of a cyclotron on the NIH premises is being considered and planned. The details related to the source, preparation, supply and delivery of the radiopharmaceuticals and the logistics of the patient throughput are being worked out.

Publications: Brooks, R.A., Sank, V.J., Talbert, A.J. and Di Chiro, G.:
Sampling requirements and detector motion for positron emission tomography. IEEE Trans. Nucl. Sci. NS-26:2760-2763, 1979

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01526-12 SN
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Mechanisms of Epilepsy		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: C.L. Li, Associate Neurosurgeon SNB NINCDS		
COOPERATING UNITS (if any) 1. Dr. Claire Parsons, University of Virginia School of Medicine 2. Dr. Vazha Okujava, Academy of Sciences, Tbilisi, 380004, USSR		
LAB/BRANCH Surgical Neurology		
SECTION		
INSTITUTE AND LOCATION NINCDS - NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <p>Epileptic discharges from <u>single cortical neurons</u> under the effect of strychnine were found to be accompanied by prolonged depolarization of the neuronal membrane. This was expected except that the depolarization was found to last for as long as 2,020 msec. The experiment also suggests that under these conditions the <u>inhibitory mechanisms</u> in the cortex were blocked or suppressed and that the prolonged depolarization with high-frequency spike discharges was triggered by impulses initiated in the centrum medianum-para-fascicularis complex of the <u>thalamus</u>.</p>		

PROJECT DESCRIPTION:

I. OBJECTIVE: To study the excitability characteristics and membrane properties of cerebral cortical nerve cells with particular reference to the mechanism of epilepsy.

II METHODS EMPLOYED:

(A) Electrical activity of cortical neurons was recorded with glass micropipette electrodes.

(B) In some experiments, the activity of two neurons were simultaneously recorded with two micropipette electrodes.

(C) With the electrode placed intracellularly, the membrane properties of the neuron were investigated.

(D) In all experiments the cortex was treated with strychnine hydrosulphate and stimulation of varying intensities and frequencies were applied to a peripheral nerve, to the unspecific intralaminar nucleus of the thalamus or directly to the cortical surface.

MAJOR FINDINGS:

(A) Neurons in an epileptic focus did not always fire synchronous in time.

(B) The majority of neurons in the epileptic focus underwent a prolonged depolarization associated with the surface epileptiform waves.

(C) The prolonged depolarization was not followed by post-excitatory hyperpolarization suggesting that the inhibitory mechanisms in the cortex were blocked or suppressed.

(D) The input resistance of the neuron was found to decrease with the polarization of the neuron.

(E) Stimulation of the cortex or of the peripheral nerve at varying frequencies sometimes, but not always, resulted in prolonged depolarization of the neuron. In most instances, when "spontaneous" prolonged depolarization of the neuron occurred, stimulation of a peripheral nerve, or the specific thalamic nucleus or of the cortex would terminate the prolonged depolarization of the cell.

(F) Stimulation of the unspecific intralaminar nucleus of the thalamus at 6-8/sec. always triggered off epileptic discharges and prolonged depolarization of the neuron in the epileptic focus.

IV. SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Epilepsy is a syndrome of a variety of neurological disorders manifested by excessive neuronal discharges. Needless to say that the best way to control epileptic attacks is to eliminate these dysfunctions in the nervous system. In many instances, however, dysfunctions cannot be readily eliminated and the epileptic attacks continue to become a threat or a displeasure to the patient. A better understanding of the mechanisms of excessive neuronal discharges will provide better therapeutic control of the epileptic attacks and better knowledge about the integrating function of the central nervous system.

V. PROPOSED COURSE OF THE PROJECT:

To continue the present study with particular emphasis in the unspecific intralaminar structures of the thalamus.

VI. PUBLICATION:

Li, C.-L., V.M. Okujava and C. Parsons: Cortical Epileptiform Activity Induced by Strychnine and Its' Response to Stimulation of Thalamic Centrum Medianum-Para-fascicularis Complex. In "Neurophysiological Mechanisms of Epilepsy" USSR Academy of Sciences. In Press.

PROJECT DESCRIPTION:

I. OBJECTIVE:

(A) To study the physiological mechanisms of pain.

(B) To evaluate the effect of conditioning somatosensory inputs upon the response of neurons to pain-fiber stimulation.

(C) To investigate the mechanisms of pain-relief by beta-endorphin and other analgesic agents.

II METHODS EMPLOYED:

(A) Adult cats under light general anesthesia were used.

(B) A peripheral somatosensory nerve (radial or saphenous) was stimulated with a negative haversine-wave pulses of varying duration and intensities. The response-thresholds and conduction velocities of various fiber components in the nerve were determined.

(C) Spontaneous neuronal activity was recorded from the nucleus reticularis gigantocellularis with micro-pipette electrodes.

(D) The neuronal activity in response to nerve stimulation was investigated.

MAJOR FINDINGS:

(A) Conduction velocities of the A-beta, A-delta, and C-fibers in a peripheral nerve exposed to sodium-deficient solutions were found to decrease in a decremental fashion. Recorded at a conduction distance of 18 mm and with the nerve in a 0.25% NaCl solution, the conduction velocity of the A-beta fibers in the nerve decreased at an average rate of 1.07 m/s per minute; of the A-delta, 0.35 m/s per minute and of the C-fibers, 0.020 m/s per minute. Thus, the A-delta fibers, which were generally known to mediate quick pain sensation, would cease to conduct in about 29 minutes; the C-fibers, which were known to mediate slow pain sensation, in 49 minutes; and the A-beta, in 54 minutes. Similar observations were obtained in nerves exposed to 0.5% NaCl solution except that the rate of decrement was slower.

(B) In the study with microelectrode recording from the nucleus reticularis gigantocellularis, neurons were found to respond to stimulation of the radial nerve. Further experiments will be carried out before conclusive statements can be made in regard to this response following a conditioning stimulus applied to the nerve or following an application of analgesic agents to the animal.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The present investigation will provide better understanding of pain mechanisms and subsequently a better treatment of pain.

PROPOSED COURSE OF THE PROJECT:

(A) To continue the present study in localizing the nuclear structures which receive pain impulses from the peripheral nerves.

(B) To study the spontaneous neuronal discharge patterns recorded from these nuclear structures.

(C) To study these discharge patterns in response to stimulation of various fiber components in a peripheral nerve.

(D) To test the neuronal discharge patterns in response to nerve stimulation by giving a conditioning stimulus or by administration of analgesic agents to the animal.

(E) To study the membrane properties, e.g., input impedance, capacitance and time constant of the neurons responding to peripheral nerve stimulation and to various pharmacological agents.

(F) Iontophoretic application of various pharmacological agents, including Beta-Endorphin, will be made extracellularly to study the change in synaptic transmission of impulses from the periphery to the central nervous system.

PUBLICATIONS:

Li, C-L. : Anatomy, Physiology and Some Surgical Treatment of Pain. American-Chinese Medical Assoc. J 15:14-18, 1978

Li, C-L. : Decremental Conduction in a Mammalian Peripheral Nerve. Acta Neurol. Scand. 52:31-45, 1979

ANNUAL REPORT

October 1, 1978 through September 30, 1979

Laboratory of Central Nervous System Studies
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT

October 1, 1978 through September 30, 1979

Laboratory of Central Nervous System Studies
National Institute of Neurological and Communicative Disorders and Stroke
Dr. D. Carleton Gajdusek, Chief; Dr. C. J. Gibbs, Jr., Deputy Chief

The Laboratory of Central Nervous System Studies comprises two major projects: (1) Neurobiology of Population Isolates--the Study of Child Growth and Development, Behavior and Learning, the Disease Patterns in Primitive Cultures; and (2) Chronic Central Nervous System Disease Studies--Slow, Latent and Temperate Virus Infections. Both projects are an outgrowth of the Study of Child Growth and Disease Patterns in Primitive Cultures. It was this parent organization that gave rise to the discovery of kuru, a heredofamilial subacute progressive degenerative disease of the central nervous system of the Fore people in the Eastern Highlands of Papua, New Guinea, and led to the demonstration that kuru is caused by a serially transmissible virus which possesses unconventional biological and biochemical properties. The successful transmission of kuru and the isolation of its virus provided the necessary techniques for the subsequent discovery of a viral etiology for some forms of presenile and senile dementias of man, particularly the Creutzfeldt-Jakob type and more recently certain forms of familial Alzheimer's disease and progressive supranuclear palsy. And, it was this study that has led to the discovery that the agents causing these diseases form a group of transmissible virus-like agents new to the field of microbiology.

Following the convening of a series of international workshops on the "Subacute Spongiform Virus Encephalopathies and the Structure of the Unconventional Viruses Which Cause Them" held in the latter part of the last fiscal year, the staff of LCNSS participated in an international symposium on "Slow Virus" sponsored by NIAID and held at the Rocky Mountain Laboratory, Hamilton, Montana. Eleven papers were presented and submitted for publication in the proceedings; they covered the origin of slow infections in humans, the worldwide epidemiology of these diseases, the pathogenesis and molecular biology of the viruses, the biological, physical and chemical properties of the viruses including the evidence for strain variations and their unusual resistance to gamma and ultraviolet radiation.

Consistent with our earlier predictions, the most challenging outcome of the discovery that some chronic progressive noninflammatory CNS diseases (sporadic, as most cases of Creutzfeldt-Jakob disease (CJD); epidemic, as kuru; or familial, as familial CJD, kuru, and some forms of Alzheimer's disease), are "slow infections" caused by viruses with incubation periods measured in years or decades, has been the realization that the etiologic agents of these infections are a new kind of micro-organism. Their unusual resistance to ultraviolet and ionizing radiation, to formaldehyde, β -propiolactone, and to heat, place them in a group unique among viruses. Their ability to produce fatal CNS disease without eliciting inflammatory responses, the failure of the course of disease or incubation period to be influenced by immunosuppression, and failure to demonstrate any antigenicity

in high titer infective virus preparations, or to find any evidence of humoral or delayed hypersensitivity reactions in the diseases, as well as an absence of response to interferon, immune interferon, or interference with interferon production, and absence of interference with known viruses, form the series of atypical biological properties which likewise differentiate these agents from any other group of microorganisms. On the other hand, by demonstrating classical virus properties, such as adaptation to new hosts, broadening of host range and reduction of incubation period, dependence on the genetic breed of the host, the presence of strains of differing virulence in wild stock viruses selected by limiting detection, and the interference of fast-growing by slow-growing strains of scrapie, are all indicative of a complex host-virus genetic interaction characteristic of more classical viruses. Attempts to delineate the chemical nature of the replicating agents, especially to determine whether they are replicated from introduced genetic information or by the induction, derepression or activation of pre-existing genetic information in the host, are the major thrusts of current investigation.

The elucidation of the structure and molecular configuration of the infectious agent of scrapie, CJD, and kuru remains the first goal of this laboratory. For two decades this frustrating problem has been a challenge to molecular biologists, biochemists, and virologists.

In the past year we have made advances in our attempts to characterize the scrapie agent: (1) Cesium chloride fractionation of the infectivity. The general trend of the infectivity distribution in the first sedimentation to equilibrium from homogeneity of the mouse scrapie agent from a mouse brain homogenate has been determined. The infectivity is banding in a broad peak centered around density 1.24. The broadness of the peak indicates a considerable heterogeneity in density. Due to the steepness of the gradient we have achieved a marked separation from other components assayed, i.e. RNA, DNA, protein and lipid. Of greatest interest is the 500 x purification with respect to total brain DNA.

Individual or combined fractions from these gradients have been assayed analytically for scrapie specific DNA, RNA and proteins by gel electrophoresis but as yet without detecting a new species of macromolecule. The highly complex protein patterns are virtually identical in normal and affected brain except for several protein deficiencies in the affected animal.

The preliminary infectivity data also indicate that the cesium chloride gradient has concentrated the infectivity relative to a sample stored in cesium chloride and not banded.

(2) Adaptation and development of the hamster 263-K strain of scrapie. Scrapie-infected hamster (strain 263-K) is a more suitable source of virus for purification studies. It is associated with a short incubation period and high initial titer of infectivity. The disease can be detected behaviorally only 55 days after a high titer passage, compared with a minimum of 180 days in the mouse system. Several titrations of hamster 263-K brain homogenates have consistently shown initial brain titers of 2-5 x

10¹⁰ infectious units/gram of brain, over 100 times the titers obtained from mice. In a detailed analysis for biochemical studies and titration purposes, the hamster system is at least two times and, for some purposes, over 500 times more efficient with respect to titration time and required animal space than is the mouse system. In terms of macromolecular distributions the hamster brain has fractionated much the same as the mouse brain. There is also a pronounced dependency of incubation time in the hamster on the dose of the agent, and this feature of the disease can be exploited to give an early indication of the distribution of the agent in fractionations, if not a quantitative assessment of infectivity.

Additional work focused on the possibility of obtaining infectious nucleic acids from extracted brain tissue. In order to enhance the potential infectivity of any naked nucleic acid recovered by our procedure we coupled the infectious assay with a transfection procedure which we had shown to be effective for herpes simplex virus. The experimental approach was to fractionate infected mouse brain homogenate following a heat inactivation step (80°C for 30 minutes) designed to inactivate any enzymes that might interfere with the recovery of infectious material.

Following heat inactivation the homogenate was digested with Protease K, then extracted with phenol in the presence of 1% sodium dodecyl sulphate (sds). The resulting three fractions (aqueous, phenol and heavy interphase) were further extracted under conditions designed to preserve the molecular nature of the material finding its way to that fraction. The aqueous phase was further extracted with organic solvents and alcohol precipitated. The phenol phase was buffer extracted to recover any material and the interphase was buffer extracted to remove the phenol. The resulting fractions were assayed for infectivity in NIH Swiss Webster mice. The results of this experiment clearly indicated that there was no infectivity associated with the nucleic acid fraction. The conditions used in these experiments would yield infectious HSV-1 DNA from infected cells but provided no scrapie infectivity. The heat and Protease K treatment had no effect on the infectious titer, however the subsequent steps destroyed virtually all of the infectivity. The only possible infectivity was in the highest concentrations of the buffer extracted interphase from the phenol extraction; the presence of infectivity in this fraction has not been confirmed by pathology. These results suggested to us that the viroid model, at least in its simplest forms, is not valid for the unconventional agents. Further studies on the scrapie system have focused on our impression that an essential, very hydrophobic protein is intimately associated with the scrapie agent and that new procedures are necessary for its isolation.

More recent studies reported in the literature indicate that at least a small percentage of the scrapie population has a DNA component of low molecular weight that is DNase sensitive which is eluted at 0.48M phosphate buffer from hydroxyapatite columns. This would suggest that the DNA molecule could be double stranded. During this year we tried to detect double-stranded scrapie-specific DNA by molecular hybridization experiments since analysis of the kinetics of DNA reassociation has proven to be a very sensitive means of detecting the presence of specific DNA sequences in mammalian genome. As a probe we used the DNA extracted from concentrated

enriched scrapie labeled with I¹²⁵ and annealed to total DNA extracted from infected and uninfected brains of the same and different species. No difference was observed between the extent of reassociation of the probe with DNA of scrapie or normal animals. Our levels of detection indicate that if the scrapie agent were a double-stranded DNA molecule its presence in the brains is below the level of 50 molecules of DNA per infective unit. We have sought also to repeat the work of others claiming to have isolated a scrapie-specific DNA. However, the preliminary results of our attempts to reproduce this much discussed procedure are showing disappointing results with less than a 1% recovery of infectivity in the high speed supernatant as opposed to the 10-90% indicated by Marsh and Malone. We are beginning to see infectivity in the acrylamide gel fractions, but the extent of this cannot yet be determined.

In yet another approach we have been comparing neurotransmitter concentrations in brains of scrapie-affected and normal mice and hamsters in the hope of identifying a particular neuronal system as the target for the infection in the brain. Comparing late scrapie mice with same age controls we have observed normal levels of catecholamines and most amino acids, but a two-fold increase in GABA levels and a nearly 100-fold decrease in 5-hydroxytryptamine (5-HT) levels. This finding prompted us to look at 5-HT levels in the blood. In the case of late hamster scrapie we observe a somewhat variable but significant decrease in blood serotonin of almost two-fold. At present these findings are being vigorously pursued: (1) to discover the time course of these changes and correlate them with behavioral changes and histopathology; (2) to narrow down by behavioral neuropharmacology, and brain microassay of neurotransmitters and enzymes the specific lesion(s) involved; (3) to identify other non-CNS indicators of these changes which may be of clinical use; and (4) to test the efficacy of 5-HT analogs as a therapy.

In a continuing effort to both characterize the agent and find ways to inactivate and/or stabilize it we have initiated the following inactivation experiments presently under titration: (1) sensitivity of scrapie to shear forces; (2) sensitivity of scrapie to osmotic shock; (3) sensitivity of scrapie to exhaustive protease treatment; and (4) sensitivity of scrapie to chlorine dioxide.

During the period covered by this report major efforts have been made to study the interaction of scrapie with the immune system of infected animals. These studies have been done in three parts. First, the search for a new antigenic component on the surface of spleen cells at various times following infection. Second, a systematic examination of the interaction of scrapie with a C3H/HeJ mouse line reported to be unique, and thirdly the identification and culture of the infectious cell population in the mouse spleen.

The search for a new antigenic component of the surface of spleen cells was based on the possibility that a new cell surface component would not be detected by the humoral immune response but would be detected by the cellular immune system. To examine this possibility, mixed lymphocyte cultures were utilized using two inbred strains of mice, Balb/C and C57BL/6. Two large

groups of animals were studied with cultures at weekly intervals over the early and late stages of infection. In every case controls inoculated with normal mouse brain were included on a 1:1 ratio. Data on the early times post infection included spleen weights to check for the enlargement reported by others. Throughout this study the results were uniformly negative with respect to both the splenomegaly and to the presence of any new cell surface component. Several cultural combinations were included to examine the scrapie-infected cells as both target cells and responder cells. It seems clear from this work that: (1) there is no new cell surface component on scrapie infected spleen cells that can be detected in mixed lymphocyte culture; (2) scrapie-infected spleen cells retain the capacity to respond to the mitogens Con A and LPS as well as respond to a heterologous H-2 determinant in mixed lymphocyte culture. These responses are identical in magnitude to those animals inoculated with normal mouse brain; (3) there is no detectable splenomegaly in scrapie infected mice within the first three months of infection and the data suggest that there is no splenomegaly throughout most infections.

Extensive studies with the C3H/HeJ strain of mouse have not confirmed the published report of other investigators that this strain of mouse, when infected with scrapie, loses its ability to mount a mitogenic response to the endotoxic protein component of E. coli LPS. This animal is genetically unable to respond to the Lipid A moiety. These studies were carried out at weekly intervals from weeks 2 through 7, since previous reports indicated the peak depression to occur at week 4. It has been reported that a marked spleen enlargement occurred, a finding also not confirmed in this work. There are only two possible explanations for the lack of agreement--one is a difference between the Chandler and C506 strains of scrapie, or that other investigators had a contaminating virus in their inocula. The plan for the future is to attempt to determine which of these is the explanation and to attempt to clarify completely if there is or is not a measurable change in the immune response of C3H/HeJ mice with scrapie.

The results of the spleen cell sub-population studies are too preliminary to report. It is clear that strain C506 gives extremely low spleen titers and that only a very small number (less than 1 in 10^5) spleen cells are infectious, whatever sub-population they are in. Extensive studies on splenic macrophages have been disappointing from the point of view of continued infectivity.

We have also explored the ability of scrapie to grow *in vitro* in well-established, 'T', 'B' and macrophage cell lines of murine origin. Two questions are being investigated: (1) does the cell have a receptor for scrapie on their cell surface?; and (2) if they do not have the receptor (assuming that scrapie agent is the free nucleic acid bound to lipid membranes), other methods have to be used to get the agent in the cell so that it could replicate. Inactivated Sendai virus and lysolecithin were used as membrane-fusing agents; DEAE-Dextran, which alters the permeability of the membrane and is used for assay infectivity of other viral nucleic acids in cell culture, was also used. Cell culture harvests from these experiments have been titrated in mice for infectivity and the results from these experiments will help us answer the two questions. Since most of the murine

cell lines used in the study have endogenous C-type viruses, it will also be interesting to see if these viruses act as helper viruses for the growth of scrapie. Attempts to grow scrapie in mosquito cells: *Aedes albopictus* mosquito--cell lines have been used to grow several groups of arboviruses. In such cells these viruses grow at 22°C without producing cytopathic effect, and infected cells become chronically infected by the virus. Virus is released from these chronically infected cells into the medium. We have infected these cells with the scrapie agent, and cell lysates at different passage levels have been inoculated into mice for the assay of infectivity. The results will be helpful to find out if, like some members of the togaviruses, scrapie agent grows in insect cells. An SV-40 transformed cell line that contained scrapie virus at the 12th passage level was serially passaged to higher levels; none of 50 pooled and cloned cultures was infectious for mice at the 30th passage level or higher. The scrapie-infected SMB line of Clarke and Haig was imported from England; five lots of this line have been prepared and aliquots stored; mutants of the cells are being prepared. A line of cells was derived from the brain of a hamster infected with the 263-K strain of scrapie; this line is also under study.

Since conventional immunological techniques have thus far failed to elicit an antigen-antibody reaction in either kuru, Creutzfeldt-Jakob disease or scrapie, we have been attempting to produce specific antibody to scrapie by hybridomas since it has been shown that cells from a mouse myeloma could be fused with splenic cells from mice stimulated with an antigen, and such fused cell clones produce specific antibody which is monoclonal for individual antigenic determinants. Such a technique facilitates antigenic analysis of complex antigens. As a control for the scrapie studies, somatic cell hybridization to produce monoclonal antibody against a major glycoprotein (P₀ 30,000 MW) associated with peripheral nervous system myelin was carried out. Thus far, 150 hybrids have been successfully grown from the 565 cultures initiated and 105 of these hybrids have been successfully cloned in semi-solid agarose and inoculated into Balb/C mice to determine infectivity of the clones as a first step in antibody production. We also measured the general immunocompetence of splenic lymphocytes in an attempt to detect alterations of the immune system of scrapie affected animals. In general splenic activation by Concanavalin A, phytohemagglutinin and lipopolysaccharide of control and scrapie inoculated mice were compared. Mitogen-induced responses of splenocytes from infected and control cultures were not significantly different. The PHA response of scrapie-infected mouse spleen cells was slightly depressed over a period of 29 to 56 days post-inoculation. Additional efforts to induce scrapie specific antibody are underway and indeed the use of several different preparations of high-titering scrapie infected hamster brain that has been subjected to (a) chemical tissue membrane modifiers, (b) purified by density gradient banding, and (c) tied up with haptens. Such mitogens are being assayed in animals rendered immunotolerant to uninfected hamster brain.

Since the demonstration of cell-fusing activity in the majority of brain extracts of scrapie mice and CJD patients (see Annual Report: October 1, 1977 through September 30, 1978) additional studies have been carried out using two different techniques. One involved the formation of multinucleated cells

and the other the formation of somatic hybrid cells. Heterokaryons were measured at 18 hours and hybrid cells after an average of 25 days. The studies employed three scrapie cases, 32 cases of transmitted CJD, two cases of untransmitted CJD, 26 cases of other neurological diseases, three transmitted cases of other than CJD and 17 patients without neurological disease. The results show a significantly higher proportion of CJD brains (61%) was positive than other neurological diseases (31.4%) or the control group (6%). Thus our earlier observations have been clearly confirmed and although the assay does not separate CJD from other neurological diseases to warrant its use as a specific diagnostic test we hope that such discrimination can be improved to the extent that the detection of cell-fusing activity might be possible utilizing serum, urine and CSF from patients and their family members as a biological marker of this disease. We shall continue to study the phenomena of cell fusing activity in an effort to elucidate the mechanism in CJD and other neurologic diseases as well as the application of this technique as a rapid means of more quickly measuring infectivity in experimentally derived fractions of purification procedures employed for scrapie and CJD.

Resistance to high concentration of formaldehyde, to heat up to 85°C, and to ultraviolet radiation at 254 nm, and an ultraviolet sensitivity at 237 nm greater than at 254 nm have been found for kuru and CJD viruses as for scrapie. These very unusual physical properties greatly emphasize our current contention that the viruses of the human diseases are closely related to the scrapie virus. Similarly, the two human agents have been shown to have the same enormous resistance to ionizing radiation (gamma rays from Cobalt Co_{60}) as is found for scrapie virus. The most direct inference from this enormous resistance is an effective size of under 100,000 daltons molecular weight. Although many possible explanations, including atypical fine structure for a nucleotide configuration and unusually efficient nucleic acid repair mechanisms have been suggested to account for such anomalous properties, the simplest explanation, namely, that in fact the agents are of such small size, may be true.

The discovery that the worldwide-distributed Creutzfeldt-Jakob disease is caused by a serially transmissible, self-replicating agent that passes through bacteria-, protozoan- and fungus-retaining membrane filters, the demonstration that the virus is widely distributed in tissues and fluids outside the CNS of affected patients and possesses the physiochemical properties as described above, has also resulted in a growing concern among medical and paramedical nursing and laboratory personnel, particularly neurologists, neurosurgeons, pathologists, and anesthesiologists, about the potential hazards involved in caring for patients with presenile dementias and handling their tissues. Concern comes largely from recent reports documenting transmission of Creutzfeldt-Jakob disease by corneal transplant, the accidental inoculation of two patients in neurosurgery with CJD-contaminated electrodes used in stereotactic electroencephalographic recording and stimulation, the suspicion that a neurosurgeon and two general practitioners may have contracted CJD from patients and the characteristic greatly over-represented among patients with CJD of a history of brain or eye surgery in the previous two years before onset of clinical disease. These concerns have further been heightened by the recent transmission of CJD to a

chimpanzee by implantation of the same silver electrodes that had caused disease in the two human patients after more than two years storage in formaldehyde vapors used for sterilization. In response to these concerns we have published precautions for conducting biopsies and autopsies and have more recently, presented a summary on the current knowledge of the pathogenicity and communicability of CJD and related subacute spongiform virus encephalopathies of man and animals which are caused by similar unconventional viruses. We have also made recommendations on the rational precautions that should be taken in caring for these patients and in handling their tissues.

During the last year, inactivation studies were made with disinfectants using mouse scrapie agent. Mouse scrapie, kuru, and CJD agents seem to have similar properties. Disinfectants used were clorox, organic iodine (Wescodyne), potassium permanganate, hydrogen peroxide, and Zepharin. Since ethylene oxide gas is commonly used in hospitals, ethylene oxide was also used. The data showed that a 1:250 dilution of $KMnO_4$ and a 5% solution of clorox are the most effective disinfectants, followed by Wescodyne and ethylene oxide which reduced infectivity by 99 percent. Under the experimental conditions used in the study hydrogen peroxide did not affect the titer of the scrapie agent at concentrations used in the hospital environment. Residual toxicity of Zepharin for mice was too high to draw any conclusions. Further studies are in progress on the CJD agent, with ethylene oxide autoclaving used for sterilization in the hospital setting. Finally, chlorine dioxide (a new generation disinfectant called Alcide) has been examined in parallel with 5% hypochlorite for inactivation activity towards both CJD, using a guinea pig-adapted strain, and scrapie, using a hamster-adapted strain. Time-dose experiments are on titration at this time, and should be completed within the year. Depending upon the results further recommendations will be made to the medical community.

In an effort to determine the method of spread of CJD virus in man, we have recently completed a comprehensive worldwide epidemiologic survey of CJD. It is shown that in the United States the average annual mortality is at least 0.26 deaths per million population. Temporal-spatial clustering of cases was not found in the United States, but reports from other countries indicate that this occurs. Fifteen percent of the cases were of the familial type, suggesting a genetic susceptibility to infection. In this survey, some evidence was found that previous surgery or pre-existing neurologic disease may be associated with an increased risk of developing CJD.

A systematic investigation of all cases of CJD dying in France during the decade 1968-1977 was completed during this year in collaboration with Dr. Francoise Cathala and members of the French Neurological Society, with a view towards clinical definition of a large and unselected case series, and to obtain some clue as to the natural mode of disease transmission. One hundred and seventy cases were discovered, of which 124, confirmed by autopsy or biopsy, were the subject of multifactor statistical analysis. The disease forms a clinical spectrum from nearly acute encephalitic type illness with a few weeks' rapid progression and death, to lingering illness of years' duration, impossible to diagnose in the absence of neuropathological verification. Types of clinical onsets, range of symptoms during the course

of illness, and symptom combinations with the highest frequencies were analyzed in detail. In addition, epidemiological data on all 170 cases were examined for the possibility of iatrogenic or case-contact types of human-to-human transmission. Apart from the approximately 10% of familial cases, no contact could be established between any two patients in France during a 10-year period, no iatrogenic transmission was discovered, no case occurred in any member of the medical profession, and those cases in paramedical professions did not occur at a higher rate than in the general population. Close examination of familial cases established that even in such families, personal contact between two subsequently affected members does not always occur, suggesting ever more strongly the participation of predominantly genetic factors in the familial type of CJD. Our epidemiological studies have already indicated that an annual incidence of nearly one case per million can be expected when newly occurring cases are actively searched out. The frequency of the disease continued to be highest in the densely populated center of Paris, raising further speculation about human-to-human modes of natural transmission. On the other hand, study of exceptionally isolated cases, which could simplify examination of the number of possible routes of acquiring the disease, still has not yielded any clues to this problem. A full-scale study of any possible association of CJD and scrapie in sheep is also under way.

A detailed analysis of the clinical features of the first 100 transmissible cases of CJD has been performed, and the results compared to the clinical features of a similar number of cases of Alzheimer's disease. There is a considerable overlap in the clinical spectrum of both diseases, and a group of patients with Alzheimer's disease with myoclonus has been delineated for further clinical and pathological evaluation. In addition, the clinical syndrome of "amyotrophic" CJD and a group of cases of "untransmissible" CJD are being studied.

Other clinical features of CJD which may be related to different strains of the virus are being examined. A manuscript is in preparation describing a small number of cases of CJD with the clinical features of progressive supranuclear palsy. The differences between the acute and chronic forms of CJD have already led to the discovery of a virus strain from a Japanese case that takes readily in non-primates and causes both gray and white matter spongiform lesions. The possibility that the virus also causes previously unrecognized childhood encephalopathies is also being investigated.

In a continuing investigation on the possible modes of natural transmission of the CJD virus, we are intensively evaluating the familial occurrence of the disease. To date, we have identified 37 families with a total of 155 affected members. CJD occurs in a pattern suggesting autosomal dominant transmission. Compared with the sporadic form of CJD, in familial CJD the age at death is slightly earlier and there is a female preponderance. The clinical and pathological features are otherwise indistinguishable. No maternal effect was found. There was some evidence for anticipation. An analysis of temporal and spatial separations between affected family members suggests that if contact transmission were occurring, incubation periods up to four decades might be expected. However, the available data do not yet allow us to distinguish between a genetic susceptibility to infection or some

form of vertical transmission. Studies are in progress determining genetic markers, such as the HLA type, of both sporadic and familial CJD, which might give us an indication of the genetic component of susceptibility to infection.

A major part of our experimental studies on CJD include the routine screening of the brains of all animals dying after inoculation with various chronic neurologic diseases, since it is now known that in the case of the squirrel monkey at least, approximately 15% of the animals die without showing clinical signs of neurological disease. The topography of the spongiform change has recently been analyzed in more than 200 squirrel monkey brains, where the results indicate that considerable variation in the severity and distribution of the lesions occur. The differences between CJD, kuru and scrapie are being examined in both primate and non-primate hosts. The unusual white matter change produced by a Japanese strain of CJD in mice is being examined.

A re-evaluation of the spongiform change in human kuru is being performed to see if the same general features as seen in human CJD also occur. The peculiar amyloid plaques that occur in 60% of kuru patients and approximately 10% of CJD patients is being investigated both structurally and at a biochemical level. The occurrence of these amyloid plaques in a virus-induced encephalopathy has great relevance to the etiology of the plaque of Alzheimer's disease.

With our demonstration of the transmissibility of scrapie disease from American sheep and English goats to several species of non-human primates, manifested by a disease in the experimental monkey that is indistinguishable from the transmissible virus dementia originating from man, we are confronted with the urgent question of the possible relationship between scrapie of sheep and the spongiform encephalopathies of man. The scrapie virus is capable of infecting all species of monkeys tested. However, the Compton (English goat) strain after passage through non-human primates no longer induces disease when inoculated back into sheep or goats. Of tremendous importance however has been the discovery that although these same strains of non-human primate-adapted scrapie virus did not induce clinical disease in mice during the more than two years they were observed, such mice did in fact have neuropathological lesions of spongiform encephalopathy in their brains and sub-inoculation of this material did induce disease in other mice. Thus, we have evidence that infected animals can remain asymptomatic and that in these animals the incubation period before onset of clinical disease may exceed the life span of the host. Moreover, the same exceptionally long incubation periods are evidenced in those few cases of kuru that have occurred in the Fore of Papua New Guinea during the past five or six years; new cases occur only in patients over 20 years of age. Thus, the biological properties of scrapie appear to be altered after passage through the primate host-behavior, not unlike classical viruses. If scrapie and the human diseases are caused by similar viruses, such altered biological properties may account for the failure of CJD and kuru viruses to induce disease in mice routinely. We have experienced difficulty in adapting the virus of CJD to mice and guinea pigs, but in recent experiments some passage lines of CJD have caused spongiform encephalopathy in both guinea pigs and mice. However,

we now have proven the transmissibility of the spongiform viruses by the oral route through feeding of virus-infected whole tissues. One out of two squirrel monkeys fed scrapie-infected hamster tissues and one out of two squirrel monkeys fed CJD-infected chimpanzee tissues have developed clinical disease typical of the spongiform encephalopathies. Two monkeys fed kuru-infected chimpanzee tissues are equivocal at this time.

The elucidation of the etiology and epidemiology of a rare, exotic disease restricted to a small population isolate--kuru in New Guinea--has now brought us to worldwide considerations that have importance for all of medicine and microbiology. For neurology, specifically, we have considerable new insights into the whole range of presenile dementias, and, in particular, to the larger problems of Alzheimer's disease, familial and senile dementias, and the processes of CNS aging. The implications of vertical transmission of slow virus infections, of conjugal transmission of these diseases, and of host genetic control of disease expression for all genetic diseases, and the relationship of these slow virus infection processes to those which may lead to neoplastic transformation are obvious.

The major problems among the degenerative diseases of multiple sclerosis, amyotrophic lateral sclerosis, and Parkinsonism remain unsolved, although there are tantalizing laboratory and epidemiological data pointing to the possible role of virus-like agents in these diseases. Perhaps the masked and defective slow infections with conventional viruses such as are seen in PML and SSPE may provide the best leads for studying these diseases.

Our scientific direction of the amyotrophic lateral sclerosis (ALS) studies at the Guam laboratory of NINCDS for the study of the ALS-PD complex in high incidence among the Chamorro people, has resulted in some 12 publications which have already appeared, or are in press, and many promising ongoing studies. These are summarized below, but they indicate our conviction that the answer to the perplexing problem of motor neuron disease (ALS) and Parkinsonism-dementia (PD) are to be found in these ethnically and geographically limited foci.

Our study of the similarly intense focus of ALS and Parkinsonism and dementia among the isolated Jakai and Auyu people of Western New Guinea, discovered during our field studies (New England Journal of Medicine, 1963), and with two recently updated reports just published (Ciba Symposium, 1977; Symposium on ALS, February 2-3, Tokyo, 1978) is proceeding with further field work this year. Once again the intense localization of the focus in a small population and limited geographic area suggests strongly a restricted environmental variable (plant toxin or mineral substance or a deficiency) coupled, perhaps with genetic factors in the population. This year's work has proven that the disease is fully environmental and that ALS and PD are related as evidenced by (1) husband and wife with classical ALS; (2) husband with pure PD, wife with classical ALS, simultaneously; (3) next door neighbor to (2) above with classical PD; and (4) two women with classical ALS in 1974 in same village and a neighbor with PD. It appears that the "rule" is that people living or drinking exclusively from small springs and rivers originating in "red-soil" lowland plain get ALS/PD. Some people of the same cultural and linguistic group, living on tidal flats and on big rivers, from

the high mountains do not get ALS/PD. With this in mind, we are covering these possibilities as well as those of an endogenous virus in an isolated population in our studies on Guam and West New Guinea.

We have increased our collaborative research with the Japanese investigators, who have been helping us on Guam by providing us each year with a young neurologist to assist in the clinical neurological surveillance and care of our patients there and in collaborative pathological, biochemical and pharmacological studies. During this reporting period, Dr. Takao Makifuchi, of the Brain Research Institute, Niigata City, Japan, took up residence on Guam as a Visiting Scientist. Also, Dr. Richard Yanagihara was recruited for Guam, and after three months of intensive preparation and developing protocols here at NIH will report to Guam in October, 1979.

The Japanese are themselves concerned with their own foci of high incidence of ALS and PD on the Kii Peninsula of the main island of Japan. The series of meetings and conferences on ALS in Japan held in March 1978 resulted in the confirmation by Dr. Hirano of the pathological identity of the Kii Peninsula PD cases with those on Guam (both demonstrating neurofibrillary tangles), and the final agreement that the two disease foci represent the same disease complex. During his 1979 field studies in West New Guinea, the Chief, LCNSS, has obtained definitive evidence that classical Guamanian ALS, PD, and ALS/PD does occur in the high incidence foci he discovered in West Irian and is very excited about resolving this problem. In addition, Dr. Gajdusek's field notes of 1979 reflect the occurrence in West New Guinea of a subacute progressive paralysis that looks like "slow-poliomyelitis" vitamin B deficiency. He has seen many cases this year and recognized it as the same disease he first saw in 1974-1976 field trips. The disease is not ALS; it can be "acute", it is often fatal, but remissions and recurrences do occur. A few cases have had beriberi-like edema with onset but most have not. That this very severe paralytic disease should occur within the ALS/PD focus is amazing. International collaboration and, most importantly, more original and innovative research concepts and more imaginative and cautious study of the various Western Pacific foci have continued and been expanded. Those studies which are underway in our collaborative project, and a bibliography of recent publications (1975-1979 in press) resulting from studies of these foci are included as an appendix to this annual report. The ongoing studies include:

- (1) Clinical variations in ALS-PD complex in Chamorros;
- (2) Human biology of ALS-PD complex and other chronic diseases in Chamorros of the Mariana Islands;
- (3) Chronic CNS disease and disability survey of Guamanian Chamorro migrants to the mainland United States;
- (4) Genetic studies of the Chamorro population, both normal and ALS-PD afflicted;
- (5) Detection of sedimentable reverse transcriptase activity in the brains of patients dying with ALS-PD;
- (6) Search for biochemical defects in ALS-PD brains by gel diffusion chromatography;
- (7) Search for nucleic acid repair mechanism defects in transformed leucocyte cell lines derived from ALS-PD patients;

- (8) Search for an ALS or PD specific antigen in brain tissues by clonal myeloma cell hybridization with spleen cells of ALS and PD from hyperimmunized animals and resultant monoclonal antibody production;
- (9) Trace aluminum and other heavy metal studies in brain, CSF, blood and other tissues of ALS-PD patients;
- (10) Evaluation of the precise nature of the cognitive and affective defects and the progression of dementia in the PD patient;
- (11) Evaluation of liver function and pathology;
- (12) Development of techniques for the unmasking of an infectious agent by in vitro techniques;
- (13) Assessment of the immunological competence of patients;
- (14) Attempts to transmit ALS-PD to non-human primates and non-primate hosts;
- (15) Major virus group seroepidemiology of the Mariana and Caroline Islands, Japan, and West New Guinea populations with relation to ALS-PD;
- (16) Pharmacologic studies of ALS-PD;
- (17) Elucidation of osteoporosis, osteoarthritis, and bone deformities in the Chamorros; and
- (18) Evaluation of the growth and development of normal Guamanian children and adolescents--a 30-year follow-up study.

The genetic studies, already well advanced, include blood group factors, red cell enzymes, serum proteins, HLA typing, and mixed leucocyte agglutinins, dermatoglyphics, anthropometry and other gene markers.

Since World War II, there has been an extensive migration from Guam of at least 15,000 Chamorros, primarily to the United States. This represents nearly one-third of the total Chamorro population of 47,000 residing on Guam. Amyotrophic lateral sclerosis has developed in 14 Chamorro migrants from Guam to the United States, Japan and Korea after periods of one to 36 years of absence from Guam. Nine of these cases have been previously reported. In another eight subjects ALS has developed within 1 to 14 years of their return to Guam after absences of many years from the islands. Parkinsonism dementia, a high incidence presenile dementia peculiar to Chamorro Guamanians, has developed in one subject 46 years after his departure from Guam. It appears that the onset of ALS in these patients after long absences from Guam will demonstrate the lower limit for the incubation periods if a toxic or infectious exposure occurring only on Guam is the cause of the disease.

Additionally, during the past two decades there has been an increasing number of cases of Guamanian ALS in long-term Filipino migrants to Guam. The average annual incidence rate of ALS in these migrants is approximately four-fold higher than the rate of ALS in the United States. Parkinsonism dementia has been clinically verified in five Filipino patients. Because of the high degree of genetic similarity between the Chamorro and Filipino peoples, which we have recently demonstrated, a detailed epidemiological survey for ALS and a clinical search for PD in the Philippine Islands would be of interest.

The clinical and pathological characteristics of long surviving cases of Guamanian ALS, that is of more than ten years duration, are currently under

study. Long surviving cases of ALS in Guam are younger, have a familial occurrence, have a different sex ratio, and show a different pattern of disease progression than those with a normal duration of disease.

Additional studies on HLA, dermatoglyphics and other gene markers, on osteoporosis and osteoarthritis, on heavy metals and other environmental toxins and on a ten-year follow-up study of the descriptive epidemiology of ALS and PD are close to completion. Further studies based on these data are in the planning stages or already underway.

Previous studies in our laboratory have shown that ALS and PD patients from Guam had diminished levels of cellular immunity as determined by diminished response to skin test antigens, lymphopenia, diminished number of 'T' cells, and decreased mitogenic response, than those of age- and sex-matched Guamanian controls. Further, ALS patients with HLA BW-35 had diminished cellular immunity and shorter mean duration of the disease. This association was found to a lesser degree among PD patients and no association was detected in the controls. Using C₁₉ binding techniques, Oldstone *et al.* have shown high frequency of immune complexes in the sera of ALS patients in the continental United States. There was evidence of immune complex deposition in some of the kidneys of the ALS patients. The nature of these immune complexes was not determined. Studies of hepatitis B in the South Pacific reveal that hepatitis B virus is endemic in most of the Pacific Islands. There is high prevalence of hepatitis B surface (HBsAg) antigenemia, and most of the population has either HBsAg or antibody to HBsAg. It is common to have found both HBsAg and anti-HBsAg in many individuals in the population. Since immune complexes are known to cause immunosuppression, we investigated the prevalence of HBsAg, anti-HBsAg, and the immune complexes due to HBsAg and anti-HBsAg in the sera of ALS and PD patients from Guam and healthy controls. Additionally, we also tested sera for the presence of hepatitis A antibody. The data showed that ALS patients have lower levels of anti-HBsAg than PD patients or controls. There was no significant HBs antigenemia or immune complexes in ALS and PD patients and controls. Almost all sera tested had antibodies to hepatitis A. These studies show that HBsAg and anti-HBsAg complexes were not responsible for the immunosuppression observed. The lower rates of HBsAg in this population may be due to sampling of older individuals.

In other areas of Micronesia, human biological field and laboratory studies continue. Studies of chronic respiratory diseases indicate that 75% of the children under five years of age were found to have asthma, while over 50% of the adults over 40 years of age were affected by chronic bronchitis, often with an asthmatic component, and typical chronic obstructive airway disease occurred in almost one-third of the male population over 50 years of age. As a result, pulmonary airway diseases constitute the most important cause of morbidity and mortality in the Western Caroline Islands.

Since chronic inflammatory neurological disease is known to follow togavirus (arbovirus) encephalitis infections of humans in Europe and Asia, sera from more than twenty American patients with chronic epilepsy and inflammatory brain disease were examined by hemagglutination for all

togaviruses known to cause encephalitis of humans in North America. None had antibodies. It seems unlikely that togavirus encephalitis is an important cause of chronic inflammatory brain disease in the United States.

A survey of togaviral antibodies in several Pacific populations confirmed earlier studies of the geographic distribution of several viruses. A possible correlation between susceptibility to Ross River Virus and one red cell Rh subtype was found in a population of Papua New Guinea. Plaque and microtiter tests have been developed for groups A and B togaviruses, and neutralization tests are being performed on selected sera.

Serum and CSF specimens from schizophrenic patients and age- and sex-matched controls were obtained from Doctors Torrey and Wineberger of St. Elizabeth's Hospital, Washington, D.C. and Constantine Sakkles of the University of Maryland Hospital, Baltimore. These specimens were tested for group A and group B arboviruses using the hemagglutination inhibition test. Viral antigens used in the test were Eastern and Western Encephalitis, St. Louis encephalitis, and California encephalitis. There was no significant association of arboviral antibodies to schizophrenia. In the light of recent reports by Tyrell *et al.* of detection of cytopathic agents from the CSF and some controls, attempts will be made to do similar studies with the CSF samples on hand.

The work on the development of animal models for the study of persistent infections has continued. A foamy virus of chimpanzees (Pan 1, also called foamy virus 6) was isolated in this laboratory over ten years ago. In the chimpanzee it appears to be a latent virus, and can at times be isolated from brain explants of healthy animals. The mechanism of viral latency has been impractical to examine, however, due to the expense and scarcity of the chimpanzee for experimental purposes. Therefore, experiments were conducted to adapt Pan 1 virus to a more convenient laboratory host, and after several preliminary studies, we succeeded in adapting the virus to the mouse. Using kidney and spleen explants from mice-infected neonatally, infectious virus has been isolated up to one month following inoculation, viral antigen has been demonstrated in the explants, and serum CF antibody has been detected. However, in no animal has it been possible to detect infectious virus or viral antigen directly in the organs themselves. We are currently studying the possibility of viral persistence for up to a year following inoculation, and evaluating the mice for any signs of disease during their natural lifetime. Integration of viral genome in the host cells is also under investigation in collaboration with Dr. Chev Kidson in Australia.

The model of lysogenicity and of subviral genetically active macromolecular structures from the study of bacterial viruses and bacterial genetics supply ample imaginative framework for an expression of our ideas of possible pathogenic mechanisms for kuru and CJD in man. The unconventional viruses of the spongiform encephalopathies tax even our imagination in relation to molecular biology gained from these studies in bacteria.

For a now-disappearing disease, kuru, in a small primitive population to have brought us this far is ample reason for pursuing intensively the challenges offered by the still inexplicable high incidence and peculiar

profusion of different neurological syndromes, pathologically distinct yet apparently related to each other, which have been discovered in the several small population enclaves we have investigated. Thus, the high incidence of ALS, ALS-PD on Guam and among a small population of people in West New Guinea, coupled with the high incidence of ALS on the Kii Peninsula of Japan, may indeed offer the best opportunity of solving the problem of this sclerosing disease which in the United States has an incidence as high as that of multiple sclerosis.

The delineation of infection as the etiology of heredofamilial and presenile and senile dementias of man was made possible only through the concomitant studies on the neurobiology of population isolates. In this area we have been engrossed in the investigation of deaf-mutism, mental subnormality and other congenital central nervous system defects associated with endemic goiter in the Central Highlands of Western New Guinea, as well as patterns of delayed puberty, slow growth rates, and of early aging in isolated Melanesian groups. Ethnic drug abuse (particularly of kava), strange patterns of psychosexual development, pseudohermaphroditism, and culturally-determined responses to pain, and roots of aesthetic expression, have all been under study. Foci in primitive population isolates of familial periodic paralysis, progressive muscular dystrophy (both the pseudohypertrophic type of Duchenne and the non-pseudohypertrophic distal type), amyotrophic lateral sclerosis and Parkinsonism, are also being investigated. Genetic studies on human evolution led to the discovery of new genetic factors among haptoglobin, hemoglobin, and red cell enzyme pleomorphisms and the definition of their biochemical structure.

The further significance of scientific investigations of small population enclaves of remote populations was even more dramatically apparent during the 1975-1976 and the current 1979 field trip of the Chief of LCNSS, with his re-evaluation of what may turn out to be one of the largest "epidemics of epilepsy" ever recorded. This continues to occur in the Wissel Lakes area of West New Guinea and is the result of cysticercosis, an infestation with the larvae of Taenia solium, the pig tapeworm, newly introduced into New Guinea. Our recent studies have led us to conclude that the natural history of cysticercosis epilepsy is not a result of death of the worm, scarring and calcification of lesions, as much of the literature suggests, but is an early sign of inflammation from new invasion of the brain by the Taenia larvae. Convulsions often occur even before the first subcutaneous nodules appear, and as the nodules increase in number, additional seizures occur. The high incidence of severe third-degree burns, which may even result in death, is a direct result of cysticercosis-induced seizures that occur during sleep, throwing the patient into the house fire. The unclothed people, living at a 2000 meter elevation, need to sleep close to the home fires on cold nights. We are able to date the first introduction of Taenia solium into the area and to plot the spread of taeniasis in pigs and man, and of cysticercosis and associated epilepsy in man, to other previously Taenia-free areas. During this year, we have learned that the cysticercosis has spread both in swine and man throughout the West New Guinea Highlands and is now in the Baliem region. With Dr. Budi Subianto, the local Indonesian medical officer, we have planned a neuroepidemiologic study aimed at elucidating the natural history of the epilepsy and acute psychoses and other neurological

complications that have occurred concomitantly with the emergence of subcutaneous cysticercosis nodules. Recently, we have adapted the ELISA test, a highly sensitive enzyme binding test for determining antigen-antibody reactions, for conducting seroepidemiologic studies of the disease. This has led to studies on the development of techniques to produce purified cysticercosis antigens for better specificity of reactions.

During this year tests were completed on serum samples from West New Guinea. Fifty-four percent (12/22) sera from patients with cerebral cysticercosis (convulsions) were positive. In contrast nearly eighty percent (8/10) of patients with systemic cysticercosis (palpable nodules) with or without cerebral involvement were positive. Higher percentage of positive patients in this group may possibly be due to exposure to a larger antigenic mass. Fourteen percent of infections were asymptomatic. The lower positive rates observed among cerebral cysticercosis patients may be due to lack of antibody response due to direct massive infection of the brain by the parasite and short incubation period prior to detection of convulsions. The study shows that the cysticercosis epilepsy epidemic in the Ekari people in West New Guinea was restricted to the Wissel Lakes area and no further spread was observed in the adjoining area. We recently obtained serum and CSF specimens from proven cases of cerebral cysticercosis in endemic areas of Mexico. The specimens were obtained from the National Neurological Institute in Mexico City. In that institution diagnosis is made by complement fixation test on CSF of the suspected cases. Comparative tests (CF and ELISA) will be made on Mexican samples to determine the specificity and sensitivity. Importance of the cerebral cysticercosis in the third world countries cannot be underestimated. ELISA test may be useful in early diagnosis treatment and control of the disease.

As previously reported, the Chief of LCNSS was invited by the Soviet investigators to participate in the investigations in the U.S.S.R. of a unique degenerative disorder of the nervous system, Vilyuisk encephalitis. This disease occurs only in the Yakut region of Eastern Siberia and has many features of a slow virus disease. In August 1979 a field study was completed, the first by any Western investigator, and much valuable information was obtained.

The development and maturation of the two major projects of this laboratory has resulted from cross-fertilization of each since their origin, and both have grown from the basic studies on child growth and development and disease patterns in primitive cultures. Although the two projects, each composed of many subsections, differ markedly in the questions they address and the techniques of investigation they employ, much of the field data collected from one project is also requisite for the studies in other projects. Both are served by the same investigators, who function as a team. These scientists derive their creative stimulus, dedication and enthusiasm to a great extent from the atypical and exotic biological, social and cultural materials presented, and the diverse, frequently unconventional, approaches of the two projects.

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01282-15 CNSS
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PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PRINCIPAL INVESTIGATORS:
D. Carleton Gajdusek, M.D., Chief, LCNSS; Clarence J. Gibbs, Jr., Ph.D., Deputy Chief, LCNSS; David M. Asher, M.D., Paul W. Brown, M.D., Ralph Garruto, Ph.D., and Lon R. White, M.D.
Michael Alpers, M.D.; Richard Benfante, M.A.; Judith Farquhar; Peter Fetchko, M.A.; Chev Kidson, M.D.; Klaus Mannweiler, M.D.; Colin L. Masters, M.D.; Steven Ono; Robert Rohwer, Ph.D.; Donald Rubinstein; Vincent Zigas, M.D.
Christoph Bernoulli, M.D.; Jacques Bert, M.D.; Francoise Cathala, M.D.; Kwang-Ming Chen, M.D.; Louis Court, M.D.; Olivia Cruz, M.D.; Arwin R. Diwan, Ph.D.; Richard Feinberg, Ph.D.; Father David Galles; Fusahiro Ikuta, M.D.; Undapmaika Kalaqune; David E. Kohne, Ph.D.; Robert MacLennan, M.D.; Jesus Raglmar; Frank Saul, Ph.D.; Wulf Schiefenhowel, M.D.; Koiye Tasa; Yushiro Uebayashi, M.D.; Fransje van der Waals, M.D.

COOPERATING UNITS (if any) AUSTRALIA: Dr. HOM King, Queen Elizabeth Hospital, Adelaide; Dr. C. Kidson, University of Queensland, Brisbane; Drs. T. Asch, N.M. Blake, R.L. Kirk, K. Omoto, S.A. Wurm, Australian National University, Canberra; Dr. C.C. Curtain, Dr. E. French, Commonwealth Science and (continued)

LAB/BRANCH
Laboratory of Central Nervous System Studies, Intramural Research Program

SECTION

INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12	8	4

CHECK APPROPRIATE BOX(ES)
 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) 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. Laboratory techniques of molecular biology, immunology, virology, endocrinology and biochemistry in these cultures and field epidemiological, genetic and clinical studies are aimed at problems more appropriately studied in small isolated primitive bands than in civilized societies. Data and specimens collected over years on expeditions to Micronesia, Polynesia, Solomon Islands, New Hebrides, New Guinea, Indonesia, S. 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 CNS function of 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 were amalgamated into the cosmopolitan community of man. Foci of high incidence prevalence kuru, ALS/PD, epilepsy, other neurological degenerations, hysterical disorders, schizizophrenia, neoplasms, goiter, cretinism, rheumatoid diseases, diabetes, asthma, chronic lung disease, malaria, filariasis, leprosy, cysticercosis and other infections are investigated.

COOPERATING UNITS: continued

Industrial Research Organisation, Melbourne; Dr. M.P. Alpers, Dr. J. Sheridan, Dr. N.P. Stanley, University of Western Australia, Perth; Dr. I. Hancock, Department of Public Health, Darwin; Dr. T.G. Aitchison, Schofields, N.S.W.

BRAZIL, SOUTH AMERICA: G. Schmutterer, Curitiba.

CANADA: Dr. O. Schaefer, Department of National Health and Welfare, Northern Medical Research, Edmonton; Dr. M. Kinsbourne, Hospital for Sick Children, Toronto; Dr. J.A. Hildes, Arctic Medical Research Unit, University of Manitoba, Winnipeg.

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FINLAND: Dr. J. Lahdevirta, Department of Medicine, University of Finland, Helsinki.

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GERMANY: Dr. R. Hassler, Prof. Kruecke, Dr. H. Stephan, Max-Planck Institute for Neurology, Frankfurt.

INDONESIA: Java--Dr. J. Sulianti-Saroso, Public Health Department, Jakarta; Dr. K. Sorenson, NAMRU-2, Jakarta; Dr. J. Dean, Summer Institute of Linguistics, Bogor; Irian Jaya--Dr. T. Gerungen, Dr. L.R. Tumada, Dr. Wasito, Public Health Department, Jayapura; Dr. A. Gunawan, Dr. A.M. Hutapea, Dr. E.A. Iswandi, Dr. B. Kawengian, Dr. L. Kristanda; Dr. D.B. Subianto, Public Health Department, Enarotali; Dr. Widodo, Public Health Department, Kapi; Dr. K. Dresser, T. Benoit, Associated Aviation, Sentani; Dr. W. Schweifenhoeval, Public Health Department, Sentani; Bishop A. Sowada, Fathers J. Donkers, D. Gallus, B. Mischke, F. Trenkenshuh, B. van Oers, Catholic Mission Center; Dr. C. Hoogeland, Aboaha.

ITALY: M. and Dr. A. Jablonko, Perugia.

NETHERLANDS: Dr. L.N. Went, State University of Leiden.

NEW HEBRIDES: Dr. Baudson, Dr. P. de Carfort, Dr. R. Greenhough, Dr. Retard, Medical Service, Port Vila; Captain J. Barley, Condominium Government, Port Vila; N. Woodward, British Residency, Port Vila.

COOPERATING UNITS: continued

PAPUA NEW GUINEA: Dr. J. Onno, Dr. A. Tarutia, Dr. J. Tuvi, Public Health Department, Port Moresby; Jack Baker, Department of Administration, Port Moresby; Dr. K. Clezy, Dr. D. Dowd, Dr. H.M. Gandy, Dr. A. Saweri, Faculty of Medicine, University of Papua New Guinea, Boroko, Port Moresby; Dr. G. Mosuwadoga, Robert Mitten, Dirk Schmit, Papua New Guinea Museum, Port Moresby; Dr. U. Beier, Institute of Papua New Guinea Studies, Port Moresby; Dr. V. Zigas, Public Health Department, Kimbe, West New Britain; Dr. J.L. Gressitt, Wau Ecology Institute, Wau, Morobe Province; Rev. H. Gericke Evangelical Lutheran Mission, Okapa, Eastern Highlands, Province; Rev. W.R. Wiesner, Reb. Stuart Merriam, Koiye, Waneri, Anua and Tosetnam, Okapa, Eastern Highlands; Undopmaina, Martin, Marawaka, Eastern Highlands, Province.

SCOTLAND: Dr. J. MacGregor, Lerwick; Drs. L. and B. Herzberg, Dundee.

SINGAPORE: Dr. M. Simons, W.H.O. Immunology Research and Training Center, University of Singapore; Dr. Kok Ann Lim, Department of Bacteriology, University of Singapore; Dr. Ivor Polunin, Department of Social Medicine, University of Singapore; Mr. Lim Chong Keat, University of Singapore.

SOLOMON ISLANDS: Dr. A. Solomon, Medical Services; Dr. P. Beck, Ministry of Health and Welfare; Drs. B. Wilkin and D.S. MacKay, Central Hospital, Honiara.

TAIWAN: Dr. P. Beasley, NAMRU-2, Taipei.

TRUST TERRITORY OF THE U.S.: Dr. M.T. Ueki, MacDonald Memorial Hospital, Palau; Peter Tigweyar, Ifulik Atev, Western Caroline Islands; Dr. P. Huitema, Ponape Hospital, Kolonia; Mr. Hiliary Tacheloil, Yap District Office, Western Caroline Islands.

U.S.S.R.: Dr. P.A. Petrov, Director, Ministry of Health, Iakutsk, A.S.S.R.; Dr. M.P. Chumakov, Dr. Luoff, Dr. K. Umanskii and Dr. L. Goldfarb, Institute of Poliomyelitis and Virus Encephalitis, Moscow; Dr. L. Fadeeva, Institute of Virology, Moscow; Dr. V. Zhdanov, Dr. A.A. Smorodintsev, Dr. V.I. Il'yenko, All-Union Research Institute of Influenza, Leningrad.

UNITED STATES: Alabama--Dr. C.J. Hoff, Department of Medical Genetics, University of South Alabama Medical School; California--J. Boykin, College of the Pacific, Valencia; Dr. P. Terasaki, Rehabilitation Center, University of California, Los Angeles; Dr. R.L. Walford, Center for Health Sciences, University of California, Los Angeles; Dr. Ted Schwartz, Department of Anthropology, UCLA, Los Angeles; Dr. S.A. Brown, School of Public Health, University of California, Berkeley; Dr. L.L. Cavalli-Sforza, Stanford University, Stanford; Colorado--Dr. S. Wiesenfeld, National Jewish Hospital, Denver; Connecticut--Dr. J. Casals, Rockefeller Laboratory, Yale University, New Haven; Delaware--Dr. R. Rodrigue, Wilmington; District of Columbia--Dr. G. Gibson, Smithsonian Institution; Hawaii--Dr. A. Diwan, University of

COOPERATING UNITS: continued

Hawaii School of Medicine, Honolulu; Drs. L. Rosen, G. Wallace and R. Tesh, Pacific Research Station, NIAID, Honolulu; Maryland--Drs. Richard T. Johnson, Guy McKhann, Donald Price, Neal Nathanson, Gerald Cole, School of Public Health and Hygiene, Johns Hopkins University, Baltimore; Dr. K. Shah, Department of Neurology, Johns Hopkins University Hospital, Baltimore; Dr. K. Brown, Dr. W.C. Leyshon, Laboratory of Developmental Biology and Anomalies, NIDR, NIH, Bethesda; Dr. P. MacLean, Laboratory of Brain Evolution and Behavior, NIMH, NIH, Bethesda; Drs. F. Neva, L.H. Miller, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda; Dr. J. Wolff, Clinical Endocrinology Branch, NIAMH, NIH, Bethesda; Dr. J. Sever, Dr. S. Houff, NINCDS, NIH, Bethesda; Dr. C. Wisseman, School of Medicine, University of Maryland, Baltimore; Dr. T.C. Raines, National Bureau of Standards, Gaithersburg; Massachusetts--Dr. P. Fetchko, E. Dodge, Peabody Museum, Salem; Dr. N. Geschwind, Neurology Unit, Beth Israel Hospital, Boston; Dr. John Enders, Dr. M. Oxman, Dr. R. Ferber, Children's Hospital Medical Center, Boston; L.K. Marshall, Boston; K. Muller, Harvard University, Cambridge; Michigan--Dr. E.A. Rodin, Department of Mental Health, Lafayette Clinic, Detroit; Dr. T.M. Ernst, Department of Anthropology, University of Michigan, Ann Arbor; New York--Dr. Roger Traub, I.B.M., Yorktown Heights; Dr. R.E. Peterson, Department of Medicine, Cornell Medical Center, New York; Dr. P. Kennedy, Program of American Studies, State University of New York, Buffalo; Dr. R. Glasse, Queens College, Flushing; Dr. S. Lindenbaum, York College, CUNY, Jamaica; Dr. Alan Lomax, Applied Social Research, Columbia University, New York; E.I. Schiefflin, Fordham University, Bronx; Ohio--Dr. A. Steinberg, Case Western Reserve University, Cleveland; Pennsylvania--Dr. D. O'Brien, Department of Anthropology, Temple University, Philadelphia; Dr. N. Chagnon, Dr. P.T. Baker, Pennsylvania State University, University Park; Rhode Island--Dr. T. Kiefer, Brown University, Providence; Washington--Dr. R. DiGiacomo, Department of Epidemiology, Dr. P. Kunstatter, Department of Preventive Medicine, University of Washington, Seattle.

VENEZUELA: L.T. Laffer and F. Melchiorri, Caracas.

- Sub-Project I: Study of the developmental 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 populations of Asia, Africa, Indonesia, Melanesia, Micronesia, Polynesia and the Arctic.
- Sub-Project IX: Experimental developmental neuropediatrics in infantile programming: an empirical approach to the language of information input into the nervous system.
- Sub-Project X: Ciphers and notation for the coding of sensory data for neurological information processing.
- Sub-Project XI: Racial distribution and neuroanatomic variations in the structure of the human brain.
- Sub-Project XII: Studies of high incidence of neurological diseases in specific racial and ethnic groups and in primitive or geographic population isolates.
- Project Description: Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures (described fully on pages 1-LCNSS/IRP through 18-LCNSS/IRP.
- Publications: Listed on pages 33 - LCNSS/IRP through 37 - LCNSS/IRP

ETHIOPIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 00969-15 CNSS

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PRINCIPAL INVESTIGATORS: D. Carleton Gajdusek, M.D., Chief, LCNSS; and Clarence J. Gibbs, Jr., Ph.D., Deputy Chief, LCNSS

Herbert L. Amyx, DVM; Tomonobu Aoki, MD; David M. Asher, MD; Sina Bahmanyar, MD; Maria-Teresa Borrás, PhD; Paul W. Brown, MD; Ralph Garruto, PhD; Patrick Gourmelon, MD; David T. Kingsbury, PhD; Yasuo Kuroda, PhD; Pyung Woo Lee, PhD; Colin L. Masters, MD; Shigeru Mori, MD; Seiho Nagafuchi, MD; Robert Rohrer, PhD; Lon R. White, MD; Richard Yanagihara, MD

Francoise Cathala, MD; Louis Court, MD; Philippe de Micco, MD; Arwin R. Diwan, PhD; Sergio Galvez, MD; Jaap Goudsmit, MD; Chev Kidson, MD; Takao Makifuchi, MD; Klaus Mannweiler, MD; Marie-Claude Moreau, PhD; Charles Morrow, MD; Ryoichi Mori, PhD; Wanda Pogodina, MD; Fransje van der Waals, MD

COOPERATING UNITS (if any)

AUSTRALIA: Michael Alpers, M.D., University of Western Australia, Perth; Eric French, Ph.D., Division of Animal Health, CSIRO, Victoria; Robert L. Kirk, M.D., Department of Genetics, Australian National University, Canberra; Eric Shaw, Ph.D., Peter Harden, Ph.D., (continued)

LAB/BRANCH

Laboratory of Central Nervous System Studies, Intramural Research Program

SECTION:

INSTITUTE AND LOCATION

National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205

TOTAL MANYEARS:

24

PROFESSIONAL:

14

OTHER:

10

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS

(a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Studies elucidate cause and pathogenesis of chronic degenerative CNS disorders with emphasis on MS, ALS, parkinsonism-dementia, Parkinson's, Pick's and Alzheimer's diseases, Huntington's chorea, supranuclear palsy, other presenile dementias, chronic encephalitis with focal epilepsy, muscular dystrophies, chronic schizophrenia, SSPE, PML, dialysis encephalopathy, and intracranial neoplasms. Even familial, apparently hereditary diseases may be slow virus infections. Subacute spongiform virus encephalopathies (kuru and Creutzfeldt-Jakob (CJD) diseases 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 brain occur. 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.

COOPERATING UNITS: continued

Red Cross Blood Transfusion Service, Brisbane.

AUSTRIA: F. Seitelberger, M.D., Neurologisches Institut der Universität Wien, Vienna.

BELGIUM: Armand Lowenthal, M.D., Laboratory of Neuropathology, l'Institut Bunge, Antwerp.

BRAZIL, SOUTH AMERICA: R. Luzzatto, Instituto de Neurocirurgia, Hospital de Neurocirurgia, Santa Casa Porto Alegre.

CANADA: J.J. Gilbert, Victoria Hospital, London, Ontario; W.P. McInnis, Victoria Hospital, London, Ontario; Hyun J. Cho, D.V.M., Ph.D., Animal Diseases Research Institute (Western), Agriculture CANADA, Lethbridge, Alberta; Theodore Rasmussen, M.D., University of Montreal; N.B. Rewcastle, Toronto General Hospital, Banting Institute, Toronto; D.M. Robertson, Queens University Hospital, Department of Neuropathology, Kingston, Ontario; A.R. Watts, Neurological Associates, Regina, Saskatchewan.

CZECHOSLOVAKIA: Helena Libikova, M.D., Institute of Virology, Slovak Academy of Sciences.

ENGLAND: Peter M. Daniel, M.D., Department of Neuropathology, Maudsley Hospital, London; Geoffrey Edsall, M.D., 5 Ellerdale Road, Hampsted, NW3, London; Mrs. Elisabeth Beck, Institute of Psychiatry, Department of Neuropathology, London; George Dick, M.D., Bland-Sutton Institute of Pathology, London; P.J.H. Fletcher, Department of Pathology, N. Staffordshire Royal Infirmary, Stoke-on-Trent; D. Haig, D.V.M., G.D. Hunter, Ph.D., Animal Research Section, Compton; M. Sim, Queen Elizabeth Hospital, Edgbaston.

FINLAND: N. Oker-Blom, Saikku, Department of Virology, University of Helsinki.

FRANCE: Françoise Cathala, M.D., Department of Neurology, Hopital de la Salpêtrière, Paris; Contamin, M.D., Hopital St. Antoine, Paris; Lhermitte, M.D., Hopital de la Salpêtrière, Paris; F. Pertuiset, Hopital de la Salpêtrière, Paris; Warot, M.D., Department of Neurology, Université de Lille Nord; Raymond Latarjet, Fondation Curie-Institut du Radium, Paris; Jacques Bert, M.D., Phillipe de Micco, Ph.D., M.D., Jacques Tamalet, M.D., University of Marseille, Marseille.

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COOPERATING UNITS: continued

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INDIA: M.S. Bhat, Department of Neurology, J.J. Group of Hospitals, Byculla, Bombay.

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PUERTO RICO: V. Mojica, M.D., V.A. Hospital, San Juan.

SCOTLAND: Alan Dickinson, M.D., Moredun Research Institute, Edinburgh; E.H. Jellinek, Neurological Unit, Northern General Institute, Edinburgh.

SWITZERLAND: F. Assaad, Medical Officer Virus Disease, World Health Organization, Geneva.

U.S.S.R.: Peter Rytik, M.D., Institute of Epidemiology and Microbiology, Byelorussian Research, Minsk; A.M. Gardashyan, M.D., Department of Immunology and Oncology, Gamelaya Institute, Moscow; V.I. Il'yenko, M.D., All-Union Research Institute of Influenza, Leningrad.

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YUGOSLAVIA: H. Vesenjok-Hirjan, M.D., Department of Virology, Medical Faculty, 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 encephalopathies.
- 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 infections 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 subacute 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 viral 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 XXVII: Study of mitochondrial 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 cysticercosis-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 auto antibodies 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 spongiform viruses on nerve cells grown in vitro.
- Sub-Project XXXVI: In vivo and in vitro studies to determine the etiology of myasthenia gravis.
- Sub-Project XXXVII: Neurophysiological study of animals experimentally infected with subacute spongiform virus encephalopathies.
- Project Description: Chronic Central Nervous System Disease Studies (described fully on pages
- Publications: Listed on pages 33 - LCNSS/IRP through 37 - LCNSS/IRP

The projects (I through XXXVII) 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 and the summary in pages 1-LCNSS/IRP through 18-LCNSS/IRP. Contractural phases of this work are being conducted at: Gulf South Research Institute, New Iberia, Louisiana; and Public Health Research Institute of New York, Otisville.

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ANNUAL REPORT

October 1, 1978 through September 30, 1979

Clinical Neurosciences Branch

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT

October 1, 1978 through September 30, 1979
Clinical Neurosciences Branch
National Institute of Neurological and Communicative
Disorders and Stroke

Susumu Sato, M.D., Acting Chief

Summary of Program Activity

As of June 30, 1979, Dr. Cosimo Ajmone-Marsan retired from the National Institutes of Health after 25 years service. Dr. Marsan became the Chief of the Electroencephalography and Clinical Neurophysiology Branch in 1954 and the Chief of the Neurosciences Branch following reorganization in 1975. The Branch has been the stage for his historical accomplishments that have led to its excellence in the field.

The activity of this Branch consists of research and clinical diagnostic service, that involves a total of 3.9 man/year (1 professional and 2.9 technical-clericals).

I. Clinical Diagnostic Service:

During this reporting period (6/1/78-6/14/79 inclusive), a total of 1008 electroencephalograms were obtained in patients who were referred to our Branch as part of their routine clinical investigation or for specific research projects from other Branches of our Institute or from other Institutes.

The distribution of these referrals according to the Institute of origin is as follows:

<u>Institute</u>	<u>No.</u>	<u>%</u>
NINCDS	512	50.8
(OPD)	(263)	(26.1)
NIMH	160	15.9
NICHD	73	7.2
NCI	94	9.3
NHLBI	63	6.3
NIAID	12	1.2
NIAMDD	74	7.3
NEI	3	0.3
MISC.	17	1.7
	<hr/> 1008	<hr/> .100.0

There is a slight decline in the total number of electroencephalograms as compared with that of the previous year. Almost half of the EEG

requests came from Institutes other than NINCDS. Among them, a significant number of recordings were performed at the patient's bedside on the Ward or in the intensive care unit. There were several referrals from the Employee Health Clinic. The service provided and continues to supply useful information for several research projects in our Branch which collaborate closely with other units, especially the section on Clinical Epilepsy of the Experimental Therapeutic Branch. It also provides material for the training in Clinical Electroencephalography so that each year one or two Clinical Associates become eligible for the American Board of Qualification in EEG.

II. Research Activity:

Of the 14 research projects mentioned in the previous report, nine had been completed or terminated due to retirement of the principal investigators. The current report includes a total of 5 projects, all of which represent the continuation of projects from the past.

a) Clinical

The analysis of clinical seizure patterns in different forms of epilepsy continues to be a main field of interest for this Branch. In a project still in course of completion, this analysis has been focused on epileptogenic processes involving the central-vertex, parasagittal region. Up to date sixty epileptic patients have been collected, 30 of whom showed typical epileptiform EEG discharges discretely localized to the central vertex region and 30 with pathologically documented (expanding or vascular) lesions in the same area. The various clinical features and seizure patterns of these two subgroups of patients are currently being analyzed and correlated. Although the principal investigators for this project left the Institute, the results of this study should be ready for publication in the near future.

In the previous report, a study of visual evoked potentials in patients with demyelinating disease and other neurophthalmological disorders was described and its results showed a high rate of abnormal VEPs in M.S. patients. Our Branch now has a renewed interest in this area of service and research, and continues to conduct the evoked response study.

A comprehensive neuropsychological test battery, consisting of standard and new experimental techniques was developed to assess cognitive and emotional defects associated with Huntington's Disease. In addition, the project was designed to study the feasibility of developing reliable diagnostic behavioral techniques to predict whether an 'at risk' individual may yield to Huntington's Disease.

The data was consistent with neurobehavioral research showing that HD patients are troubled by pervasive deficits in perception and memory, in solving visuomotor tasks and in utilizing spatial cues. Moreover, patients with mild, early stage symptoms displayed considerable difficulty in perceiving and encoding visual, auditory and tactile stimuli. With

specialized techniques, HD patients were impaired in recognizing forms presented dihapically and in recalling words delivered under special auditory conditions. Clinically, the HD patients complained about an inability to plan, organize and schedule personal and other activities.

In addition to the cognitive weaknesses, the HD patients were concerned about emotional and social changes. The personality tests depicted the HD patients as prone toward schizophrenic reaction, with depression. Embedded in the disorder were high anxiety, admitted concern about suicidal ideation and a variety of psychoeducational difficulties.

The second phase of the study, which deals with the establishment of valid and reliable predictors is being processed as additional normal subjects are evaluated.

b) Experimental Research:

For many years, the main interest of the section of Clinical Neurophysiology has been the pathophysiology of epilepsy and, specifically, the investigation of neuronal mechanisms which are at the basis of epileptiform activity. This interest is reflected in two experimental projects. These studies utilized the experimental model of focal epilepsy in cat by means of topical penicillin and were designed to investigate the mode of action of this epileptogenic agent. In the previous projects, it had not been possible to prove that such an action is primarily dependent upon a competitive antagonism between penicillin and GABA.

The present studies re-examined this aspect of the problem and utilized the multi-barrel micropipette technique for extracellular recording from cortical and hippocampal neurons before and following microiontophoretic application of various amino acids and of the epileptogenic agent in question.

Following topical penicillin application, the cerebellum and olfactory bulb demonstrated a decrease in both the GABA inhibitory effect and the endogenous inhibition, as compared to the lack of observable effects on the hippocampal units tested. However, surface interictal and ictal discharges developed in the hippocampus, but in neither the olfactory bulb or the cerebellum. Systemic penicillin injection was associated with an increased unit firing rate in the hippocampus and the cerebral cortex with minimal changes in the GABA inhibitory effects and endogenous inhibition. Penicillin iontophoresis suggested two mechanisms of action: a decrease of GABA induced and endogenous inhibition, and direct increase of excitability in neurons under investigation. These findings suggested a critical population of directly activated cells may be required to generate epileptiform activity, whereas the decrease of inhibitory activity might not have a main role in producing epileptiform activity.

These projects were completed and the results will be published soon.

Other Activities, Honors etc.

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00100-26 CN															
PERIOD COVERED October 1, 1978 thru September 30, 1979																	
TITLE OF PROJECT (80 characters or less) Epileptogenic Mechanisms in the Brain of Man and Other Primates																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 33%;">P.I.:</td> <td style="width: 33%;">J.M. Van Buren, M.D.</td> <td style="width: 15%;">Associate Chief</td> <td style="width: 10%;">CN</td> <td style="width: 9%;">NINCDS</td> </tr> <tr> <td></td> <td>C. Ajmone-Marsan, M.D.</td> <td>Chief</td> <td>CN</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>C.R. Lake, M.D.</td> <td>Clinical Associate</td> <td>LCS</td> <td>NIMH</td> </tr> </table>			P.I.:	J.M. Van Buren, M.D.	Associate Chief	CN	NINCDS		C. Ajmone-Marsan, M.D.	Chief	CN	NINCDS		C.R. Lake, M.D.	Clinical Associate	LCS	NIMH
P.I.:	J.M. Van Buren, M.D.	Associate Chief	CN	NINCDS													
	C. Ajmone-Marsan, M.D.	Chief	CN	NINCDS													
	C.R. Lake, M.D.	Clinical Associate	LCS	NIMH													
COOPERATING UNITS (if any) LCS, NIMH Division of Endocrinology, National Naval Medical Center																	
LAB/BRANCH Clinical Neurosciences																	
SECTION Functional Neurosurgery																	
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205																	
TOTAL MANYEARS: 2	PROFESSIONAL: 1.8	OTHER: 0.2															
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) <p>In support of the noted protocols, it was possible to record with the <u>floating microelectrode</u> in three patients since September 1977. No other protocols were supported due to lack of material. The cortical NADH fluorometry project has been cancelled due to lack of support.</p>																	

Project Description:

Objectives:

1. To study causal mechanisms of epileptic seizures in man and other primates.
2. To study the electrographic characteristics of epileptogenic activity in the brain of man and other primates.
3. To study the approved methods of surgical therapy for these lesions and develop new therapeutic methods.
4. To make use of opportunities in diagnosis and therapy for the study of neurophysiological and neuropsychological problems.

Methods Employed:

1. Clinical Neurological, contrast and radiologic examination.
2. Neurophysiologic examination (macro-and micro-electrode, NADH fluorometric methods).
3. Evaluation of changes in histology and CSF neurotransmitters.
4. Neuropsychological examination of speech and cognitive function.

Major Findings:

None

Proposed Course of Project:

This project was terminated because the principal investigators left the Institute.

Publications:

Van Buren, J.M., Lewis, D.V., Schuette, W.H., Whitehouse, W.C., and Ajmone-Marsan, C. (1978) Fluorometric observations in human epilepsy. Neurosurgery 2: 114-121

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 00200-25-CN

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Involuntary Movements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Fedio	Psychologist	CN NINCDS
OTHER:	T. Chase	Neurologist	ETB NINCDS
	C. Cox	Psychologist	CN NINCDS
	G. R. Frederick	Psychologist	CN NINCDS

COOPERATING UNITS (if any)

--Experimental Therapeutics Branch, NINCDS

LAB/BRANCH

Clinical Neurosciences

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.1

PROFESSIONAL:

0.6

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An emotional and cognitive profile of individuals with Huntington's Disease, or those classified as 'at risk' for Huntington's Disease was derived from comprehensive neuropsychological assessment. The evaluation extended into memory/learning and perceptual areas, and included personality and emotional measures, utilizing standard and experimental tasks. The investigation attempted to identify the intellectual and emotional traits of at-risk individuals, and with additional study, to develop reliable and sensitive predictive indicators for Huntington's Disease. The behavioral data will be collated with biochemical and neuroradiologic measures.

Evaluation of the 'at risk' individuals has been completed; normative data are being gathered.

Project Description:

Objectives:

To develop sensitive neuropsychological indicators in individuals classified as 'at risk for Huntington's Disease'. The project was designed to yield a three-fold purpose: 1) evaluate various perceptual, memory and behavioral defects associated with HD; 2) to provide an objective assessment of cognitive, emotional and behavioral dimensions for individuals who are classified as risk candidates for Huntington's Disease; 3) to establish a reliable diagnostic technique of being able to predict whether an 'at risk' individual may yield to Huntington's Disease.

Methods Employed:

A comprehensive neuropsychological test battery, comprised of standard and new experimental techniques was utilized. The major areas addressed in the project include personality assessment, anxiety and psychosomatic traits, and affective balance. In cognitive areas, standard psychometric tests of memory and intelligence were used, supplemented by laboratory measures of spatial orientation, high speed perception and attention, various learning and memory devices.

Major Findings:

The data were consistent with neurobehavioral research describing wide spread cognitive and emotional impairment in individuals with Huntington's Disease. HD patients showed pervasive deficits in perception and memory, in solving visual spatial integrative tasks, and in utilizing spatial directional cues. Moreover, patients with mild, early stage symptoms displayed considerable difficulty in perceiving and encoding visual, auditory and tactile stimuli.

With specialized techniques (tachistoscopic recognition), HD patients showed elevated thresholds in identifying verbal and patterned material. Similarly, using specialized interhemispherical competitive techniques, HD patients were impaired in recognizing forms presented dihaphtically, and in recalling words delivered dichotically. Clinically, the patients also complained about an inability to plan, organize and schedule activities and to remember; this pattern of deficits supports the hypothesis for alleged frontal disturbances.

In addition to cognitive deficits produced by Huntington's Disease, the patients presented troubled emotional and personality changes. The group showed abnormal profiles on the MMPI and other selected tests, schizophrenia and depression being identified as benchmarks of aberrant processes. Embedded in the disorder, HD patients also showed high overt anxiety, admitted concern about suicidal rumination and about a wide range of personal and social difficulties.

Significance to Biomedical Research and the Program of the Institute:

In view of the neuropsychiatric complications associated with Huntington's Disease, the project represents a behavioral approach to early identification of 'at risk' candidates. The data will be collated with neuroradiographic and biochemical assessments in an effort to try to develop a meaningful and reliable predictive indicator for individuals susceptible to Huntington's Disease. The research will also provide an empirical profile of individuals classified as 'at risk' and measure the impact of Huntington's Disease on personal-social and educational factors within the families.

Proposed Course of the Project: To secure and evaluate an adequate number of normal control individuals for purposes of statistical comparison with the 'at risk' population.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00304-24 CN
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PERIOD COVERED
October 1, 1978 thru September 30, 1979

TITLE OF PROJECT (80 characters or less)
Lesions Upon Function and Structure of the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J.M. Van Buren	Associate Chief	CN	NINCDS
OTHER:	R.C. Borke	Biologist	CN	NINCDS
	C. Sandri, Ph.D.	Acting Chief, EM Sect.	Institut für Hirnforschung	
	K. Akert, M.D.	Director	"	Zürich Zürich
	C.L. Li, M.D.	Physiologist	ET	NINCDS
	D. King, M.D.	Clinical Associate	CN	NINCDS
	W. Schuette, E.E.	Electrical Eng.	DRS	NIH

COOPERATING UNITS (if any)
Institute fur Hirnforschung, Zurich Switzerland
Experimental Therapeutics Branch, NINCDS; DRS, NIH

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.1	PROFESSIONAL: 1.1	OTHER: 1.0
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CHECK APPROPRIATE BOX(ES)
 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER
 (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) In the EM lab personnel training has progressed well, and in the last six months thin sections can be cut and stained reliably. 2) Work correlating fiber-size histograms and the action potential in several peripheral nerves of various structure has provided satisfactory material which is currently under analysis. 3) Late (18 month) changes in the pia-arachnoid membrane related to air exposure and low energy UV irradiation are being evaluated with material currently in process. 4) A serial section study of a case of chronic dyslexia following an occipital lobectomy with minor damage to the posteromedial extremity of the angular gyrus disclosed that the posterolateral inferior portion of the pulvinar is related to this cortical region. Previous anatomical studies showed in case of chronic receptive aphasia with a lesion in the supramarginal gyrus and posterior temporal cortex that the degeneration in the pulvinar affected the anterosuperior portion.

Project Description:

Objectives:

This project is directed toward the study of basic neuroanatomy and correlated neurophysiology making use, where possible of human pathological material and the opportunities afforded by the operative treatment of neurological disease.

Methods Employed and Major Findings:

Our hopes of being able to pursue topographical neuroanatomical studies using techniques to demonstrate specific enzymes (i.e., the glutamic acid decarboxylase label of Roberts) has not proved feasible due to lack of local personnel and the logistical difficulties of distant collaboration. Thus, we have turned to thin section and freeze-etch electron microscopy which seems within the capabilities of our personnel and facilities. The new construction will remove our large processing laboratory and our ability to serially section whole human brains. Training of personnel in thin section EM has continued and sections of research quality have been available on a regular basis for the past six months.

1. Correlation of fiber size spectra and the action potential.

Satisfactory material for histograms has been obtained in three animals with section and resuture of the saphenous nerve and tibial nerves from 6-15 months duration. In addition an animal with an unsutured saphenous section (12 months) and normal nerves has been processed (saphenous, tibial, vagus, and greater splanchnic).

The action potentials from all nerves have been recorded. Some 500-600 electron micrographs have been made suitable for preparing histograms (4800x for myelinated and 12,000x for unmyelinated) of fiber size. Construction of the fiber size histograms has just begun with the Zeiss particle size analyser.

Attention will be focused upon correlation of the characteristic action potentials and the fiber size histograms in the normal and resutured nerves. Since there has been no analysis of such correlations with the size of unmyelinated fibers and after regeneration, this aspect is of particular interest.

2. Studies of experimental meningocerebral cicatrix.*

In six cats the lateral gyri were exposed bilaterally, the right side irradiated for 60 minutes with low energy (355 nM) UV irradiation (200-700 $\mu\text{W}/\text{cm}^2$) and the other side simply exposed to air. There was equal irrigation with Ringer's solution on both sides. Two cats died over the months. In the three weeks which the freeze-etch apparatus was working in this lab, three of the cats were processed after perfusion. Thin sections through the same areas will also be correlated. This material remains to be prepared and photographed.

3. An anatomical study of a case of dyslexia.

A case of chronic dyslexia, dyscalculia, and dysgraphia was studied in which the thalamus and brain stem were available in serial section and selected levels of the left hemisphere were likewise prepared in myelin and cresyl-violet.

Examination of the left parieto-occipital region showed degeneration in the central portions of the external and internal sagittal strata. The tapetum was intact. Degeneration in the lateral geniculate body was complete except for a thin layer of retained cells along the anteromedial and the anterolateral aspects. This appeared related to the retention of the most anterior portion of the left calcarine cortex. In the pulvinar, there was degeneration in the posteroinferior pole which spread laterally to the external medullary lamina and the posteroinferior aspect of the n. ventrocaudalis (n. ventralis posterolateralis). In the paraventricular region of the pulvinar, a small area of degeneration spread forward to the posterior aspect of the n. medialis (n. medialis dorsalis).

In comparing this case with one previously reconstructed in which a chronic predominantly receptive aphasia followed a lesion of the supramarginal gyrus, the pulvinar degeneration associated with the receptive aphasia lay in the anterosuperior lateral portion of the pulvinar whereas a predominantly dyslexic speech defect was associated with degeneration in the posteroinferior lateral portion of the pulvinar.

Proposed Course of Project:

This project was terminated due to retirement of the principal investigator and his associates.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 00907-18 CN
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PERIOD COVERED
October 1, 1978 thru September 30, 1979

TITLE OF PROJECT (80 characters or less)

Regeneration of the Spinal Cord after Trauma (Previous Title: Regintegration and Regeneration after Trauma to the Brain and Spinal Cord in Man and Animals)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI.:	Ayub K. Ommaya	Acting Chief	CN	NINCDS
	Carrie Walters	Medical Officer	CN	NINCDS
Other:	James Reed	Clinical Associate	CN	NINCDS
	Lawrence Mononen	Staff Fellow	CN	NINCDS
	John Doppman	Chief	DR	CC, NIH

COOPERATING UNITS (if any)
Diagnostic Radiology Dept., Clinical Center, NIH

LAB/BRANCH
Clinical Neurosciences Branch

SECTION
Section on Applied Research

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A study of the use of peripheral nerve grafts, pulsed radiofrequency and other modalities to induce regeneration of the spinal cord in monkeys. An anatomical, physiologic, pathologic and neurologic study using histologic, neurophysiologic and clinical techniques.

Project Description:

Objectives:

A study of spinal cord trauma and regeneration in the rhesus monkey from an anatomical, histological, vascular, physiological and neurologic point of view with special emphasis on the use of peripheral nerve-grafts and other therapy which could affect the outcome of "complete" lesions treated at clinically significant intervals after the acute injury phase.

Methods & Materials:

A. Production of the spinal cord lesion: Standard laminectomy and extradural crush injury to the cord. Determination of the level depends upon the location of the feeding artery in the lumbar region, usually L1 or L2. The animals are then divided into 4 groups: 1. Crush only, 2. crush plus delayed peripheral nerve graft (PNG), 3. crush plus PNG plus electromagnetic energy therapy (EET), 4. crush plus EET only.

B. Peripheral Nerve Graft: At timed intervals of 1, 2, 3 and 4 weeks the dura and pia are opened using microsurgical techniques and the necrotic cord tissue is removed with microsuction. Segments of the monkey's own peroneal nerve are inserted longitudinally into the cavity. Care is taken not to disturb any vessels which were not destroyed by the initial injury. The pia is then closed using 10 "0" silk and the dura closed using 6 "0" silk. The wound is closed in layers using 4 "0" silk.

C. Electromagnetic energy via Diapulse: The Diapulse device generates radio waves at 27.12 mega-Hertz which is in the 11 meter band. Each pulse lasts 65 microseconds. The animals undergoing this form of treatment are treated for 30 minutes twice a day at settings of frequency = 400 and penetration = 6.

D. Spinal cord angiography: In collaboration with Dr. Doppman from the Department of Radiology, spinal cord angiography is performed pre-crush (control), immediately post-crush (to determine what, if any, adverse effects resulted from the surgery) and before the animal is sacrificed.

E. Sensory evoked responses and neurological status: Animals will be monitored weekly for any evidence of subclinical return of the sensory system and simultaneous serial neurologic examination will be made.

F. Histologic examination of the spinal cord cut in longitudinal sections:

- a. Luxol/PAS combination stain for myelin and axons.
- b. Gomori's Trichrome for connective tissue.
- c. H & E - routine stain.

G. Nursing care of the paraplegic rhesus monkey.

Much of our time and effort during the first 7 months was devoted to developing a satisfactory technique of nursing the paraplegic monkey. We first tried padding the primate chairs with commercially available foams such as "Rest On," "Bye, Bye Decubiti," etc. This was not successful. An attempt was then made to modify the chairs in such a way that they could rotate through an arc of 180° allowing us to position the monkey on his abdomen, on his back or anywhere in between. While this prolonged the life of the monkey, it still ultimately resulted in severe decubiti which in turn led to sepsis and death. The third attempt at postoperative nursing care appears to be the most satisfactory. This consists of a commercially available baby's playpen, well padded with foam rubber. We now have one monkey which has survived 3 months without any serious problems and a second monkey entering the 8th week.

Major Findings:

Seven monkeys have survived over twelve months. Four have received peripheral nerve grafts and three are controls. Evoked potential studies and histologic evaluations are in progress.

Proposed Course:

Due to administrative interference the future of this work is clouded.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01025-17 CN
PERIOD COVERED October 1, 1978 thru September 30, 1979		
TITLE OF PROJECT (80 characters or less) Tumors of the Nervous System		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
P.I.:	A.K. Ommaya	Acting Chief CN NINCDS
OTHER:	C. Walters	Medical Officer CN NINCDS
	J. Reed	Medical Officer CN NINCDS
	P. Kochie	Neuropathologist CN NINCDS
	P. Chretien	Senior Investigator SURG NCI
	D. Poplack	Pediatric Oncologist PO NCI
	D. Sadowsky	Statistician OBE NINCDS
COOPERATING UNITS (if any) Office of Biometry and Epidemiology, OD, NINCDS Surgery Branch, NCI; Radiation Oncology and Pediatric Oncology, NCI		
LAB/BRANCH Clinical Neurosciences Branch		
SECTION Section on Applied Research		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) A clinical study of the effect of <u>intratumoral chemotherapy</u> using 8-Azaganine compares this method given in combination with systemic CCNU after surgery and radiotherapy to the effect of adjuvant <u>chemotherapy</u> with CCNU given alone. In a pilot study, our methods have increased the mean survival time to approximately twice that reported by other centers using CCNU alone after surgery and radiotherapy. Dosage of therapeutic agents used for local intrathecal chemotherapy is being monitored in a primate model for chronic intrathecal chemotherapy. Our immunologic approach is currently embodied in a Phase I study of treatment with Thymosin. Based on in-vitro studies with Thymosin in our gliomas patients we have completed a Phase I study comparing the in-vitro effects of Thymosin Fraction V and Thymosin α 1 in 14 patients. Further work has been stopped due to administrative interference.		

Project Description:

Objectives:

1. To develop surgical and immunochemotherapeutic methods for the treatment of human malignant gliomas and other "inoperable" tumors of the nervous system.
2. To evaluate immunologic parameters of patients undergoing therapy of brain tumors.
3. To develop animal models suitable for evaluation of immunotherapy and chemotherapy trials.
4. To improve the quality and quantity of survival of patients with malignant brain tumors.

Methods Employed:

1. Microneurosurgical and intravital staining techniques to enhance maximal reduction of tumor cell mass.
2. Patients with histologically verified glioblastoma multiforme and malignant astrocytomas (Grade III and IV) are selected. The extent of neurological deficit and intracranial mass anatomy is established clinically and neuroradiologically, including evaluation by computerized axial tomography before and after maximal tumor resection and treatment with radiotherapy.
3. Cerebrospinal fluid reservoirs and intratumoral cysts are inserted to allow evacuation of tumor bed contents and for infusion of chemotherapeutic agents or agents to induce intratumoral delayed hypersensitivity reactions.
4. Patients are randomized into a prospective, controlled study to evaluate combined chemotherapy with CCNU and 8-Azaguanine versus chemotherapy with CCNU alone. (Both groups receive the tumor cyst implant).
5. The program of combined chemotherapy utilized oral CCNU, 130 mg/sq meter body surface, and intratumoral 8-Azaguanine, 100 mg. by infusion, the oral drug being given for 6 doses, one dose per 6-8 week period or until onset of liver or marrow disturbance. The intratumoral drug is given once a week for 6 weeks and then once a month for one year, then once a month indefinitely after that.
6. A murine glioma model has been developed which can reliably induce intracerebral tumors in mice and provide large numbers of cells for immunotherapy of that tumor. This animal model was also used to test varying combinations of immunotherapy, chemotherapy, radiotherapy and to check the

effect of splenectomy on tumor growth with and without therapy.

7. A model of human glioma in nude mice was being developed in collaboration with Dr. David Houchens of Battelle Institute, but did not receive fiscal support from IRP, NINCDS.

8. A model for chronic intrathecal chemotherapy in the rhesus monkey has been developed and studies of methotrexate and 8-Azaguanine toxicity are now under way.

9. Serial measurement of lymphocytes in tumor patients is being used to assess immune competence and to assay in-vitro the rationale of using Thymosin as an adjuvant immunotherapy.

10. Thymosin Fraction V and Thymosin α 1 have been given to 14 patients in a Phase I trial.

Major Findings:

Long term survival data on our glioma patients receiving local chemotherapy with 8-Azaguanine confirm this technique as being practical and productive of >75%, 2 year survival with no decrement in quality of survival. Our Phase I Thymosin Trial in 14 patients has shown no toxic effect in a 4 week exposure. Measures of cellular immunity after this therapy were encouraging.

Proposed Course:

Due to administrative interference the future course of our brain tumor studies has been stopped.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01245-14-CN												
PERIOD COVERED October 1, 1978 to September 30, 1979														
TITLE OF PROJECT (80 characters or less) EEG Learning Correlates Using Scalp and Intracranial Depth Electrodes														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">P. Fedio</td> <td style="width: 35%;">Psychologist</td> <td style="width: 15%;">CN NINCDS</td> </tr> <tr> <td></td> <td>W. Sheriff</td> <td>Computer Programmer</td> <td>TD NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>M. Buchsbaum</td> <td>Research Medical Officer</td> <td>AP NIMH</td> </tr> </table>			PI:	P. Fedio	Psychologist	CN NINCDS		W. Sheriff	Computer Programmer	TD NINCDS	OTHER:	M. Buchsbaum	Research Medical Officer	AP NIMH
PI:	P. Fedio	Psychologist	CN NINCDS											
	W. Sheriff	Computer Programmer	TD NINCDS											
OTHER:	M. Buchsbaum	Research Medical Officer	AP NIMH											
COOPERATING UNITS (if any) <p style="text-align: center;">Technical Development, NINCDS Adult Psychiatry, NIMH</p>														
LAB/BRANCH Clinical Neurosciences														
SECTION Office of the Chief														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) <p>Central processing of information by the human brain was monitored by averaged evoked response techniques. The electrographic recording of left and right brain activity during learning and perception in normal subjects was compared with that of neurosurgical patients. Suspect disturbances in brain-behavior relations in psychiatric patients were evaluated, relating left brain dysfunction to ideational disorders, right brain to emotional problems.</p>														

Project Description:

Objectives:

To identify brain mechanisms in man which regulate perception, and the storage and retrieval of information; to evaluate the significance of brain dysfunction in psychiatric patients by electrophysiological techniques.

Methods Employed:

A series of language and spatial tasks, designed to evaluate left or right brain processes, were used. Electroencephalographic (EEG) activity was recorded from scalp electrodes positioned over the posterior temporal-parietal regions of the left and right hemispheres. Included for study were neurosurgical patients who had undergone unilateral removal of the temporal lobe, and psychiatric patients exhibiting affective or ideational thought disorders.

Major Findings:

All electrographic test runs were conducted off-line and the evoked potential data for cognitive parameters is currently being processed. The study involving neuropsychiatric patients is in progress.

Significance to Biomedical Research and the Program of the Institute: Behavioral data available from epileptic patients following unilateral temporal lobectomy reveal significant perceptual and learning deficits which are related to the laterality of surgery and to the specific character of the material. The technique employed in this project affords a more precise method for outlining cortical and subcortical systems in the human brain which mediate learning and memory. The research also provides physiologic and behavioral data for the comparison of neurologic and psychiatric patients in order to identify possible brain dysfunctioning in schizophrenia or psychosis.

Proposed Course of the Project: A computer has been acquired, and will be programmed to provide off-line analysis of data. Specialized neuropsychological tasks will be developed, and applied in the study of patients with neuropsychiatric disorders.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01424-13-CN																
PERIOD COVERED October 1, 1978 to September 30, 1979																		
TITLE OF PROJECT (80 characters or less) Response Modulation by the Limbic System in Man: Neuropsychological and Physiological Changes																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">P. Fedio</td> <td style="width: 20%;">Psychologist</td> <td style="width: 30%;">CN NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>G. R. Frederick</td> <td>Psychologist</td> <td>CN NINCDS</td> </tr> <tr> <td></td> <td>C. Cox</td> <td>Psychologist</td> <td>CN NINCDS</td> </tr> <tr> <td></td> <td>A. Holtzman</td> <td>Psychologist</td> <td>CN NINCDS</td> </tr> </table>			PI:	P. Fedio	Psychologist	CN NINCDS	OTHER:	G. R. Frederick	Psychologist	CN NINCDS		C. Cox	Psychologist	CN NINCDS		A. Holtzman	Psychologist	CN NINCDS
PI:	P. Fedio	Psychologist	CN NINCDS															
OTHER:	G. R. Frederick	Psychologist	CN NINCDS															
	C. Cox	Psychologist	CN NINCDS															
	A. Holtzman	Psychologist	CN NINCDS															
COOPERATING UNITS (if any) None																		
LAB/BRANCH Clinical Neurosciences																		
SECTION Office of the Chief																		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205																		
TOTAL MANYEARS: 1.1	PROFESSIONAL: 0.6	OTHER: 0.5																
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) <p>Emotional and sensory characteristics are studied in patients with <u>temporal lobe epileptic foci</u>. Patients and raters independently complete true-false questionnaires which probe specific behavioral and emotional traits, and permit analysis of distortions in self perception. In a separate investigation, patients rate various <u>emotions</u> displayed by <u>facial</u> expressions, and learn words with different <u>emotional</u> connotations. <u>Temporal</u> epileptic patients are compared with matched normal subjects and patients with other neurologic illnesses. Patients with a right temporal focus are compared with left temporal epileptics. Statistical analyses are employed to codify behavioral and sensory profiles of right and left temporal epileptic subjects. The research examines the role of the anterior <u>temporal lobe</u> in establishing <u>limbic associations</u> and differences between the <u>left</u> and <u>right hemispheres</u> in regulating emotions and sensory experiences in man.</p>																		

Project Description:

Objectives:

1. To identify cognitive profiles of epileptics who have undergone unilateral temporal lobectomy, for the relief of intractable seizures; to evaluate relationships between seizure patterns and frequency, and cognitive and behavioral parameters.
2. To evaluate the role of the temporal lobe in 'emotional perception and learning'. A procedure was developed to assess how accurately temporal lobe patients identify various emotional states, and whether there may be a selective memory deficit for emotionally laden or neutral stimuli.

Methods Employed:

Specialized neuropsychological techniques and procedures are being designed and evaluated, and will include tests to identify perceptual and memory processes, the role of language encoding to facilitate recall, the adaptive strategy developed and utilized by brain-injured patients.

Two additional procedures, a verbal and nonverbal, were developed to assess emotional perception and memory. The verbal task consisted of 18 words, assigned to 3 emotional categories: neutral, positive and negative. A memory paradigm was employed.

The nonverbal task utilized human faces portraying a neutral state and various emotional expressions. The subjects were required to identify the emotions expressed by the faces.

Major Findings:

The study is being redesigned and has not yielded patient data. New procedures and techniques are being developed and will be submitted to a pilot study before actual testing with neurologic patients.

Significance to Biomedical Research and the Program of the Institute:
By identifying specific behavioral sequelae of a temporal lobe focus, these observations further neuroanatomical understanding of emotional processes. The results may be interpreted as a consequence of enhanced sensory-limbic associations. This interpretation regarding the effects of temporal lobe epilepsy in human subjects is consistent with extensive animal experimentation on sensory-limbic disconnections. The findings quantitatively support an asymmetry of emotional processing within the right and left hemisphere of man.

Proposed Course of the Project: Testing of additional psychiatric and neurologic contrast groups (nontemporal epileptics) is planned.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01658-12-CN												
PERIOD COVERED October 1, 1978 to September 30, 1979														
TITLE OF PROJECT (80 characters or less) Hemispheric Development and Specialization of Intellectual Functions														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">P. Fedio</td> <td style="width: 20%;">Psychologist</td> <td style="width: 30%;">CN NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>G. R. Frederick</td> <td>Psychologist</td> <td>CN NINCDS</td> </tr> <tr> <td></td> <td>J. L. Rapoport</td> <td>Medical Officer</td> <td>BP NIMH</td> </tr> </table>			PI:	P. Fedio	Psychologist	CN NINCDS	OTHER:	G. R. Frederick	Psychologist	CN NINCDS		J. L. Rapoport	Medical Officer	BP NIMH
PI:	P. Fedio	Psychologist	CN NINCDS											
OTHER:	G. R. Frederick	Psychologist	CN NINCDS											
	J. L. Rapoport	Medical Officer	BP NIMH											
COOPERATING UNITS (if any) Biological Psychiatry Branch, NIMH														
LAB/BRANCH Clinical Neurosciences														
SECTION Office of the Chief														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS: 1.6	PROFESSIONAL: 0.6	OTHER: 1.0												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) <p>The disabling effects of <u>brain damage</u> in man were evaluated on a broad range of <u>perceptual</u>, <u>learning</u> and <u>memory</u> functions. Changes in the intellectual behavior of neurologically handicapped individuals were evaluated before and after <u>brain surgery</u> and during <u>electrical stimulation</u> of the surface and depths of the brain. In contrast to these cases with confirmed brain injury, the effects of peripheral, <u>sensory deficits</u> were assessed in terms of possible neuropsychological dysfunctioning, and <u>communicative disorders</u>.</p>														

Project Description:

Objectives:

1. Outline brain mechanisms which support speech and language functions and code information to be held for immediate (short-term) or for delayed (long-term) memory; assess the effects of injury to these brain regions on communication skills and memory.
2. Compare the effects of brain injury with abnormal developmental conditions and assess neuropsychological pattern of specific psychiatric disorders, viz., obsessive compulsive disorders among children.
3. Examine the role of the temporal lobe in guiding visual behavior under altered or distorted conditions.

Methods Employed:

1. The laterality and general outline of cortical zones instrumental in basic speech and language functions were mapped by stimulation of the cortex during neurosurgical treatment of epileptic patients. The behavioral tests utilized photographs of common objects with instruction to name and remember the object.

Major Findings:

The ability to identify objects and to remember their names was studied during electrical stimulation of the exposed surface of the brain in patients undergoing surgery for the relief of epilepsy. This speech mapping procedure identifies by stimulation those cortical areas of the brain that are essential for the preservation of language.

The general findings conform to established observations that language and related verbal processes rely upon an intact left brain. In the posterior temporo-parietal regions (Wernicke's area) of the left hemisphere, we were able to map a distinct zone which is indispensable for identifying verbal material. There was a disruption of basic language processes, the patient being unable to name simple objects.

Additional comparisons between the disruptive effects of left and right brain stimulation showed different effects, the left being more readily disrupted than the right. There were interhemispheric differences to stimulation during speech mapping and pattern perception. Object naming was more disrupted by left brain stimulation than pattern discrimination was by right brain stimulation. Moreover, verbal memory was more easily upset by left brain stimulation than nonverbal memory by right brain stimulation. Taken together, these data suggest that comparable stimulation levels were more disruptive to left brain than right brain mechanisms during cognitive performance. This may indicate that the structure-function relations within each

hemisphere may be different, and the topographic neural organization of visuospatial functions on the right may demand a diffuse, less sophisticated system than the discrete, specialized verbal system on the left.

Significance to Biomedical Research and the Program of the Institute: These investigations contribute to the basic understanding of the development and organization of structural-functional relationships in the human central nervous system. This research advances clinical knowledge of the relationships between brain dysfunctions and amnesia, dysphasia, dyslexia and specific behavioral or adaptive responses.

Proposed Course of the Project: A battery of tests is being designed to examine adaptive strategies used by neurologic patients to compensate for visuomotor or language disorders. Visual and auditory tasks will be developed to further delineate immediate and long-term memory impairment in patients with lateralized cortical and subcortical lesions.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02213-04 CN
PERIOD COVERED October 1, 1978 thru September 30, 1979		
TITLE OF PROJECT (80 characters or less) Neuron Response to Axon Injury in the Immature and the Mature Rat		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT P.I.: Rosemary Borke Biologist CN NINCDS OTHER: M.W. Brightman, Ph.D. Section Head LNNS NINCDS		
COOPERATING UNITS (if any) Laboratory of Neuropathology and Neuroanatomical Sciences, NINCDS		
LAB/BRANCH Clinical Neurosciences		
SECTION Functional Neurosurgery		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.1	OTHER: 0.8
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The results indicate distinct differences with maturation upon the <u>neuronal glial reaction</u> of the <u>hypoglossal nucleus</u> to <u>axonal injury</u> in rats. Up to some ten days postnatal, the neuronal membrane engulfs the <u>presynaptic terminals</u> in contact with its membrane and the proliferative <u>perineuronal glial phase</u> is delayed to 1-3 weeks post-operative. In the mature neuron (21 days postnatal) the <u>microglia</u> proliferate earlier and lift the <u>presynaptic terminals</u> off the neuronal somata.		

Project Description:

Objectives:

1. To compare the ultrastructural features of retrograde responses to nerve crush, ligation and transection in young adult (21 days postnatal) and immature rat (7-10 days postnatal).
2. To study the sequence of events of the progressive changes in the perikaryon capacity to respond to axonal injury.
3. To study the ultrastructural mechanism by which neurons are switched to a different metabolic program from that operating during the cortical maturation period of neurons.
4. To compare the glial reaction to axon injury in immature and mature animals.
5. To correlate the cell body responses with axonal regeneration in young adults (21 days postnatal) and in immature animals (7-10 days postnatal).
6. To study the membrane events of the axon and cell body of neurons subjected to nerve crush, ligation, and transection.

Methods Employed:

1. Surgical techniques of ligation, nerve crush, and nerve transection of hypoglossal nerve in rats.
2. Transmission Electron Microscopy of the ultrastructural changes associated with axon regeneration and retrograde reaction of the cell body.
3. Electron staining using tannic acid to delineate alterations in surface membrane coats of synapses on soma and dendrites of hypoglossal nucleus cells.
4. Freeze fracture techniques of hypoglossal nucleus and nerves in normal and injured neurons to study the membrane events associated with the retrograde responses.
5. Neurophysiological stimulation techniques as needed to test functional regeneration of axons.

Results:

Albino male rats 7, 10, and 21 days old were subjected to hypoglossal nerve crush, ligation, and transection. The hypoglossal nuclei on the operated and nonoperated sides were compared after survival time

of 1-3 days, 7-13 days, and 20-40 days (total of 155 experimental animals to date). 84 control and sham operated animals of each postnatal age and corresponding post-operative survival have served as comparison. All animals were perfused and prepared for thin section EM and thick section 1 micron quantitative analysis.

Results indicate a progressive change in the character of cell body and glial response with neuronal maturity.

In the mature neuron (21 day animals) the microglia proliferate 1-3 days post-operatively and invade the hypoglossal nucleus to surround the reacting cell soma. Presynaptic nerve terminals are lifted off the neuronal membrane (1-7 days).

In immature neurons (7 and 10 day animals) the proliferative perineuronal glial phase is delayed (7-20 days) and during the early post-operative period (1-7 days) the neurons appear to send out cytoplasmic protrusions which surround dendrites and presynaptic terminals in contact with its membrane. The fate and time course of these protrusions is currently under study.

Neuronal soma and dendritic profiles demonstrating degeneration and concurrent regenerative features are present in the XII nerve nucleus on the side of the experimental lesion. These are thought to be morphologic manifestations of plasticity of the CNS.

Proposed Course of Project:

This project was terminated due to retirement of the principal investigator and associate.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02269-03 CN
PERIOD COVERED October 1, 1978 thru September 30, 1979		
TITLE OF PROJECT (80 characters or less) Photic Flash Visual Evoked Potentials in Clinical Neurology and Neuro-Ophthalmology		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT P.I.: L.E. Brody, M.D. Clinical Associate CN NINCDS S. Sato, M.D. Acting Chief CN NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Neurosciences		
SECTION Clinical Neurophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.2	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) An analysis of the morphology, amplitude and latency of <u>visual evoked potentials</u> in patients with various neurological and neuro-ophthoLogic problems will be made.		

Project Description:

Objectives:

The use of visual evoked potentials in the evaluation of patients with suspected clinical diagnosis of demyelinating disease and lesions in the optic pathway is wide-spread. The practical value of this diagnostic test is confirmed in this study.

Methods Employed:

Photic flash VEP tests have been performed in patients with various neurological diseases and data collection continues. Data is collected on-line using a CAT 1000 Signal Averager and plotted with an XY recorder.

Major Findings:

None

Significance to Biomedical Research and the Program of the Institute:

Evoked potentials are useful in the detection of occult lesions in the nervous system, and in establishing the presence of visual function in patients with extensive neurologic disease. The study of evoked potentials is still in a correlative stage. The extensive and varied patient population in NINCDS would provide an opportunity to study evoked potentials in variety of disease entities.

Proposed Course of Project:

This project will continue.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02270-03 CN
PERIOD COVERED October 1, 1978 thru September 30, 1979		
TITLE OF PROJECT (80 characters or less) Clinical and EEG Features in Cases of Midline (Parasagittal) Epileptiform Abnormalities and Pathologic Lesions		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT P.I.: C. Ajmone-Marsan, M.D. Branch Chief CN NINCDS N. Olmos-Lau, M.D. Clinical Associate CN NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Neurosciences		
SECTION: Clinical Neurophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.6	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) A correlative study of the clinical, electrographic and seizure patterns of 20 pathologically proven midline (<u>parasagittal</u>) <u>lesions</u> has been carried out. A similar group of 30 cases with electrographic interictal and ictal <u>epileptiform discharges</u> originating from the vertex region (CZ electrode) has been selected for comparison of their <u>seizure pattern</u> and behavior to differentiate this as a group from the <u>tumor</u> cases.		

Project Description:

Objectives:

To analyze and describe the clinical and electrographic manifestations in cases where midline electrographic EEG features are found and to determine if certain clinical clues can separate the cases with structural pathology.

Methods Employed and Major Findings:

The files of the EEG department were reviewed, 65 cases with possible CZ active epileptiform discharges were selected. A further review of the records reduced this number to 30 suitable cases with exclusive or primary discharges from the vertex. At this time an evaluation and analysis of the clinical seizure patterns and course is being done. These cases have been selected because of their absence of demonstrable pathology. Four patients underwent electrode implants, they will be described in greater detail. Another review of the EEG files yielded 30 cases referred with possible parasagittal pathology. A review of their records showed 18 cases with pathologically documented lesions and 2 cases with neuroradiologically demonstrated pathology. There were 14 tumors, 8 meningiomas, 6 gliomas, 4 vascular malformations, 1 trauma, and 1 degenerative disease. An analysis of their seizure patterns, EEG findings and clinical presentation has been done. These findings will be compared to those of the first group to determine if any definite conclusions in the comparison of the two groups can be drawn.

Significance to Biomedical Research and the Program of the Institute:

This study is providing useful information on the clinical spectrum of parasagittal seizures, their role of the EEG in the diagnosis of lesions in the parasagittal region and results of surgical treatment.

Proposed Course of the Project:

This project has been terminated because the principal investigators left the Institute.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02320-02 CN
PERIOD COVERED October 1, 1978 thru September 30, 1979		
TITLE OF PROJECT (80 characters or less) The Mechanisms of the Epileptogenic Action of Penicillin in Different Neuronal Structures with Reference to Inhibitory Mechanisms		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT P.I.: T. Yamauchi, M.D. Visiting Scientist CN NINCDS S. Newman, M.D. Research Associate CN NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Neurosciences		
SECTION Clinical Neurophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.2	PROFESSIONAL: 1	OTHER: 1.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Feline hippocampal pyramidal cells were studied with compound multibarrel micropipettes using the technique of combined <u>penicillin (PCN)</u> and <u>amino acid iontophoresis</u> . PCN iontophoresis increased the unit firing rate in most cases, and decreased the duration of electrically induced <u>post-discharge inhibition</u> . Conversely, following topical PCN-induced interictal surface discharges, unit firing was inhibited for a longer duration (2-10sec) than following electrically induced post-discharge inhibition (50-370msec). <u>GABA suppressed D-l-homocysteic acid</u> induced and spontaneous unit firing, but failed to completely suppress topical PCN-induced unit firing occurring between surface interictal discharges.		

Project Description:

Objectives:

To determine the mechanisms of PCN induced epileptogenesis by various routes of PCN administration in several inhibitory neuronal structures.

Methods Employed:

Extracellular single unit activity was recorded from feline hippocampal pyramidal cell with compound multibarrelled micropipettes. D-1-homocysteic acid (DLH) was applied iontophoretically to stimulate cell activity. PCN was applied by iontophoresis and/or topically to the surface of hippocampus, and interaction with GABA was examined.

Major Findings:

Extracellular recordings of 64 hippocampal cells predominantly located in the CA₁ region from 30 cats were analyzed.

After topical penicillin application to the hippocampal surface paroxysmal discharges began to appear within 6 min, and gradually become more regular in occurrence and more stereotyped in form than in the initial stages. Changes in the unit firing rate were examined during the whole course of developing epileptiform activity. The firing rate initially decreased in the majority of cells, and failed to increase even during the periods immediately preceding interictal and ictal surface events.

The unit responses to both intermittent and continuous GABA iontophoresis was a depression in unit firing incidence, except for the bursts patterns associated with the surface interictal or ictal discharges.

The duration of endogenous inhibition was minimally and transiently increased immediately after topical penicillin application. However, no change in inhibition was noted after these initial effects.

These results suggest that the large pyramidal cell may not have a main role in primary production of epileptiform activity, and also support the proposed requirements of an activated small cell population for epileptogenesis.

Proposed Course of the Projects:

This project has been completed. The results will be published soon.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02321-02 CN
PERIOD COVERED October 1, 1978 thru September 30, 1979		
TITLE OF PROJECT (80 characters or less) Prolonged Penicillin Iontophoresis and Evolution of Interictal Discharges in Somatosensory Cortex		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT P.I.: S. Newman, M.D. Research Associate CN NINCDS T. Yamauchi, M.D. Visiting Scientist CN NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Neurosciences		
SECTION Clinical Neurophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.2	PROFESSIONAL: 1	OTHER: 1.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The response of cells from the feline <u>somatosensory cortex</u> was investigated with compound multibarrel micropipettes filled with amino acids (<u>D-1-homo-cysteic acid</u> (DLH), GABA) and <u>penicillin</u> (PCN). Drugs were applied by <u>iontophoresis</u> . Typical unit spikes derived from 0.3mm to 2.0mm of cortex were excited by DLH and inhibited by GABA. Although topical PCN application resulted in classical surface interictal and ictal discharges, cataionic iontophoresis of PCN (100nA) most commonly produced the following responses: 1) Initially a decrease in unit firing rates. 2) Return to the pre-PCN unit firing rate (3-10 min). 3) Development of grouped unit spiking with longer duration PCN iontophoresis (10-20 min). 4) Transient increase in firing rate following the end of PCN iontophoresis. The unit response pattern to PCN was not clearly related to electrode configuration, form, tip size, ejecting current, carrier ions, or pH. Therefore, the iontophoretic characteristics of PCN effecting its release were evaluated.		

Project Description:

Objectives:

To determine the mechanism of action of PCN-induced epileptogenesis by investigation of single cell responses preceding and during development of interictal surface discharges.

Methods Employed:

Simultaneous surface and extracellular microelectrode recording from feline somatosensory cortex using compound multibarrel micro-pipettes filled with DLH, GABA, NaCl, and PCN were performed. Unit spikes were affected by iontophoresis of DLH and GABA over short-time periods. PCN low-dose iontophoresis was continued for an average 20-30 min.

Major Findings:

Extracellular recordings from 96 hippocampal cells located predominantly in the CA₁ region of 56 cats were analyzed.

Penicillin iontophoresis reversibly increased the firing rate of 49 of 52 cells within 3 min after beginning penicillin iontophoresis. In 40 of 52 cells so tested, iontophoretically applied GABA almost completely suppressed cell firing. Within 1-2 min after the onset of penicillin iontophoresis, cell firing began again, and the rate gradually increased. This also occurred during continuous GABA iontophoresis.

Intermittent GABA iontophoresis during continuous penicillin iontophoresis permitted calculation of changes in the time for 50% inhibition of firing rates. GABA-induced inhibition was decreased; however, this was not closely correlated with the increased cell firing rates induced by continuous penicillin iontophoresis. Furthermore, electrically induced stimulation of pyramidal cells was accompanied by a decrease in the duration of endogenous inhibition during the first 2-3 min of penicillin iontophoresis, probably mediated by the axon collaterals of basket cells.

Iontophoresis of penicillin suggests 2 mechanisms of action may occur: 1) a decrease of inhibition, and 2) a direct increase of pyramidal cell excitability. The exceptional occurrence of a surface epileptiform discharge supports the concept that the nonspecific antagonism of penicillin versus GABA inhibition action may not be the essential factor, but that a critical population of directly activated cells may be required in order to generate overt epileptiform phenomena.

Proposed Course of the Project:

This project has been completed. The results will be published.

Project Description:

Objectives:

1. To study the mode of recovery of cognitive and other higher integrative functions of the central nervous system after trauma.
2. To discover the mechanism controlling the mode of recovery which is hypothesized to be the neural basis for cognitive coding.
3. To apply the knowledge gained in 1 and 2 above to improve the quality of recovery after injuries to the central nervous system.

Methods Employed:

1. Serial neurologic and neuropsychologic testing of verbal and non-verbal communicative behavior.
2. Serial auditory, visual and somatosensory testing using standard clinical, audiologic and evoked potential techniques.
3. Serial documentation of morphologic-physiologic changes in the brain using transmission and emission computerized tomography.
4. Intraoperative neurophysiologic and neuropsychologic testing of cortical functions in patients undergoing surgery for brain tumors and other focal lesions.
5. Development of a computer based 3-dimensional graphic technique for visualization of patterns of brain functional changes as measured by methods 2,3 and 4 above.

Major Findings:

1. A unified hypothesis for cerebral asymmetry and cognitive functions has been developed and is to be tested in our proposed studies.
2. Clinical observation on the mode of recovery of patients with head injury have supported our centripetal theory of cerebral concussion. Such observations have also indicated that the recovery process appears to recapitulate the ontogenetic as well as some of the phylogenetic aspects of development of neural functions.

Significance to Biomedical Research and the Program of the Institute:

There have been no previous systematic studies of the mode of reintegration of cognitive and communicative functions after neural trauma. This study will be the first to provide such data and form the basis of an inquiry into the nature of cognitive coding in the brain.

Proposed Course:

Due to administrative decisions the future of this program is bleak.

Publications: None

ANNUAL REPORT

October 1, 1978 through September 30, 1979

Developmental and Metabolic Neurology Branch
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT

October 1, 1978 through September 30, 1979
Developmental and Metabolic Neurology Branch
National Institute of Neurological and Communicative Disorders and Stroke
Roscoe O. Brady, Chief

Our approaches to the understanding and treatment of neurological disorders prospered in several important directions during FY 79. Major research efforts of the Branch were directed along the following four lines. 1. Basic and clinical investigations of the metabolism of complex lipids and mucopolysaccharides in normal and pathological conditions. 2. The organization and function of lipids in cell membranes and their role as biotransducers for agents that regulate cell growth. 3. Characterization of myelin glycoproteins and their role in brain development and in demyelinating diseases. 4. The function of enzymes on the external surface of cells with regard to the pathogenesis of traumatic shock. The salient accomplishments in these areas in the past year are summarized in this report.

I. INHERITED METABOLIC DISEASES

A. Enzyme Replacement Therapy for Lipid Storage Diseases

Results obtained in our continuing investigation of enzyme replacement therapy indicate that this modality will eventually become a realistic procedure for the treatment of heritable metabolic disorders such as Gaucher's disease. This conclusion is supported by improvement of the clinical condition of two boys with Gaucher's disease who were injected with purified human placental glucocerebrosidase. In the case of the younger boy, this salutary effect was independently observed by the child's pediatrician, Dr. Margaret Hilgartner, Professor of Pediatrics at New York Hospital (quoted with Dr. Hilgartner's permission). On the basis of these encouraging findings, we have undertaken a long-term prospective course of enzyme infusion at bimonthly intervals in these patients. The intravenously administered enzyme has not caused an un-toward reaction in the recipients, and highly sensitive tests indicate that antibodies were not produced against the enzyme.

In related experiments conducted during the past year, we discovered that pretreatment of Gaucher patients with corticosteroids for a brief period prior to infusion of the enzyme markedly enhanced the beneficial effect of the enzyme. In the two adult females with Gaucher's disease who were treated in this fashion, there was a 30 and 47% reduction of accumulated glucocerebroside in their livers, respectively. A similar decrease of glucocerebroside in the circulation was also observed. Treatment with steroid alone was without effect. These reductions in the quantity of the pathological lipid are the largest we have obtained to date. We anticipate that further decreases of accumulated lipid will be obtained by this regimen and we expect that evidence of improvement of the patients' clinical status will follow.

B. Delivery of Enzymes to the Central Nervous System

We are continuing our investigation of temporarily altering the blood-brain barrier so that enzymes can gain access to the brain in order to treat metabolic disorders that affect the central nervous system. Studies with monkeys indicate that opening the barrier is feasible in primates, and in fact, it appears that it can be accomplished with greater facility in these animals than in the rodents previously used because of well-developed blood vessel catheterization procedures. We are currently monitoring the physiological and anatomical effects of various barrier modification schedules to assess the reasonableness of this procedure for therapeutic trials in humans. So far, we have not observed pathologic changes in the brains of the experimental animals.

A second important observation made in FY 79 was that neuronal cells, and apparently only these cells in the central nervous system, have high-affinity receptors on their surfaces for hexosaminidase A, the enzyme lacking in Tay-Sachs disease. The presence of these receptors greatly increases the likelihood that exogenous hexosaminidase A will be taken up by these cells. This finding provides great encouragement for pursuing enzyme replacement trials in metabolic disorders that involve the nervous system.

C. Mucopolysaccharide Storage Diseases

Two principal new findings were made in the Branch during the past year in this area. The first was the discovery that in addition to the accumulation of mucopolysaccharides in nerve cells of patients with these disorders in every instance where the central nervous system was damaged, there was an accompanying pathologic accumulation of glycolipids, particularly gangliosides. Ganglioside accumulation has long been known to cause brain damage in disorders such as Tay-Sachs disease. The quantity of gangliosides that accumulate in patients with mucopolysaccharidoses such as Hurler's disease, Hunter's disease and Sanfilippo disease was of the order of magnitude that occurs in the lipid storage diseases. The gangliosides probably accumulate in the tissues of patients with mucopolysaccharidoses because the latter substances interfere with the enzymes required for the breakdown of gangliosides. Ganglioside accumulation was seen in patients with mucopolysaccharidoses only where brain damage had occurred. It therefore seems likely that this ancillary accumulation of gangliosides plays a significant role in the neuronal damage that occurs in these disorders. This demonstration has important implications for the development of successful strategies for the treatment of mucopolysaccharide storage diseases.

The second major contribution in this area was the pharmacological production of an animal model with mucopolysaccharidosis. It was found that the injection of Suramin, a trypanocidal drug in wide use throughout the world, causes brain damage in rodents. There was a 5 to 6-fold increase in the quantity of mucopolysaccharides in the brains of these animals plus an accumulation of gangliosides analogous to the

situation in patients with mucopolysaccharide storage diseases. Suramin was shown to inhibit the activity of iduronate sulfate sulfatase, a key enzyme in mucopolysaccharide degradation. The activity of this enzyme is deficient in patients with the Hunter syndrome. The availability of this animal model should greatly facilitate studies on the pathogenesis of the mucopolysaccharidoses and the development of enzyme replacement procedures for these disorders.

II. CELL MEMBRANE RECEPTORS FOR ENVIRONMENTAL SIGNALS

We have continued our investigations on the biological roles of gangliosides in cell membranes. These compounds are information transducers for external signals that affect the metabolism and growth of cells. Gangliosides are particularly well suited for this activity since their carbohydrate chains extend out from the cell surface while the remainder of the molecule is anchored in the lipid bilayer of cell membranes. The velocity and extent of response of cells to various external factors is a function of the type and number of ganglioside molecules in their membranes and the efficiency of coupling of gangliosides to cytoskeletal components. An important function of gangliosides discovered in collaboration with investigators in NIDR is the interaction of these glycolipids with the external cell protein called fibronectin (cell surface protein). Fibronectin anchors cells to collagen matrices. The connecting links between the collagen-fibronectin complex and the cell membrane appear to be certain higher ganglioside homologues. These particular gangliosides are precisely the substances that we previously demonstrated to be lacking in the membranes of tumorigenic virus-transformed cells and cells made neoplastic with chemical carcinogens or X-irradiation. Thus, we now appear to be in position to explain the following well known properties of transformed cells: (i) the lack of adhesion of these cells to substrata; (ii) the increased motility of transformed cells; and (iii) their tendency to grow in much higher cell density than non-transformed cells. This finding is of impressive significance in considering the pathologic changes in the social behavior of malignant cells.

III. MULTIPLE SCLEROSIS

We believe we have achieved a major breakthrough in multiple sclerosis in FY 79. The prediction in previous reports from the Branch that the myelin-associated glycoprotein (which was discovered by this laboratory), may be early involved in the destructive process in multiple sclerosis received extraordinarily strong confirmation during the past year. This glycoprotein was found to be highly antigenic and high antibody titers were raised to this material. In a collaborative study with LNNS, the antibody was used for immunocytochemical localization of the myelin-associated glycoprotein. The glycoprotein was found to be located between the axolemma and the inner part of the myelin sheath of nerves. In studies with tissue sections obtained from brains of patients with multiple sclerosis, it was found that this glycoprotein is the first detectable brain component to disappear at the periphery of the expanding multiple sclerosis plaque. The destruction of this compound occurs even in areas of the brain that appear histologically normal. The high susceptibility of myelin glycoprotein to breakdown under these conditions suggests that it is likely to be intimately involved in

the pathogenesis of multiple sclerosis. Elucidation of the factors that cause its destruction should provide important clues towards understanding the cellular pathology of multiple sclerosis.

IV. TRAUMATIC SHOCK

Work on the activity of enzymes on the external surfaces of cells has indicated a potentially important role for these ectoenzymes in maintaining normal physiology. In particular, studies have indicated that the level of adenosine triphosphate (ATP) becomes pathologically elevated in plasma of patients in traumatic shock. When the quantity of ATP in the plasma exceeds the capacity of enzymes on cell surfaces to break this material down, an irreversible increase in cell permeability occurs causing still further release of ATP perpetuating the pathological process. If this observation is substantiated by further experimentation, it seems likely that infusion of enzymes that destroy ATP (ATPases) may have therapeutic value in the treatment of shock.

V. CONCLUDING STATEMENT

During the year important contributions were made to the etiology and therapy of inherited metabolic disorders; a realistic explanation was provided for the pathologic growth pattern of tumorigenic cells; a potentially extremely important discovery was made concerning the destruction of a brain component in multiple sclerosis; and insight has been obtained in the pathogenesis of traumatic shock. I therefore feel that research objectives of the Branch were achieved in a particularly successful fashion in FY 79.

PERIOD COVERED
 October 1, 1978-September 30, 1979

TITLE OF PROJECT (80 characters or less)
 Inborn errors of metabolism, cerebral degeneration and brain dysfunction of diverse etiology.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John A. Barranger, M.D., Ph.D., Acting Chief, Clinical Investigations and Therapeutics		DMN NINCDS
Other:	George Constantopoulos, Ph.D.	Staff Biochemist	DMN NINCDS
	Charles Chang, M.D.	Clinical Associate	DMN NINCDS
	Jan K. Steusing	Research Assistant	DMN NINCDS
	Nancy Krett	Guest Worker	DMN NINCDS
	Roscoe O. Brady, M.D.	Chief	DMN NINCDS

COOPERATING UNITS (if any)
 None

LAB/BRANCH
 Developmental and Metabolic Neurology Branch

SECTION
 Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION
 NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.7	OTHER: 0.1
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project incorporates a large number of diverse conditions, ranging from inborn errors of metabolism, to cerebral degenerations, to the brain dysfunction of diverse etiology. The unifying factors are cerebral involvement, determination of pathogenesis and therapeutic trials. The methodological approach includes clinical, and genetic appraisal, brain pathology through cerebral biopsies when indicated, post mortem obtained material, tissue culture as well as bio-chemical determination of content and composition of basic compounds and estimation of respective enzyme activities.

Project Description:

Objectives: The majority of chronic neurological disorders are first recognized during childhood. Because of the chronicity and long duration of the handicaps these diseases constitute a formidable medical and social problem. Taken together, mental retardation (frequently familial) birth defects, cerebral degenerations and inborn errors of metabolism affecting the nervous system amount to over four million people in the U.S.A. Our main objectives are: 1) to study the pathogenesis and etiology of these diverse disorders which are frequently of genetic origin, 2) to devise special diagnostic tests including identification of heterozygotes, 3) to institute therapeutic modifications of the respective disorders, 4) explore preventive measures and prenatal diagnosis.

Patient Material: Total of 98 inpatients and 45 outpatients were studied. Patients with the following disease categories were admitted for investigation: sphingolipidoses, mucopolysaccharidoses, ceroid lipofuscinosis, various somatic hereditary syndromes, familial mental retardation, spinocerebellar degeneration, and congenital pyruvic and lactic acidosis.

Methods Employed:

- 1) Neurologic, developmental and genetic assessments of the patients were made, including family study when appropriate.
- 2) Determination of profiles of lipids, amino acids, proteins, mucopolysaccharides and carbohydrates in various tissues, and when appropriate, in urine and cerebrospinal fluid.
- 3) Assay of enzyme activities in the peripheral blood leukocytes and platelets of genetic diseases studied; also, preparation of the karyotypes using banding techniques.
- 4) Establishment of skin fibroblast tissue cultures in patients with genetic disorders for study of enzyme activity and turnover studies using radioactive substances. In the case of certain sphingolipidoses and mucopolysaccharidoses, radioactively labelled substrates or elements are added to respective tissue cultures. In the first instance the catabolism, in the second, synthesis of the respective involved substances are followed.
- 5) Employment of invasive techniques, if required, for definitive diagnosis. Brain and liver biopsies are performed and the tissues are used for biochemical, chemical, enzymatic and electron microscopic studies.
- 6) Therapeutic modification of the diseases is attempted whenever possible. For this purpose we use pharmaceuticals, hormones, plasma or formed blood elements transfusion, dietary modifications and, where appropriate, enzyme replacement.
- 7) In case the patient with a metabolic disease dies, samples of the organs and other tissues are stored frozen for future chemical and enzymatic studies; the fresh specimens of tissue are immediately fixed or processed for histochemical and electron microscopic studies.

Major Findings:

For the past 10 years a considerable portion of our efforts and resources were directed to elucidation of pathogenesis of mucopolysaccharidoses. The most important contributions have been in 2 areas, 1) determination of the content and composition of mucopolysaccharides in the body fluids and tissues of patients with different types of this disorder; 2) the demonstration of glycolipid abnormalities in those types of mucopolysaccharidoses that have mental retardation as a complication. The material was obtained by biopsy or at autopsy.

- 1) Multidisciplinary studies were conducted on the brain and other tissues of patients who died with the antemortem diagnosis of mucopolysaccharidoses (MPS) of one of the following types: Type IS, Scheie disease; type I, Hurler disease (MPS-I); and type II, Hunter disease. The principal new finding in the brain of the patient with MPS-IS is the presence of lesions in the periadventitial mesenchymal tissue of the white matter, similar to those of MPS-I, while the nerve cells in MPS-IS are histologically normal, in contradistinction to MPS-I, in which the neuronal abnormality is severe. Electron microscopical studies of the brain in MPS-I demonstrated numerous complex membranous inclusions in the neurons, whereas the neurons in MPS-IS contained only a small number of lipofuscin-like inclusions and typical lipofuscin granules. There was a three-fold increase of glycosaminoglycans (GAG) in the brain of MPS-I, but only a slight increase in the MPS-IS; GAG in the liver and spleen of all patients was noticeably increased. α -L-iduronidase activity was not detectable in the brain and liver of patients with MPS-I and MPS-IS, thus suggesting a similar enzymatic defect.
- 2) Histochemical and electron microscopic studies of the brains including leptomeninges with large blood vessels from 7 patients with MPS I, II, IIIA and IS showed marked increase in mesenchymal elements and generalized presence of characteristic lesions around cerebral veins and arteries. The periadventitial space was greatly distended and filled with viscous fluid and numerous mononuclear cells containing large cytoplasmic vacuoles; these cells stained positively for glycosaminoglycans (GAG). In contrast, the neurons showed only a slight increase of GAG over the normal controls but contained an excessive amount of glycolipid-like material. The amount of GAG in the leptomeninges including large blood vessels was 10.8, 6.5, 4.5 and 2.2 times greater in patients with MPS I, II, IS and IIIA respectively, than the mean from the unaffected controls. Dermatan sulfate (DS) accounted for most of the GAG increase in MPS-I, II and V (mixed excretors of DS and HS), and HS in MPS-IIIA (HS excretor). We conclude that the mesenchymal elements contribute substantially to the content of GAG, as dermatan sulfate, in the combined brain tissue.

- 3) Concentrated efforts were made to introduce therapeutic modifications of selected inborn errors of metabolism. In an attempt to delineate the pathogenesis and clinical variability of the sphingolipidoses, and in order to proceed logically with therapeutic modalities, principally enzyme replacement, these disorders have been studied in depth. Gaucher's disease has been particularly closely scrutinized. Suggestions from the literature and observations of our patients have prompted us to investigate the significance of disturbances of liver function, lung function, immune response, cardiac function, and reticuloendothelial function. Specifically, correlation of the severity of disease and angiotensin converting enzyme levels, pulmonary function testing, pulmonary macrophage concentration of storage product, clotting abnormalities, macrophage mobility, mitogenesis of white cells, Kupffer cells phagocytic capacity, electroencephalographic changes, hepatic blood flow, BSP uptake, and degenerative changes of the eye among other parameters have been assessed. Results of some of these studies are cited in appropriate listed publications.
- 4) The ability of the liver to clear intravenously administered enzyme depends upon specific receptor sites which recognize the carbohydrate portion of the glycoprotein enzyme. Hepatic parenchymal cells and Kupffer cells have different receptors in the sense that they are specific for different carbohydrate units, e.g., galactose, N-acetylglucosamine etc. We have been most interested in identifying the carbohydrate keys on the enzymes responsible for directing it to specific cell types. In the case of the enzyme in Gaucher's disease, we have shown that exposing galactose terminals hidden in the molecule by sialic acid results in directing the enzyme to hepatic parenchymal cells. Further modification of the molecule by removing single sugar moieties results in improving uptake by Kupffer cells. This is important in Gaucher's disease, for example, where the storage is predominantly in Kupffer cells. Other methods of directing the activity of infused enzymes to specific cell types are being investigated including the use of liposomes, red cell ghosts, and binding of the enzymes to specific carbohydrate carriers.
- 5) Patients presenting with myoclonus are being investigated. Four patients with the diagnosis of Lafora body disease have been identified. Clinicopathologic correlation has been made. The diagnostic value of the liver pathology has been confirmed. The nature of the biochemical defect is being investigated. Preliminary characterization of the storage material in liver and identification of a previously unknown urinary polysaccharide have been accomplished.
- 6) Patients with ataxia are being investigated for biochemical disorders. Two patients with ataxia have been demonstrated to have "ragged red fibers" in their muscle mitochondria. The precise biochemical lesion is being pursued. Other cases of hereditofamilial ataxia currently

being actively tested are pyruvate dehydrogenase deficiency, hexosaminidase variants, and other variants of the sphingolipidoses.

- 7) Development of an in vitro model of enzyme replacement is being studied. Currently being investigated is the usefulness of pulmonary macrophages and Kupffer cells isolated from Gaucher patients and sustained in culture.
- 8) Modification of the blood-brain barrier results in the entry of macromolecules such as enzymes into brain interstitial fluid. We have further demonstrated that catalytically active enzymes are taken up and incorporated into the lysosomes of neurons and to some extent glia. The possibility of enzyme replacement in the central nervous system is being investigated. Furthermore, the receptors on neurons for macromolecules are being described. Studies designed to describe the processing of macromolecules by brain are being carried out.

Significance to Bio-Medical Research and the Program of the Institute:

Because the majority of infections affecting man are now under quite satisfactory control, the time has come for increased attention to accord a measure of control to such common disorders as hereditary diseases, congenital malformations, mental retardation and degenerative conditions affecting the nervous system. Improved methodology makes it now feasible to advance our knowledge and institute some control in certain of these crippling chronic disorders. Prevention and therapy include prenatal diagnosis, enzyme infusion, dietary modifications and institution of certain eugenic measures. Since many of the disorders affect exclusively or predominantly the nervous system the study of etiology and pathogenesis as well as institution of therapeutic trials are of importance in furthering the main mission of our Institute.

Proposed Course of the Project: During the next years increasing emphasis will be given to the underlying genetic mechanisms of the hereditary diseases and to therapeutic modifications of respective disorders. We expect to evaluate the usefulness of complexed and immobilized enzymes in the treatment of various diseases. Further, the possibility of plasmapheresis as therapeutic tools in storage disorders will be investigated.

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PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Metabolism of Complex Lipids of Nervous Tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	A. E. Gal	Organic Chemist	DMN	NINCDS
	J. W. Kusiak	Senior Staff Fellow	DMN	NINCDS
	F. S. Furbish	Senior Staff Fellow	DMN	NINCDS
	J. A. Barranger	Acting Section Head	DMN	NINCDS

COOPERATING UNITS (if any)

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Tufts University Medical School, Boston, Massachusetts
National Center for Toxicological Research, Jefferson, Arkansas

LAB/BRANCH

Developmental & Metabolic Neurology Branch

SECTION

Enzymology and Genetics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

6.6

PROFESSIONAL:

5.6

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Procedures are developed for the purification of enzymes from tissues such as human placenta that are lacking in patients with Gaucher's disease, Fabry's disease, Tay-Sachs disease and Niemann-Pick disease and mannosidosis. The effects of enzyme replacement therapy in patients with these disorders is under investigation. Procedures are developed for the diagnosis of patients with these disorders, the detection of heterozygous carriers of these genetic traits, and for the detection of heterozygous carriers of these genetic traits, and for the monitoring of pregnancies at risk for each of these diseases.

Project Description:

Objectives: (1) To elucidate the biosynthetic pathways for the formation of long chain fatty acids, cerebroside, ganglioside and sphingomyelin; (2) to study the control mechanisms which regulate these processes; (3) to study the metabolic fate of sphingolipids in normal and lipodystrophic disease states, and (4) to provide diagnostic and therapeutic procedures for the amelioration and control of the lipid storage diseases.

Methods: Glucocerebroside and galactocerebroside labeled with ^{14}C in either the hexose or fatty acid portion of the molecule have been synthesized. ^{14}C -labeled sphingomyelin and gluco- and galactopycholine have been similarly prepared. Ceramide-trihexoside and ceramide tetrahexoside (globoside) specifically labeled with radioactive hydrogen- ^3H have been prepared. The metabolism of these labeled materials has been investigated *in vivo* and *in vitro*. Human placenta has proved to be a convenient and rich source of sphingolipid hydrolases. Isolation of these enzymes for therapeutic replacement trials is a major continuing portion of this project.

Major Findings: (1) Enzyme replacement in Gaucher's disease and Fabry's disease holds promise as an effective therapeutic procedure for the amelioration of these disorders. A long lasting reduction of blood glucocerebroside, the accumulating lipid in Gaucher's disease was obtained in patients infused with purified human placental glucocerebroside. Patients with Gaucher's disease have subnormal activity of this enzyme in their tissues. We have developed a method for the purification of glucocerebroside on a large scale in a form that is suitable for administration to humans. Enzyme replacement trials in Gaucher's disease are underway with this preparation. The clinical course of the disease was greatly improved in two young boys who received the enzyme. Accordingly, they will be followed on a prospective long-term course of enzyme replacement. Other trials resulted in dramatic reductions in the quantity of accumulated glucocerebroside in the liver of two adult patients who received a short course of corticosteroid prior to administration of the enzyme. In the first patient, a 47% decrease in hepatic glucocerebroside was achieved, and in the second, there was a 30% reduction following intravenous administration of the purified enzyme. Furthermore, enzyme replacement was well tolerated and none of the patients developed antibodies to the exogenous glucocerebroside.

(2) We have developed a method for the purification of sphingomyelinase, the enzyme lacking in Niemann-Pick disease, also from human placental tissue. At the present time, it is very difficult to obtain sufficient quantities of this enzyme for replacement therapy trials. However, in collaboration with the National Center for Toxicological Research, we have discovered a strain of Balb/c mice with an autosomal recessive

neurological degenerative disorder characterized by the accumulation of several sphingolipids. These animals exhibit persistent deficiency of sphingomyelinase and glucocerebrosidase that is evident immediately after birth. We propose to utilize this important animal model for studies of enzyme replacement therapy.

(3) Since our original trial of enzyme replacement in patients with Tay-Sachs disease in 1972, it has been apparent that such individuals would not be benefited by the intravenous administration of the required enzyme without additional measures. This constraint is imposed by the blood-brain barrier which prevents the entrance of molecules as large as enzymes into the central nervous system. The blood-brain barrier has now been opened temporarily by intracarotid infusion of hypertonic solutions of mannitol or arabinose. Under these experimental conditions, a clear augmentation of mannosidase activity occurred in the brain after intravenous injection of the enzyme. This increase in brain mannosidase was fully equivalent to the physiological level of this enzyme. This demonstration of the accessibility of exogenous enzyme to the central nervous system is a major accomplishment towards enzyme replacement therapy for the multiplicity of heritable disorders that cause brain damage. The exogenous enzyme is readily taken up by neuronal cells *in vivo*, a critical prerequisite for enzyme replacement in disorders that cause nerve cell damage. Furthermore, we have recently demonstrated the presence of high affinity receptors for hexosaminidase A (the enzyme lacking in Tay-Sachs disease) on the membranes of neurons. The presence of these receptors greatly increases the likelihood that the exogenous enzyme will be effectively taken up by these cells.

(4) We continue to serve as a center for the diagnosis of patients and detection of carriers for all of the lipid storage diseases and much of our effort is devoted to the monitoring of pregnancies at risk for heritable metabolic disorders. During the past year, we performed more than 260 diagnostic assays for physicians and genetic counselors from all over the world.

Significance: Enzyme replacement appears promising for the treatment of Gaucher's disease and Fabry's disease. It is expected that the deleterious clinical course in these patients will be ameliorated by this form of therapy. The ability to introduce enzymes into the central nervous system has profound implications for the treatment of genetic disorders that cause brain damage.

Proposed Course: We will continue to carry out and monitor the long-term effects of enzyme infusion in patients with Gaucher's disease, Fabry's disease and other disorders. Studies of enzyme replacement with purified sphingomyelinase will be carried out in the animal model of Niemann-Pick disease. We shall attempt to design systems to deliver these exogenous enzymes in a clinically useful fashion.

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11. Pentchev, P. G., Kusiak, J. W., Barranger, J. A., Furbish, F. S., Rapoport, S. I., Massey, J. M., and Brady, R. O.: Factors that influence the uptake and turnover of glucocerebrosidase and α -galactosidase in mammalian liver. In Gatt, S., Freysz, L., and Mandel, P. (Eds.): Enzymes of Lipid Metabolism, New York, Plenum Publishing Corp. 1978, pp. 745-752.

12. Schneider, E. L., Pentchev, P. G., Hibbert, S. R., Sawitsky, A., and Brady, R. O.: A new form of Niemann-Pick disease characterized by temperature-labile sphingomyelinase. J. Med. Genet. 15: 370-374, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01309-14-DMN
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PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Biosynthesis and Function of Glycosphingolipids and Other Glycoconjugates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: P. H. Fishman, Ph.D., Research Biochemist	DMN	NINCDS
OTHER: R. O. Brady, M.D., Chief	DMN	NINCDS
M. Omodeo-Sale, Ph.D., Visiting Fellow	DMN	NINCDS

COOPERATING UNITS (if any)
Laboratory of Molecular Biology, NINCDS
Laboratory of Cellular Metabolism, NHLBI

LAB/BRANCH
Developmental and Metabolic Neurology Branch, IRP

SECTION
Enzymology and Genetics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.8	PROFESSIONAL: 1.8	OTHER: 1.0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Gangliosides appear to be important recognition molecules on the cell surface. The membrane receptor for cholera toxin is the ganglioside G_{M1}. The turnover of G_{M1} is blocked in cells exposed to the toxin. The turnover of the two components of the toxin are dramatically different. The B component, which binds to G_{M1}, is metabolized much slower than the A₁ component, which activates adenylate cyclase. Butyric acid induces cholera toxin receptors in several cultured cell lines. The increase in toxin binding corresponds to an increase in G_{M1} content. Gangliosides inhibit the attachment of cells to collagen; cell attachment is mediated by fibronectin, a cell surface glycoprotein. The most effective inhibitors are G_{T1} and G_{O1a}. The gangliosides have been shown to interact directly with fibronectin.

Project Description:

Objectives: To investigate the function of membrane glycosphingolipids in the regulation of cell proliferation, cell morphology, hormone action and toxin sensitivity; to explore the regulation of glycosphingolipid biosynthesis during development and differentiation and relate these findings to anabolic heritable disorders; to determine the underlying mechanism of altered glycosphingolipid biosynthesis in neoplastic tissues; these studies are being extended to other membrane glycoconjugates.

Methods: The glycosphingolipid composition of cultured cells and tissues is determined by extraction and purification of this class of lipids followed by separation of individual glycolipids on thin-layer chromatograms. Metabolism in cultured cells is determined by adding radiolabelled sugars to the culture medium and isolating the labelled glycosphingolipids. Biosynthesis in vitro is analyzed by assaying the activities of the glycosyltransferases involved in glycosphingolipid synthesis. Surface glycoconjugates are labelled by selective oxidation with galactose oxidase or periodate and subsequent reduction with sodium borotritide. Gangliosides radiolabeled in specific portions of the molecule are prepared by specific enzymatic reactions. Thus [¹⁴C]-N-acetylneuraminyllactosyl-ceramide ([¹⁴C]-GM₃) is synthesized from lactosylceramide and CMP-[¹⁴C] sialic acid with the specific sialyltransferase activity.

Binding of toxins and hormones to cells, cell membranes and liposomes is determined with [¹²⁵I]-labeled proteins and either filtration or centrifugation techniques. Levels of cyclic AMP and adenylate cyclase activity are measured with a modified cyclic AMP protein binding assay.

Oligosaccharides are prepared from gangliosides by ozonolysis and alkaline fragmentation. The oligosaccharides are purified by ion exchange and gel filtration chromatography. Purity and composition is assessed by thin layer and gas liquid chromatography.

Major Findings:A. Interaction of Fibronectin with Gangliosides

Fibronectin is a major cell surface glycoprotein which mediates cell attachment and is diminished in many malignant cells. Fibronectin binds to a specific region on collagen and thus mediates the attachment of fibroblasts to the collagen substratum. Fibronectin appears to be loosely associated with the cell surface and the nature of the surface binding sites for fibronectin are not yet known. Gangliosides block the fibronectin-mediated attachment of cells to collagen-coated culture dishes. Of the various gangliosides tested, Gp_{1a} and G_{T1} were the most potent whereas monosialogangliosides were relatively ineffective. Oligosaccharides derived from the gangliosides were inhibitory whereas the ceramide portion was not. Treatment of the

gangliosides with sodium periodate, which modifies the sialic acid residues, destroyed the inhibitory activity. When the cells were treated with periodate, they remained viable but no longer attached. Gangliosides did not prevent fibronectin from binding to collagen. Using immunological techniques, direct binding of fibronectin to gangliosides in solution or incorporated into liposomes could be demonstrated. Thus, fibronectin may bind to specific gangliosides or structurally related sialoglycoconjugates on the cell surface. Since previous studies had demonstrated a loss of complex cellular gangliosides from many malignantly transformed cells, there may be a coordinate loss of cellular adhesion components that potentiates abnormal behaviour both in culture and in vivo.

B. Turnover of Cholera Toxin and Its Receptor, Ganglioside G_{M1}

Previous studies have established that G_{M1} is the cell surface receptor for the *Vibrio cholerae* toxin, which mediates its effects by irreversibly activating adenylyate cyclase. We have been working with a line of transformed mouse fibroblasts, NCTC-2071, which lack G_{M1} and do not respond to cholera toxin. When G_{M1} is added to the culture medium, the cells take up the ganglioside and now respond to the toxin. Cells were exposed for 20 h to ³H-G_{M1}, washed and incubated with fresh medium with and without cholera toxin. In the absence of cholera toxin, the cell-associated G_{M1} had a half-life of 1 day. In addition, some of the G_{M1} became converted to G_{D1a}. In the presence of the toxin, there was little loss of cell-associated G_{M1} even after 72 h and synthesis of G_{D1a} was prevented. Similar results were obtained with rat glial C6 cells, which have very few cholera toxin receptors (7000 per cell). These cells also take up ³H-G_{M1}. After 24 h, only 53% of the G_{M1} was recovered from the cells whereas in the presence of cholera toxin, over 90% was recovered.

NCTC-2071 cells treated with G_{M1} or human fibroblasts which have endogenous G_{M1} bind ¹²⁵I-cholera toxin. After incubating the cells for 2 h at 37°C with the labeled toxin, unbound toxin was removed and the cells were incubated with fresh medium. At different times, the cultures were analyzed for distribution of the radioactivity. The material released into the culture medium was dialyzable and not precipitated with trichloroacetic acid (TCA) and probably represents iodotyrosine. Most of the cell-associated radioactivity was precipitated by TCA and had a half-life of 24-36 h. It was further analyzed by SDS-polyacrylamide gel electrophoresis, which can separate the toxin into its A₁ and B components. It was found that the A₁ component turned over much more rapidly than the B component. Adenylyate cyclase remained activated for as long as the A₁ component could be detected in the cells. These results are consistent with the following model. Cholera toxin through its B component binds to ganglioside G_{M1} on the cell surface. The A component separates from B-ganglioside complex, which remains on the cell surface and is only very slowly metabolized. The A component penetrates across the membrane

and becomes converted to A_1 , which in turn activates adenylate cyclase and itself becomes metabolized.

Induction of Cholera Toxin Receptors in Cultured Cells by Butyric Acid

Previous studies indicated that exposure of HeLa cells caused an induction of ganglioside G_{M3} . We now find that HeLa cells treated with butyrate bind up to 50-fold more ^{125}I -cholera toxin than untreated cells. The increase in toxin receptors was concentration and time dependent and reversible. Although the number of receptors per cell increased from 14,000 to 1 million, the apparent affinity of cholera toxin for control and butyrate-treated cells was similar. The butyrate-treated cells, however, were much more responsive to the toxin. After 1 h exposure to cholera toxin, cyclic AMP accumulation was stimulated 16-fold in the former cells and only 2-fold in the latter cells. Butyrate also caused an increase in toxin receptors in rat glial C6 cells and Friend erythrocytic cells. Whereas both butyrate and dimethylsulfoxide induce erythroid differentiation in the latter cells, only butyrate caused an increase in toxin binding. In addition, the appearance of toxin receptors preceded the appearance of hemoglobin in the erythrocytic cells. In both HeLa and Friend cells, butyrate induced a corresponding increase in ganglioside G_{M1} , the toxin receptor. Thus, butyrate appears to be a specific inducer of ganglioside biosynthesis in several lines of cultured cells.

Significance: These studies are providing information on the function of membrane glycosphingolipids. Ganglioside G_{M1} has been shown to be the receptor for cholera toxin. Other gangliosides may provide binding sites for fibronectin, a cell adhesion protein. In addition, cholera toxin is proving to be a potent probe for investigating the metabolism of gangliosides.

Proposed Course: The project will be continued with emphasis placed on the role of gangliosides in cell attachment. Further work will be done on the effects of cholera toxin and butyrate on ganglioside metabolism.

Publications:

1. Brady, R. O. and Fishman, P. H.: Gangliosides as biotransducers of membrane-mediated information. In Silverstein, S. C. (Ed.): -- Transport of Macromolecules in Cellular Systems. Berlin, Dahlem Konferenzen, 1978, pp. 69-84.

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4. Fishman, P. H.: Mechanism of action of cholera toxin: events on the cell surface. In Field, M., Schultz, S. G. and Kimberg, D. V. (Eds.): Disturbances in Intestinal Salt and Water Transport. Washington, American Physiological Society, in press.
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6. Fishman, P. H., Brady, R. O., Henneberry, R. C. and Freese, E.: Alterations of surface glycoconjugates and cell morphology induced by butyric acid. In Harmon, R. E. (Ed.): Cell Surface Carbohydrate Chemistry. New York, Academic Press, Inc., 1978, pp. 153-180.
7. Fishman, P. H., Moss, J., Richards, R. L., Brady, R. O. and Alving, C. R.: Liposomes as model membranes for ligand-receptor interactions: studies with cholera toxin and glycolipids. Biochemistry 18: in press.
8. Fishman, P. H., Quarles, R. H., and Max, S. R.: Gangliosides. In Touchstone, J. C. and Sherma, J. A. (Eds.): Densitometry in Thin Layer Chromatography: Practices and Applications. New York, John Wiley & Sons, Inc., 1979, pp. 315-327.
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11. Pacuszka, T., Moss, J., and Fishman, P. H.: A sensitive method for the detection of G_{M1} ganglioside in rat adipocyte preparations based on its interaction with cholera toxin. J. Biol. Chem. 253: 5103-5108, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01457-13 DMN
PERIOD COVERED <p style="text-align: center;">October 1, 1978 through September 30, 1979</p>		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">The Chemical Synthesis of Radioactive Sphingolipids</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: A. E. Gal, Head, Neurochemical Methodology Section DMN, NINCDS OTHER: F. J. Fash, Bio. Lab. Technician DMN, NINCDS		
COOPERATING UNITS (if any) <p style="text-align: center;">None</p>		
LAB/BRANCH <p style="text-align: center;">Developmental and Metabolic Neurology Branch</p>		
SECTION <p style="text-align: center;">Neurochemical Methodology Section</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Md. 20205</p>		
TOTAL MANYEARS: <p style="text-align: center;">0.6</p>	PROFESSIONAL: <p style="text-align: center;">0.3</p>	OTHER: <p style="text-align: center;">0.3</p>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <p>Sphingolipids containing <u>radioactive isotopes</u> were synthesized and used for <u>metabolic studies</u> and as diagnostic tools in sphingolipidoses. ¹⁴C and ³H labels were introduced by <u>synthetic and semi-synthetic techniques</u>, <u>gas exposure</u>, and a new approach: <u>functional group exchange</u>.</p>		
23 - DMNB/IRP		

Project Description:

Objectives: To prepare sphingolipids labeled with radioactive isotopes. The compounds are used for metabolic studies and as diagnostic tools in investigations related to hereditary lipid storage diseases.

Methods and Major Findings: A multitude of approaches were used in labelling glycolipids such as chemical synthesis, partial synthesis, minor synthetic modifications, functional group exchange and tritium gas exposure. These methods could be classified into two categories: specific and non-specific labelling. The ideal approach is the specific labelling which consist of the tagging of a complex molecule at a pre-determined atom. Total synthesis is the best way to accomplish this but up to now only few sphingolipids have been synthesized. We synthesized sphingosine, psychosine and galactocerebroside specifically labelled by total synthesis. However, our main effort is directed toward methods which would allow specific labelling of atoms yet would not necessitate tedious syntheses. A promising technique which we developed is called the functional group exchange. A chemical group such as an acetyl or carboxyl is split from a molecule and is replaced with a similar but radioactive one. With this approach we could prepare aminosugars even gangliosides. Using the approach-minor synthetic modification: we prepared asialo ganglioside, Tay-Sachs ganglioside and ceramidetrihexoside. In this approach oxidation and reduction of an alcohol group in the molecule with a radioactive reducing agent would reestablish the original lipid in radioactive form. The lipids used as starting material for this approach were isolated from human tissues. Tritium gas exposure a non-specific approach, was repeatedly used for labelling ceramide dihexoside, dihexoside and globoside. By this method all the non-labile hydrogen atoms in a molecule become radioactive. This procedure is relatively simple but the purification of the resulting compounds is complex. Also this type of compound requires more elaborate extractions for enzyme assays.

L-glucosylceramide was synthesized. This compound is a stereoisomeric analogue of D-glucosylceramide that occurs in nature and accumulates in pathological quantity in the organs and tissues of patients with Gaucher's disease. The properties of L-glucosylceramide that have been examined so far have been found to be indistinguishable from the naturally occurring glycolipid. However, L-glucosylceramide is completely refractory to enzymatic hydrolysis by purified placental glucocerebrosidase and enzyme(s) present in whole tissue extracts.

Significance: The compounds are indispensable for the detection, identification and isolation of enzymes connected to lipid storage diseases. Also studies related to qualitative and quantitative determination of enzymes in animal or human tissues necessitate these labelled substrates. Prenatal diagnoses are of rising importance.

These labelled compounds play a key role in these diagnostic procedures. As a therapeutic approach, this branch initiated replacement therapy by the administration of the missing enzyme in hereditary diseases. The monitoring of the enzyme levels during and after this therapeutic procedure was done by the use of these radioactive substrates. It would be also of great interest to develop new methods which would allow relatively easily and inexpensively preparation of these compounds for the use of clinicians and for researchers who are not connected to a large research center. It is anticipated that L-glucosylceramide will be uniquely useful substance for exploring pathogenetic processes in animal analogues of Gaucher's disease.

Proposed Course: Work on this project continues in three major directions: 1. Glycolipids will be labeled by using the above mentioned techniques with ^{14}C and Tritium. 2. The approach using "minor synthetic modification" will be extended and used on lipids which were not prepared at all or not prepared by this technique. Also the replacement of the enzymatic oxidation will be explored. 3. Work will continue on the development of the technique: labeling by functional group exchange.

Publications:

Gal, A. E., Pentchev, P. G., Massey, J. M. and Brady, R. O.: L-Glucosylceramide. I. Synthesis, properties, and resistance to catabolism by glucocerebrosidase in vitro. Proc. Natl. Acad. Sci. USA (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 01480-12 DMN

PERIOD COVERED October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Metabolism of Neurohumoral Transmitter Substances in Marine Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: E. G. Trams, Chief, Physiology and Metabolism Section, DMN NINCDS
OTHER: N. Salem, Staff Fellow DMN NINCDS
C. Lauter, Chemist DMN NINCDS
W. Taft, Director, Mote Marine Lab.
J. Doherty, Toxicology Branch, EPA
S. Patton, Borland Prof., Div. Food Science, Penn. State Univ.
A. A. Benson, Assoc. Director, Scripps Institute of Oceanography

COOPERATING UNITS (if any)

Mote Marine Lab., Sarasota, Florida
Hazard Evaluation Division, Environmental Protection Agency, Washington, D. C.
Division, Food Science, Penn. State University, University Park, PA.
Scripps Institute of Oceanography, LaJolla, CA.

LAB/BRANCH

Developmental and Metabolic Neurology Branch, DMN

SECTION

Physiology and Metabolism

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD. 20205

TOTAL MANYEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to explore the great variety and abundance of the marine environment for molecular models of neurobiology. In particular, to investigate species or phenomena which display an amplification or simplification of human physiology or pathology. Typical examples studied in this project are: sea urchin embryos in membranogenesis and development, CNS degeneration in spawning Pacific salmon, and use of the large nerve bundle of the lobster walking leg. We have localized membrane ATPases of sea urchin gametes using covalent probes and plasma membrane preparations. We discovered that DDT and certain other pesticides inhibit divalent cation-stimulated ATPases. Both covalent probes and pesticides prevent cell division of sea urchin embryos.

Project Description:

Objectives: To exploit the great variety and abundance and the biochemical specialization of oceanic life forms for the study of neurobiology. Such models have been explored by many investigators (e.g. squid giant axon, torpedo electroplax, tetrodotoxin) and we have introduced several others during the course of this project. Of the latter, considerable interest has been evidenced by scientific and lay community in use of the migrating Pacific salmon as a model for the aging process and for certain degenerative diseases. Another example was the use of developing sea urchin embryos in membranogenesis. We have attempted to determine whether the toxicity of the pesticide DDT is related to the inhibition of membrane ATPases. We also sought to relate the effects of covalent probes and pesticides on cell division to their action on membrane ATPases.

Methods: The primary mission of the Section on Physiology and Metabolism is to probe the molecular basis of bioelectrogenesis and neurochemical transmission. In the exploration of marine organisms we have therefore made use of model systems which allowed a facile interrogation of plasma membranes. One of the classical systems of embryology, the sea urchin embryo, has been the primary subject matter of this study. Collection of gametes, fertilization and quantitation of embryo development were performed by usual methodology.

A second system studied was plasma membranes prepared from lobster walking leg nerves. We developed our own method for plasma membrane purification using isopycnic and density gradient centrifugation, as existing methods gave unsatisfactory purification. ATPase assays were performed on the various systems using radiolabelled ATP.

Major Findings: We have continued the comparative aspects of the study of cell surface enzymes. One of the goals of this study was to determine if ecto-ATPase of mammalian cells was identical with the ATPase of lobster nerve. The lobster nerve enzyme had been shown to be inhibited by the pesticide DDT and this inhibition was suggested as the molecular basis of the pesticide toxicity. Therefore, we compared the DDT sensitivities as well as other properties of lobster nerve membrane ATPase and the mammalian enzyme. The lobster nerve membrane ATPase was optimally stimulated by Mn^{2+} with low sensitivity to Ca^{2+} as was C6 glioma cells. The crustacean enzyme was sensitive to DDT at $10^{-5}M$ but the mammalian enzyme was not. However, the lobster enzyme had the same sensitivity to DDE, a compound structurally related to DDT but not as effective in nerve conduction blockade.

The second system of concentrated study during the reporting period was sea urchin gametes and embryos. This system was chosen as it provides an easily obtainable source of homogeneous cells which can be induced to divide in a rapid and synchronous manner. The fertilization process is also of particular interest to us as membrane fusion followed by metabolic activation of the target cell was an early evolved mode of

intercellular communication fundamentally similar to chemical transmission in the brain. We have exploited this system to study the localization and properties of ATPases and their role in cell division. As molecular tools we have employed covalent (aryl halides) and non-covalent (pesticides) chemical probes.

Sea urchin eggs, sperm or fertilized eggs were treated with the cross-linking agents DFDNB, FDNB or TNBS. Covalent modification of membrane components with the permeant probes DFDNB and FDNB prevented division but the impermeant TNBS had no effect. Sperm pre-treated with 20 μ M DFDNB or FDNB showed a loss of mobility and of their ability to fertilize and induce cell division when added to untreated eggs.

A series of pesticides were tested for their effects on cell division and ATPase. The mitoside, Plictran, inhibited cell division; treated eggs could still be fertilized. Carbaryl had some effect on division, but lindane, kepone and chlorpyrifos had no effect. When sperm were treated with Plictran, kepone or chlorpyrifos, they lost their ability to fertilize untreated eggs. DDT was partially effective, but carbaryl and lindane had no effect. These effects on cell division were correlated with their effects on ATPases. Kepone and toxaphene inhibited egg plasma membrane ATPase, DDT was a slightly more potent inhibitor than its structural analogue DDE, a finding which correlates with the greater potency of DDT in blocking nerve conduction. Plictran effects on ATPases were extremely interesting as opposite effects were obtained depending on the divalent cations present. The data suggest that there is an allosteric cation binding site at which Ca^{2+} competes with Mg^{2+} .

Proposed Course: These studies have produced several promising leads. We hope to determine which structures or molecules are modified by covalent reaction that are crucial for cell division. We found that NEM-modified sperm can fertilize eggs which subsequently do not divide. This phenomenon may provide an important clue as to the molecular sequence of events following fertilization. The opposite effects on ATPase obtained with the pesticide, Plictran, depending upon the presence of Ca^{2+} or Mg^{2+} suggest that this pesticide may be a useful tool to study ecto-ATPase regulation and divalent-cation stimulation. We will attempt to extend these studies with ^{45}Ca binding and ion transport experiments using cultured mammalian cells.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01481-12 DMN
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Studies on the Composition and Metabolism of Cellular Membranes.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: E. G. Trams, Chief, Physiology and Metabolism Section, DMN NINCDS OTHER: N. Salem, Staff Fellow DMN NINCDS C. Lauter, Chemist DMN NINCDS		
COOPERATING UNITS (if any) Department of Anatomy and Embryology, University College, London, England		
LAB/BRANCH Developmental & Metabolic Neurology Branch		
SECTION Physiology and Metabolism		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The objective of this project is to elucidate the relationship between molecular composition and topographic arrangements of membrane building blocks with reference to plasma membrane functions. Bioelectrogenesis, transport, and many metabolic phenomena are based on the proper associations of membrane proteins and lipids. In particular, the function of membrane ecto-enzymes has been the objective of this study. We have found that these phosphoesterhydrolases are part of a regulatory system involved in traumatic shock. A self-sustaining increase in extra-cellular ATP is proposed as the biochemical lesion in shock. <u>Molecular probes</u> are used to modify plasma membranes of intact cells of CNS-derived tissue cultures. Modified membranes are used to elucidate the chemical composition of enzyme active sites and also associations with membrane lipids. Synaptosome-like vesicles released from cell cultures have a lipid composition distinct from parent cells.		
31 - DMNB/IRP		

Project Description:

Objectives: To elucidate the molecular composition and topographic arrangement of membrane components and their role in defining the physiological functions of the plasma membrane. Furthermore, to inquire if cell pathology in certain diseases of the CNS is associated with derangements of the function of such membrane components.

Methods: Cultured cell lines from a variety of CNS cells are employed. Established clones of murine and human neuroblastoma, gliomas as well as several primary cell cultures developed in our laboratory are grown. The major use of these cultures is the investigation of cell surface biochemistry.

High Pressure Liquid Chromatography is used to separate and quantitate nucleosides and nucleotides. Covalently reacting chemical probes are used to label plasma membrane constituents in situ. Enzyme activities are measured using radiolabelled substrates. Lipids and proteins labelled are analyzed after separation by classical extraction techniques. Fatty acid profiles are determined by gas-liquid chromatography.

Major Findings:(1) In a collaborative study with the University College London we have conducted a series of experiments relating to the function of adenine nucleotides in the purinergic nervous system. In particular, we have reviewed reports in the literature that the administration of ATP and its congeners might have therapeutic value in peripheral vascular disease, coronary insufficiency and hypertension. From our previous experience with ecto-enzymes it appeared unlikely that plasma levels of nucleotides could be maintained following various routes of administration. We have now measured the metabolic fate of ATP and its primary metabolites following intragastric or intravenous administration in a variety of animal species. ATP, ADP, AMP and adenosine are all potent vasoactive agents, but we have observed that the half-life of each, following a so-called therapeutic dose, was of the order of a few seconds. Species variation in the activity of ecto-phosphoester hydrolases varied by nearly two orders of magnitude. Activity in man and in a primate was low, while lower vertebrates had very high activity. We have concluded that any observed "therapeutic" effect ascribed to ATP etc. cannot be due to persistence of the agent in the plasma compartment. Possible other modes of action are now under investigation.

(2) During these experiments we have found that addition of ATP to final concentrations of 10^{-5} or 10^{-4} M in blood increased RBC permeability significantly. We had observed a similar phenomenon previously when addition of ATP to cultured nerve cells produced a marked increase in membrane permeability. As a consequence of the increased membrane permeability, we found that RBC cytoplasmic ATP leaked from the cells, and in effect, maintained ATP (purine nucleotide) levels in the plasma compartment. Thus, under certain conditions, when RBC ecto-ATPase activity fails to metabolize added ATP at an adequate rate and the

permeation of additional ATP is proceeding, the phenomenon becomes self-sustaining and ATP levels in the blood plasma compartment are maintained at pathologically high levels. We have proposed that under such conditions, the strong vasodilatory action of purine nucleotides will have deleterious effects on the organism. We believe that this phenomenon may account in part for the metabolic events and physiological consequences occurring in traumatic shock. Release of adenine nucleotides (presumably derived from cytoplasmic ATP) had been observed many years ago in battle casualties. Animal experiments verified the clinical observations. We postulate that in cases of irreversible shock, the plasma level of purine nucleotides becomes high enough to induce increased membrane permeability and a consequent leakage of cellular ATP as described above. If the capacity of the catabolic cell surface enzymes is exceeded, the pharmacologic effects of ATP in the plasma will persist causing sustained peripheral vasodilation, fall in blood pressure, and tissue hypoxia resulting in irreversible traumatic shock. If our hypothesis can be verified by observations in shock-trauma cases, we suggest that exogenous ATPases may have therapeutic value by reducing plasma ATP levels below the deleterious concentration.

(3) We have continued our studies of covalent chemical modification of the plasma membrane in intact cells. We are particularly interested in the associations of specific phospholipid species with membrane ecto-enzymes. We observed a marked specificity of aryl halides with respect to their inhibition of enzymes in the cultured cells. Nucleotides that are substrates protected the ecto-enzymes from this inhibition, a finding implicating active site alkylation as the molecular basis of the inhibition.

In an attempt to determine the type of chemical moiety at the active site of ecto-ATPase, we have experimented with covalent reagents which modify amino, sulfhydryl or other groups with some specificity. A major finding was that N-ethylmaleimide, a sulfhydryl blocking agent, inhibits ecto-ATPase and this inhibition could be prevented by the presence of substrate. In contrast a cleavable crosslinking reagent of the imidoester series inhibited ATPase but no protection was afforded by substrate. We have tested several other imidoesters, anhydrides, and sulfhydryl oxidizing, reducing and cross-linking reagents. Where inhibition was obtained, we have obtained information concerning the type of functional group involved by attempting to chemically reverse the reaction. We conclude that sulfhydryl and possibly also amino groups are at the active site of ecto-ATPase but that the site may be located on the interior of the membrane and therefore inaccessible to many externally acting reagents.

(4) Experiments with the effects of zinc and cobalt on ecto-5'-nucleotidase revealed that zinc was extremely inhibitory while cobalt was stimulatory. Zinc also potentiated the aryl halide inhibition of the ecto-enzyme, whereas cobalt protected the enzyme from inhibition.

Manganese afforded some protection. Surprisingly, calcium and magnesium had little effect on aryl halide inhibition of ecto-enzymes. These studies may have relevance to the toxicity of these metals.

(5) We previously demonstrated that synaptosome-like vesicles are released from monolayer cultures of neuronal and glial-derived cells. We have analyzed the lipid composition of the released "exosomes" and the parent glioma cells in order to trace the origin of the released particles. The results demonstrated that phosphatidylinositol levels are much lower in exosomes while sphingomyelin levels are much higher than in the parent cells. Fatty acid analysis showed a marked increase in palmitic and polyunsaturated acids at the expense of oleic acid. Therefore the composition of the exosomes was markedly different from the intact cells indicating that exosomes are not cellular debris. Analysis of plasma membranes should reveal whether exosomes originate from this site. In the course of these studies we also found that transformed glial and neuronal cells in culture have quite different lipid composition than normal brain cells.

Proposed Course

We will pursue experimentation on the nature and formation of biocatalysts on the cell surface. We will extend our studies of exosomal particles with emphasis on tracing their origin within the cell. To this end, we are establishing spinner cultures in order to prepare plasma membranes from cultured glioblastoma cells. We will complete and extend the studies of specific chemical modifying reagents and the effects of various metals on these reactions. We plan a careful analysis of lipids and proteins modified by the reagents and hope to identify origin(s) of the ecto-enzymes by specific labelling procedures. We shall examine in critical detail the effects of adenine nucleotides on homeostasis.

Significance:

If certain cases of traumatic shock can be shown to be due to excessive levels of adenine nucleotides in the circulation (Item 2, preceding section), the administration of exogenous ATPase may become a useful therapeutic procedure in these circumstances.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01808-10 DMN
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Glycoproteins of Myelin in Development and Disease		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: R. H. Quarles, Chief, Myelin and Brain Development Section		DMN NINCDS
OTHER: R. O. Brady, Branch Chief		DMN NINCDS
L. J. McIntyre, Visiting Associate		DMN NINCDS
D. A. Figlewicz, Guest Worker		DMN NINCDS
D. Johnson, Visiting Fellow		DMN NINCDS
S. Sato, Visiting Fellow		DMN NINCDS
COOPERATING UNITS (if any) Cellular Neuropathology Section, LNNS, NINCDS Veteran's Administration Hospital, Portland, Oregon		
LAB/BRANCH Developmental and Metabolic Neurology Branch, NINCDS		
SECTION Myelin and Brain Development		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20205		
TOTAL MANYEARS: 6.1	PROFESSIONAL: 4.0	OTHER: 2.1
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Purified central nervous system myelin contains a small amount of a small amount of a high molecular weight glycoprotein, referred to the <u>myelin-associated glycoprotein (MAG)</u> . This glycoprotein is selectively localized in the <u>periaxonal portion of intact myelin sheaths</u> . <u>Peripheral myelin</u> also contains a small amount of periaxonal MAG, but, in addition the major structural protein of compact peripheral myelin (P_0) is glycosylated. Since membrane glycoproteins are known to be involved in recognition and contact phenomena, these myelin-related glycoproteins are being studied with regard to their roles in <u>glial-axonal interaction</u> and <u>myelin compaction</u> . MAG of myelin undergoes a decrease in molecular weight during <u>development</u> which correlates well with myelin maturation. The chemical and immunological properties of mature and immature MAG are being determined. Since glycoproteins are known to be <u>cell surface antigens</u> and <u>receptors for viruses</u> , the possible involvement of MAG in the <u>autoimmune or viral aspects of multiple sclerosis demyelinating diseases</u> is being investigated.		

Project Description:

Objectives: To investigate the biochemistry of cells of the nervous system with particular regard to glycoprotein components and their roles in myelination and demyelination. Other myelin and oligodendroglial proteins and lipids will also be examined with the ultimate objective of understanding the molecular mechanisms of myelin formation and breakdown. Emphasis will be placed on the major myelin associated glycoprotein of the CNS and its role in demyelinating diseases such as multiple sclerosis.

Methods: Specific radioactive sugar precursors are used to label CNS and PNS glycoproteins. Myelin and other subcellular fractions are purified by differential centrifugation on sucrose gradients. Purified myelin is subfractionated into light, intermediate, and heavy fractions with different biochemical and morphological properties. Density gradient centrifugation is also used to isolate other oligodendroglial derived membranes (ODM). Enzyme markers are used to characterize the different subcellular fractions. The membrane-bound proteins and glycoproteins are fractionated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Double label counting techniques are used for detecting the labelled glycoproteins on gels and revealing small differences between samples. Densitometric scanning of gels stained with Fast Green for proteins or periodic acid-Schiff reagent for glycoproteins is used for quantitation of individual protein components. Glycoproteins on gels are also detected by binding of radioactive lectins. Quantitation of individual lipids is carried out by thin-layer chromatographic separation and densitometric scanning of the TLC plates. Purification of the major myelin-associated glycoprotein involves solvent fractionation, preparative polyacrylamide gel electrophoresis, and column chromatographic techniques. Molecular fragments of the isolated glycoprotein are prepared by mild proteolytic or chemical cleavage. Gas liquid chromatography and colorimetric procedures are used for quantitation of individual sugars in glycoproteins. Rabbits are immunized with purified glycoproteins and antibodies are detected by Ouchterlony immunodiffusion or radioimmunoassay using (³H) fucose-or ¹²⁵I-labeled antigen and anti-rabbit IgG serum.

Major Findings: The principal emphasis has continued to be on the isolation and characterization of the major myelin-associated glycoprotein (MAG). The protein is purified by the lithium diiodosalicylate (LIS)-phenol extraction procedure followed by preparative polyacrylamide gel electrophoresis in the presence of SDS. Alternatively, the electrophoresis can be replaced by gel filtration on Sepharose CL-6B in the presence of the neutral detergent, ammonyx.

A substantial amount of work went into determining the specificity of antisera prepared to the purified MAG. All the experiments indicate that the antiserum is highly specific for this particular glycoprotein and does not react with other glycoproteins or glycolipids. One of the most impressive experiments was the demonstration that the antiserum could selectively precipitate MAG from a heterogeneous mixture of fucose-labeled glycoproteins in the whole CNS myelin fraction. This highly specific antiserum is being used for immunocytochemical localization of MAG in collaboration with the Section on Cellular Neuropathology. In addition to showing a highly specific localization of MAG in oligodendrocytes and myelin within the CNS, these studies have shown that as myelin sheaths thicken during development, the MAG remains confined to the periaxonal portions of the sheaths. Also it was shown that MAG first appears in membranous bodies within oligodendroglia cytoplasm before the appearance of myelin. The antiserum prepared to rat MAG also reacts with MAG of bovine and human brains. Furthermore, it is possible to immunocytochemically stain paraffin sections of human brain for MAG. The human MAG is also localized in the periaxonal portions of myelin sheaths, and application of the method to multiple sclerosis tissue showed a loss of MAG staining at the periphery of growing plaques in areas which appeared normal by basic protein staining or other morphological criteria. This suggests that alteration of MAG may accompany or precede any previously described pathological change in multiple sclerosis.

The availability of the specific antiserum to MAG has also led to an important finding with regard to the peripheral nervous system (PNS). Previous biochemical studies had shown that the major Po protein of peripheral myelin is glycosylated but had not revealed MAG in PNS myelin. Therefore, the immunocytochemical finding that MAG antiserum stained Schwann cells and the periaxonal region of myelin on the trigeminal ganglion and sciatic nerve was initially surprising. However, we have now been able to make use of the selective immune precipitation to show that a glycoprotein with properties very similar to CNS MAG is in peripheral myelin. The PNS MAG had been masked in previous biochemical experiments by the very large amount of the Po glycoprotein. Thus, MAG is localized in the periaxonal region of both CNS and PNS myelin where it undoubtedly performs a similar function. Antisera to the Po glycoprotein and P₂ protein of peripheral myelin have also been prepared and used for immunocytochemical studies in collaboration with LNNS. These studies show that the Po glycoprotein is uniformly present throughout all peripheral myelin sheath, whereas P₂ appears to be present in only a proportion of the sheaths and particularly concentrated in Schmidt-Lanterman clefts.

Chemical analyses of the purified CNS MAG are in progress. Initial results indicate that it consists of two-thirds polypeptide and one-

third carbohydrate. The close structural similarity of the mature glycoprotein and the slightly larger immature form has been further indicated by reaction of the specific MAG antisera with the immature form and by very similar peptide maps that are produced by protease digestion of the two forms. Preliminary results indicate that the amino-terminus of the mature MAG is lysine.

Additional studies on myelinating tissue culture systems have been pursued as a preliminary to using the advantages of *in vitro* systems for studying the mechanism of myelinogenesis. In addition to the cerebellar explants reported last year, we have now examined aggregating culture systems in collaboration with LNNS. This culture system has the advantage of yielding large amounts of material for biochemical analysis. The cultures produce myelin which was isolated in sufficient quantity to show the presence of basic protein, proteolipid, 2'3'-cyclic nucleotide phosphodiesterase and typical myelin lipids. As we had previously shown for cerebellar explants, the [³H]fucose labeled glycoproteins in the purified myelin fraction were heterogeneous and it was difficult to discern MAG directly on SDS gels. However, we were able to demonstrate the presence [³H]-MAG by combining selective LIS-phenol extraction with specific immune precipitation as we had done for peripheral myelin. In the cerebellar explant system, it was shown that tunicamycin specifically inhibits glycoproteins in myelinogenesis. Finally, in collaboration with the Veteran's Administration Hospital in Portland, Oregon, we have demonstrated that MAG antiserum does not demyelinate cerebellar explants, but may have weak myelination inhibition activity.

Significance: The availability of specific antisera to MAG and the Po glycoprotein provides powerful new tools for studying the structure and function of these glycoproteins. It should permit the development of quantitative radioimmunoassays for these glycoproteins. With regard to myelin formation, there is considerable evidence in the literature indicating that cell surface glycoproteins are involved in recognition and cell-cell interactions. The periaxonal localization of MAG in the CNS suggests that it could be involved in oligodendroglia-axonal interactions. Its presence in the periaxonal portion of peripheral myelin sheaths suggests that a similar mechanism may mediate Schwann-cell-axon interactions in the PNS. Such a common mechanism in the CNS and PNS is consistent with recent evidence from other laboratories indicating that Schwann cells or oligodendrocytes are capable of myelinating axons from either the PNS or CNS. Demyelinating diseases such as multiple sclerosis are believed to involve autoimmune or viral processes. Membrane glycoproteins are known to be cell surface antigens and receptors for viruses. Therefore, it is reasonable to suppose that MAG could be directly involved in the pathogenesis of demyelinating diseases. Our demonstration that this glycoprotein is highly antigenic, and especially our finding that rabbits with experimental allergic encephalomyelitis induced by whole myelin have antibodies to MAG, enhance

this possibility. One hypothesis is that in multiple sclerosis a viral induced change in the sugars of MAG alters its antigenicity subjecting it to autoimmune attack. Certainly the immunocytochemical observation that loss of MAG staining is one of the earliest events in the developing plaque is strongly suggestive of an important role for this glycoprotein in the pathogenesis of multiple sclerosis. For these reasons, determination of the chemical and immunological properties of MAG will increase our understanding of the molecular mechanism underlying myelinogenesis and the pathogenesis of demyelination.

Proposed Course: In addition to purifying intact MAG, we will isolate fragments of the glycoprotein prepared by proteolytic or chemical cleavage. The smaller fragments may be easier to work with than the intact glycoprotein with mol. wt. 90,000. The carbohydrate and polypeptide portions of MAG and its fragments will be analyzed with the ultimate objective of determining its overall molecular structure. In these studies, emphasis will be placed on elucidating the precise chemical reason for the developmental decrease in its molecular weight and the way in which MAG interacts with other myelin constituents in the membrane.

Full advantage will be taken of the specific antisera prepared to MAG and the Po glycoprotein. Additional rabbits will be immunized with these glycoproteins. We will attempt to develop quantitative radioimmunoassays for MAG and Po. Immunocytochemical studies in collaboration with LNNS will continue with the goals of more precisely localizing these glycoproteins at the electron microscope level and understanding their roles in myelinogenesis and demyelinating diseases. The effects of MAG antisera and tunicamycin (the drug which inhibits glycosylation of proteins) on myelinating tissue cultures of CNS will be studied with the goal of elucidating the function of MAG in myelin formation. Finally, after immunological procedures are adequately worked out in experimental animals, multiple sclerosis patients will be tested for cellular and humoral immunity to MAG and for the release of MAG or its fragments to the cerebrospinal fluid.

Publications:

1. McIntyre, R. J., Quarles, R. H., Webster, H. deF. and Brady, R. O.: Isolation and characterization of myelin-related membranes. J. Neurochem. 30: 991-1002, 1978.
2. McIntyre, L. J., Quarles, R. H. and Brady, R. O.: Regional studies of myelin-associated glycoprotein in rat central nervous system. Brain Res. 149: 251-256, 1978.
3. McIntyre, L. J., Quarles, R. H. and Brady, R. O.: Lectin binding proteins in central nervous system myelin: Detection of glycoproteins of purified myelin on polyacrylamide gels by [³H] concanavalin A binding. Biochem. J. in press.

4. Quarles, R.H.: Glycoproteins in myelin and myelin-related membranes. In: Margolis, R.U. and Margolis, R.K. (Eds.): COMPLEX CARBOHYDRATES OF NERVOUS TISSUE, New York, Plenum Press, 1979, pp. 209-233.
5. Quarles, R. H., McIntyre, L. J. and Sternberger, N. H.: Glycoproteins and cell surface interactions during myelinogenesis. Society for Neuroscience Symposia, Vol. IV, Aspects of Developmental Neurobiology, in press.
6. Quarles, R. H., McIntyre, L. J. and Pasnak, C. F.: Lectin binding proteins in central nervous system myelin: Binding of glycoproteins in purified myelin to immobilized lectins, Biochem. J., in press.
7. Quarles, R. H., Webster, H.deF., Sakuragawa, N., Everly, J. L., Trapp, B. D. and Pasnak, C. F.: A biochemical comparison of *Xenopus laevis* myelin and mammalian myelin from the central and peripheral nervous systems. J. Neurobiol. 9: 217-288, 1978.
8. Sternberger, N. H., Quarles, R. H., Itoyama, Y. and Webster H. deF.: Myelin-Associated Glycoprotein Demonstrated Immunocytochemically in Myelin and Myelin-forming cells of developing rat. Proc. Natl. Acad. Sci., USA. 76, 1510-1514, 1979.
9. Trapp, B. D., McIntyre, L. J., Quarles, R. H., Sternberger, N. H. and Webster, H.deF.: Immunocytochemical localization of PNS myelin proteins: P₂ protein is not a component of all PNS myelin sheaths. Proc. Natl. Acad. Sci. USA., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02162-05 DMN
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Synthesis of Compounds Analogous to Glycolipids		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: A. E. Gal, Head, Neurochemical Methodology Section DMN, NINCDS OTHER: F. J. Fash, Bio. Lab. Technician DMN, NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Neurochemical Methodology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.4	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Glycolipid analogues of sphingolipids were synthesized that yield a <u>chromogenic moiety</u> on enzymatic hydrolysis. These compounds are used as reagents for the <u>diagnosis of Niemann-Pick disease, Gaucher's disease and Krabbe's disease</u> . The chromogenic analogues are also useful for the identification of <u>heterozygous carriers</u> of these disorders and for the <u>pre-natal diagnosis</u> of these diseases.		

Project Description:

Objectives: The compounds to be synthesized in the framework of this project are molecules similar to glycolipids which when cleaved enzymatically provide a chromophore useful for the diagnosis of lipid storage diseases and for the identification of heterozygous carriers.

Methods and Major Findings: Work is underway on the synthesis of a substrate for the chromogenic diagnosis of Farber's disease, a disorder characterized by a deficiency of ceramidase. This compound will be chemically related to 2-hexadecanoylamino-4-nitrophenyl phosphorylcholine (HNP). This substance resembles sphingomyelin but has a benzene ring instead of the aliphatic chain. Due to its nitrophenyl moiety, it yields an intense yellow color upon enzymatic cleavage. It is a reliable chromogenic substrate for assaying sphingomyelinase activity in diverse human tissue samples. It is used for the diagnosis of homozygotes and detection of heterozygous carriers of Niemann-Pick disease. This compound was synthesized by Calbiochem and Koch-Light and is commercially available from these manufacturers. We have developed a simplified synthesis of HNP using phosphorylcholine as the starting material. This improvement was realized because of the availability of free phosphorylcholine for which we developed a practical method of synthesis. Based on the chemistry of HNP, research on non-radioactive sphingolipid substrates was extended to other lipidoses. Compounds were synthesized which could be used as substrates for measuring gluco- and galactocerebrosidase activities in tissue extracts. Thus, 2-hexadecanoylamino-4-nitrophenyl glucoside was shown to be a useful compound for the diagnosis of Gaucher's disease and 2-hexadecanoylamino-4-nitrophenyl galactoside can be used for the diagnosis of Krabbe's disease.

Significance: The new compounds were thoroughly tested and they have been found to be reliable for the diagnosis of lipid storage diseases. These findings constitute a major breakthrough because the previously required radiolabeled products are scarce, expensive, and not widely available. The chromogenic substances can be used and easily handled by practitioners and clinical chemists with no danger of radioactive contamination and they eliminate the necessity of costly and complex radioactive detection techniques.

Proposed Course: Based on the concept demonstrated by this project, additional compounds will be synthesized with chromophoric moieties for the detection of other enzyme deficiency disorders.

Publications:

Johnson, W. G., Gal, A. E., Miranda, A. F. and Pentchev, P. G.: Diagnosis of adult Gaucher's disease. Use of a new chromogenic substrate, 2-hexadecanoylamino-4-nitrophenyl- β -D-glucopyranoside in cultured skin fibroblasts. Clin. Chim. Acta (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02163-05 DMN
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Development of Special Analytical Methods and Preparative Techniques to Investigate the Etiology and Therapy of the Sphingolipidoses		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: A. E. Gal, Head, Neurochemical Methodology Section DMN NINCDS OTHER: F. J. Fash, Biol. Lab. Technician DMN NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Neurochemical Methodology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.3	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) New <u>analytical</u> techniques were developed and used in enzymatic research and in <u>clinical investigations of lipidoses</u> . The lipid content in human tissues, the diagnosis of lipid storage diseases by <u>gas, thin-layer chromatography</u> and other techniques were studied at the <u>microgram level</u> . Also preparative work was done and used in connection with further synthetic work and for the <u>preparation of lipid standards</u> .		

Project Description:

Objectives: To develop techniques by which the separation and chemical analysis of biologic materials related to sphingolipidoses can be advanced. This work involves the following approaches: 1. Improvement of techniques leading to the separation of enzymes. 2. Development of ultramicro analytical methods for the determination of lipids in biological materials.

Methods: The development of methods for the determination of lipids in small samples of biological materials of human origin such as erythrocytes, leukocytes, fibroblasts, serum, cerebrospinal fluid, urine or biopsy samples from kidney, liver and brain. The individual sphingolipids are present usually only in submicrogram quantities in these samples. For the separation of such lipids, thin layer and gas chromatographic procedures combined with column-liquid chromatography was used.

Quantitative evaluation was made by scanning of the thin-layer plates or by gas chromatography. Much work was done in areas not covered by existing literature references.

Major Findings: Gas chromatography of glucose originating from lipids could not be determined previously. This problem was solved by us. Also a new thin-layer chromatography system was developed which resulted in more reliable results using only small amounts of specimen. A novel technique was developed in which lipids present in the same sample (but not attacked by the exogenous enzyme) were used as internal standards. Improved analytical techniques showed practical results particularly in the studies related to replacement therapy of enzymes where the decrease of lipid levels in the liver and erythrocytes of patients was established and through these procedures an evaluation of the therapeutic effect of enzyme administration can be assessed.

Significance: The purification of the missing enzymes required for the therapy of the lipid storage diseases is a complex, tedious, and costly procedure. The identification of accumulated lipids in human tissues for the diagnosis and control of inherited lipid diseases is dependent on the sensitivity of the analytical techniques. The importance of accuracy in working with trace amounts of material in biological specimens necessitates improved techniques at the submicrogram level.

Proposed Course: Much more work has to be done in relation to the improvement of microanalytical procedures; for example, the ultramicrodetermination of aminosugars and sialic acid needs further development. Some of the existing methods are too complex and their simplification will be investigated. The application of other techniques including high speed (or pressure) liquid chromatography or the use of mass spectroscopy will be explored.

Publications:

Gal, A. E., Pentchev, P. G., Barranger, J. A., Dambrosia, J. M., and Brady, R. O.: The distribution of glucocerebroside in the liver of patient with Gaucher's disease. Anal. Biochem. 95: 127-132, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02366-01DMN
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Regulation of Hormone-Responsive Adenylate Cyclase		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: P. H. Fishman, Ph.D., Research Biochemist		DMN NINCDS
OTHER: J. Hagmann, M.D., Visiting Fellow		DMN NINCDS
J. B. Parent, Ph.D., Guest Worker		DMN NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Enzymology and Genetics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20205		
TOTAL MANYEARS: 2.3	PROFESSIONAL: 2.3	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINDRS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) 1. Avian embryo <u>myoblasts</u> do not bind beta-adrenergic antagonists or respond to agonists although they have adenylate cyclase activity. Coincident with fusion to form myotubes, the muscle cells acquire <u>beta-receptors</u> which become functionally coupled to adenylate cyclase. 2. <u>Colchicine</u> , which disrupts microtubules, causes cells to become more responsive to stimulators of adenylate cyclase. Agents which promote microtubule assembly reduce cellular responsiveness. Prolonged exposure of cells to beta-agonists results in <u>desensitization</u> (loss of hormone response), which can be blocked by colchicine. 3. Accumulation of <u>cyclic AMP</u> does not occur in cells treated with <u>cholera toxin</u> for 24 h at or below 15°C whereas accumulation occurs within 35 min in cells incubated at 37°C. When briefly exposed (20 or 30 min) at 37°C to toxin and then shifted to 15°C, the cells accumulate cyclic AMP. If exposed for only 10 min, the cells do not respond to the toxin. Antitoxin blocks activation of adenylate cyclase in cells preincubated with toxin at 15°C but not at 37°C. Thus, cholera toxin action appears to involve a time and temperature dependent <u>transmembrane</u> event.		
47 - DMNB/IRP		

Project Description

Objectives: To investigate the molecular mechanisms involved in the regulation of hormone-responsive adenylate cyclase systems; to examine hormone-responsive adenylate cyclase during development and differentiation; to develop an overall model for this example of transmembrane signalling; to relate these findings to various metabolic disorders.

Methods: Quail embryo muscle cells are isolated from the pectoral muscle. Myoblasts are isolated from primary cultures after 24 h by selective treatment with trypsin; myoblasts are preferentially released while other cell types remain attached to the culture dishes. Secondary cultures are grown for 3-5 days during which time myogenesis occurs. Fusion of myoblasts to form myotubes is monitored by staining the cultures and examining them under the microscope. Guinea pig macrophages are induced in the peritoneal cavity with thioglycollate, removed and washed free of contaminating erythrocytes. Rat glial C6 cells are an established cell line. Cells grown in monolayer culture on plastic dishes or in suspension are incubated with hormones and other effectors and levels of cyclic AMP are measured with a modified cyclic AMP protein binding assay. Adenylate cyclase activity in cell lysates is determined in the presence and absence of hormones and other effectors. Binding of hormones to intact cells and cell membranes is measured with radioactively labeled ligands. Bound ligand is separated from free by rapidly washing the monolayer cultures or by filtering the cells and membranes on small filters by means of a vacuum manifold. Specific binding is determined by correcting for radioligand bound in the presence of excess unlabeled ligand.

Major Findings:A. Development of a Beta-Adrenergic Responsive Adenylate Cyclase During Muscle Cell Fusion

We are investigating the biochemistry of the plasma membrane of quail embryo muscle cells as they undergo myogenesis in culture. The myoblasts are completely unresponsive to beta-adrenergic agonists such as isoproterenol, which does not stimulate cyclic AMP production in intact cells or adenylate cyclase activity in membrane preparations. Following cell fusion, the intact myotubes accumulate cyclic AMP when stimulated with agonists; and adenylate cyclase activity in membrane preparations. Following cell fusion, the intact myotubes accumulate cyclic AMP when stimulated with agonists; and adenylate cyclase activity in myotube membranes is stimulated by the hormone. The myoblasts do respond to cholera toxin, another activator of adenylate cyclase and possess a fluoride- and guanine nucleotide-stimulated adenylate cyclase. Using radioactively labeled antagonists, we could not detect any specific beta-adrenergic receptors in the myoblasts. As the cells fuse, the number of beta-receptors increases to 1000 per cell equivalent and the increase parallels the fusion process. Thus, during myogenesis, the muscle cells, which contain a plasma membrane-bound adenylate cyclase, acquire specific cell surface beta-receptors; and these

receptors become functionally coupled to the adenylate cyclase in the differentiated myotube. These observations have potential significance for Duchenne's muscular dystrophy as other investigators have reported that the dystrophic muscle cells do not respond to epinephrine.

B. Modulation Of Hormone-Responsive Adenylate Cyclase by Cytoskeletal Organization

The mechanism(s) whereby hormone receptors are functionally coupled to adenylate cyclase in the plasma membrane have not yet been elucidated. We have found that exposure of macrophages to the antimicrotubule drug colchicine causes the cells to round up and to accumulate up to 10-fold more cyclic AMP than control cells when stimulated with isoproterenol, prostaglandins and cholera toxin. Conversely, when the cells are allowed to spread out on tissue culture dishes, they become less responsive to these stimulators of adenylate cyclase. Agents such as MIF (migration inhibitory factor), chemotaxins, butyrate, and dithiothreitol, which promote microtubule polymerization and cell spreading also decrease cellular responsiveness whereas colchicine reverses the inhibitory effects of these agents on adenylate cyclase stimulation. When cells are exposed to isoproterenol for 1-2 h, washed, and rechallenged with fresh hormone, they no longer respond; they have become desensitized. If colchicine is present during the initial incubation, the cells still respond when challenged with fresh agonist and do not become desensitized. Preliminary results indicate that colchicine has similar effects on the response of macrophages to an inflammatory stimulus as well as of other cell types to specific hormones.

C. Temperature Dependence of Cholera Toxin Action

Cholera toxin activates adenylate cyclase in intact cells after a discrete lag period whereas in disrupted cells, activation occurs without any lag period. Current concepts envision that the lag period represents the time required for the A component of the toxin to traverse the plasma membrane and enzymatically modify the adenylate cyclase complex. Rat glial C6 cells exposed to cholera toxin at 37°C begin to accumulate cyclic AMP after 35 min and their adenylate cyclase becomes activated after 10 min of exposure. At or below 15°C, the toxin-treated cells do not accumulate cyclic AMP even after 24 h. Above 15°C, cyclic AMP accumulates with increasing temperature. At 20° and 24°C, the lag periods were 2 and 1 h, respectively. Cells were incubated with cholera toxin for up to 30 min at 37° and then shifted to 15°C. Cells incubated at 37°C for 20 or 30 min accumulated cyclic AMP at 15°C; the rate of accumulation was linear with time at 15°C and was 3-fold greater for the latter cells. Cells exposed to the toxin for only 10 min at 37°C did not accumulate cyclic AMP when shifted to 15°C. As indicated above, 10 min is insufficient time for activation of adenylate cyclase by the toxin. Addition of anti-cholera toxin antibodies prior to adding the toxin to the cells completely blocked activation of adenylate cyclase at 37°C. If the antitoxin was added 20 sec after the toxin, inhibition was only partial; if added after 15 min, there was no inhibition. However, when cells were incubated with toxin at or below 15°C for 15 min and then shifted to 37°C in

the presence of antitoxin, inhibition was complete. Separate experiments with ^{125}I -labeled cholera toxin indicated that once the toxin was bound to the cells the antitoxin was not effective in displacing the toxin. When added first, the antitoxin prevented the labeled toxin from binding to the cells. These results are consistent with the proposed model for toxin action. At or below 15°C , the lipid bilayer may be too rigid to allow the A component of the toxin to penetrate and interact with the adenylate cyclase complex. Once this process has been initiated at 37°C , it can continue even at 15°C .

Significance: These studies are providing information on the molecular mechanisms involved in regulating adenylate cyclase activity. Muscle cells which have adenylate cyclase activity acquire specific beta-receptors during differentiation and are thus able to respond to epinephrine. Cytoskeletal organization can modulate the response of adenylate cyclase to hormones and, in turn, other external effectors can alter cytoskeletal structure. Activation of adenylate cyclase by cholera toxin involves a temperature-dependent transmembrane event.

Proposed Course: This is a new project and the initial results obtained so far are very promising. Therefore, the project will continue with emphasis placed on the role of cytoskeletal organization on other hormone-responsive cell types and on desensitization of hormone response, and on the biochemical basis of this phenomenon. Additional techniques will be developed to directly demonstrate the transmembrane event involved in cholera toxin action.

Publications:

None.

ANNUAL REPORT

October 1, 1978 through September 30, 1979

Laboratory of Neuropathology and Neuroanatomical Sciences
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1978 to September 30, 1979
Laboratory of Neuropathology and Neuroanatomical Sciences, IRP
National Institute of Neurological and Communicative
Disorders and Stroke

Igor Klatzo, Chief

The LNNS has continued to pursue the main research objectives and in some instances notable achievements can be recorded.

The Section on Cerebrovascular Pathology has continued studies on selective features of the blood-brain barrier in cerebral ischemia and a striking abnormal passage of norepinephrine was observed in gerbils 72 hours following release of one hour long occlusion of the common carotid artery. This was different from the abnormal penetration of dopamine and serotonin, as well as leakage of serum proteins. These studies further indicate the important role the noradrenergic system may play in the pathophysiology of cerebral ischemia.

In evaluation of various therapeutic approaches the effect of hypothermia on survival of ischemic gerbils was studied in larger series of animals. It was established that almost a 100% rate of survival (versus 32% control) could be achieved when hypothermia was induced at the time of or shortly before the carotid occlusion and the cooling was ineffective when applied more than 1 hour after the ischemic insult. The time relationships between the ischemic injury and the effect of various other metabolic depressants was studied in collaboration with the Section on Neurocytobiology and the findings are reported below.

In view of the prospects that this Institute will undertake a leading role in the field of computed tomography and in the development of emission tomography, a collaborative project with the Section on Neuroradiology and Computed Tomography was initiated. This project concerns the correlation between CT attenuation and specific gravity measurements in experimental vasogenic brain edema (VBE). Cryogenically induced VBE is studied in the rhesus monkey utilizing the EMI CT1010 scanner with narrow beam collimation. The CT scanning has been correlated with the spreading of the Evans blue tracer and the determination of specific gravity from small pieces of edematous and control brain tissue taken following the sacrifice of the animals. A precise correlation between CT images and specific gravity measurements has been established and this data, unquestionably, will be of considerable value for interpretation of clinical CT scanning.

In connection with this work a major project on the mechanisms in the resolution of VBE was undertaken in cats. Here, the VBE produced by cold lesion has been studied correlating the specific gravity measurements with the localization of the extravasated serum proteins by the immunocytochemical methods. Our studies revealed that there is a striking relationship between the resolution of edema as measured by specific gravity, which reflects the water content in the tissue, and the intracellular uptake of serum proteins

by the glial cells, primarily by the astrocytes. A hypothesis is suggested that the main mechanisms for the resolution of the VBE depends on reduction of colloidal osmotic pressure in the extracellular space, due to intracellular uptake of proteins, and this releases the water to diffuse away and leads to the reestablishment of the movement of fluid between blood and brain tissue according to the Starling's law. These studies will be pursued further on the electron microscopic level.

The Section on Neurocytobiology was successful in establishing the mono-layered cell culture derived from dissociated cells of isolated cerebral capillaries which can provide a model for the study of normal and altered endothelial function. Especially, individual pathomechanisms responsible for disturbances of the BBB occurring in various cerebrovascular disorders can be elucidated under strictly controlled conditions in vitro. The cultures derived from the isolated capillaries reveal growth of cells individually or as elongated sheets in which the activity of alkaline phosphatase, γ -glutamyl transpeptidase, butyryl cholinesterase and uptake of L-dopa were demonstrated by histochemical and fluorescent techniques.

The procedure developed by the Section for studies on isolated brain capillaries was used to investigate the mechanisms of endothelial uptake of norepinephrine. Normally this monoamine does not cross the BBB but is taken up and metabolized in the cerebral endothelium. The mechanism of such uptake was investigated in the isolated capillaries using ^3H metaraminol, a norepinephrine analogue which is neither metabolized by MAO nor COMT.

The endothelial uptake of norepinephrine and its analogues was also studied in isolated capillaries derived from the brains of gerbils subjected to 15 minute bilateral carotid occlusion and sacrificed at various time intervals. Such capillaries showed an increased uptake of norepinephrine, metaraminol and 5-hydroxytryptamine in comparison to the capillaries isolated from normal animals and in both instances ^3H monoamine uptake was equally susceptible to inhibition by a respective unlabeled substrate. The increased uptake of norepinephrine and its analogues in capillaries subjected to ischemia paralleled to a great extent an abnormal BBB passage of norepinephrine found in ischemic gerbils in vivo. These similarities in the behavior of the microvessels suggest that an increased permeability of the BBB with regard to norepinephrine observed in ischemia may be related not only to altered capillary metabolism but also to an increased uptake of the monoamines.

In another research project the effect of naturally occurring central nervous system depressants [γ -hydroxybutyrate (GHB) or γ -butyrolactone (GBL)] on the clinical course of cerebral ischemia was studied in Mongolian gerbils. The effectiveness of these compounds was manifested by increased survival rate of gerbils subjected to bilateral common artery occlusion for 15 minutes. Both compounds acted similarly and in contrast to hypothermia were useful not only in prevention but they were effective when applied in the late post-ischemic period. The mechanism of action of these agents in ischemia is still unclear, although they have, like hypothermia, a sparing effect on brain

energy metabolism. It is possible that the improvement in the outcome of cerebral ischemia could be mediated by neurotransmitters, which GBL and GHB affect under physiological conditions. GBL or GHB had also a markedly beneficial effect on the treatment of ischemic cerebral edema. Administration of either of these chemicals resulted in almost complete restoration of normal specific gravity values of brain within one week of reestablished circulation following 15 minutes of bilateral carotid occlusion. The effect of these compounds was independent of the time of injection, whether given 2 minutes prior to or 2 or 3 hours after clip release. These findings suggested that GBL or GHB do not prevent the development of ischemic cerebral edema but are beneficial for its treatment.

The Section on Experimental Neuropathology has pursued its two major projects. 1) Studies concerning the effect of dimethylsulfoxide (DMSO) on the histochemical demonstration of glycogen in the central nervous system (CNS). A strikingly enhanced glycogen staining was observed in rather focal fashion. This factor as well as some toxic cytological responses of the tissue limit the broad application of DMSO as a histochemical technique for glycogen.

The second project of the Section was concerned with elucidation of the pathomechanisms involved in the occurrence of petechial hemorrhages following oil embolism. Conditions under which the hemorrhages occur were investigated by comparing histological pictures from the brain tissue fixed by immersion with findings in material fixed by perfusion. The preliminary observations indicate that morphological characteristics as well as moment of occurrence of these hemorrhages after embolization may vary in the two types of fixation.

The major research effort in the Section on Cellular Neuropathology involved the use of immunocytochemical methods to localize myelin proteins, BP (basic protein or P_1), P_0 (peripheral myelin glycoprotein), P_2 (peripheral myelin small basic protein) and MAG (myelin-associated glycoprotein) in normal and diseased nervous tissue. The most important new findings were: 1) Myelin-associated glycoprotein (MAG) is found in immature oligodendroglia before myelination begins; in the adult, MAG is located periaxially. MAG also was detected in developing Schwann cells and periaxonal regions of peripheral myelin sheaths. The periaxonal localization of MAG suggests that it may have an important role in the formation and maintenance of CNS and PNS myelin sheaths. 2) In multiple sclerosis lesions, chronically demyelinated areas contained almost no MAG or basic protein (BP). Regions undergoing demyelination showed abnormal distributions of both MAG and BP. Finally, the changes in MAG immunostaining extended into histochemically normal white matter beyond the margin of altered BP staining. These observations suggest that in multiple sclerosis, immunoreactivity of periaxonal MAG is altered before myelin breakdown begins. 3) In the PNS, all myelin sheaths are stained uniformly by antisera to peripheral myelin glycoprotein (P_0) and BP. Another lower molecular weight basic protein (P_2), however, only was found in some myelin sheaths. Generally, larger sheaths contained P_2 but the proportion of sheaths containing P_2 depended on the nerve examined,

the species, and the developmental stage studied. Selective localization of a myelin protein in some sheaths and not others is a finding of major importance. It may be extremely useful in assessing abnormalities in myelin formation and patterns of demyelination observed in peripheral neuropathies.

The main interest of the Section on Functional Neuroanatomy has continued to be in understanding synaptic transmission and development. The rapid-freezing technique developed in this Section has been improved and applied to capture fleeting structural changes in functioning synapses. By freeze-fracturing rapid-frozen neuromuscular synapses, it has been possible to see, and count, synaptic vesicles fusing with the plasmalemma of synaptic terminals at several different levels of transmitter secretion. It turns out that each quantal secretory event results from the fusion of one synaptic vesicle with the plasmalemma. Since the temporal resolution of rapid freezing in the machine used by this section is less than 2 msec, as measured by a capacitance method developed here, the fate of synaptic vesicle membrane after vesicles fused with the synaptic plasmalemma could be followed. In less than 0.1 sec, the vesicle membrane is completely flattened out into the plasmalemma. Components of the vesicle membrane, appearing as particles after freeze-fracturing, then spread out randomly, finally to be collected a second later in little particle islands which are then incorporated into coated vesicles. The ultimate fate of these components of the vesicle membrane is to be reincorporated into synaptic vesicles. This finding of particle recycling extends earlier work of the section showing that local recycling of synaptic vesicles replaces those lost during synaptic activity.

Synaptic vesicles are so small and the initiation of exocytosis so rapid that visualization of its initial stages has been elusive. In order to see this process in more detail, amoebocytes from *Limulus* were frozen at various times after inducing them to secrete. These cells have large secretory granules which are secreted over a few seconds after exposure to endotoxin so it was possible to see the very first sign of exocytosis, a tiny hole in the plasmalemma which subsequently widens. This finding is of interest because it is incompatible with the current idea that exocytosis begins as a broad approximation between the secretory granule and the plasmalemma which then thins and bursts. The presented results require instead that a very local disruption in the adjacent lipid bilayers be considered the initial event in exocytosis (in these cells). A second important feature of these findings is that the plasmalemma puckers in to contact the secretory granule just before exocytosis begins, and filaments are associated with this process. This suggests that, at least in this cell, a contractile process may be associated with initiation of exocytosis.

The availability of a successful rapid freezing technique, which lowers the temperature near the surface tissues to -80°C in two msec, makes feasible a variety of other types of experiments. This technique was used to immobilize calcium ion in neural tissues, so that subsequent cryochemical techniques can be used to fix calcium at its natural location. The calcium is then detected

with an electron probe X-ray spectrometer. In frog muscle, calcium is found in the sarcoplasmic reticulum at rest but disappears after stimulation. In squid axon, where calcium enters with the action potential, it is sequestered in cisternae near the axolemma as well as in mitochondria. Similarly, in the synapse, the calcium which enters during prolonged electrical stimulation is sequestered in cisterns of endoplasmic reticulum. Thus, these studies of various neural tissues are identifying a type of intracellular organelle, in addition to mitochondria, which stores and reseals calcium. This year we have set up an electron probe and established calibration standards, which were not previously available so that determinations of calcium can be quantitative.

The Section on Neurocytology is concerned with five projects: 1) the interactions between an undisturbed brain surface and transplanted ganglia, 2) the identity, development and function of intramembranous assemblies of particles within astrocytes, 3) the enzymatic activity within metabolically stimulated neurosecretory neurons, 4) the localization and switch from non-neuronal enolase to neuronal enolase, in developing neurons and glia, and 5) the passage of large and small tracers across cerebral vessels.

1) Mature superior cervical ganglion (SCG) allografted to the undamaged surface of recipient brains can survive for at least 14 months. When the SCG fragment was placed against the developing cerebellar cortex, whole laminae of multipotential external granule cells (EGC) were arrested many months after they would have migrated to their normal adult residence in the internal granule layer. Contrary to our expectations of an invasion of SCG neurites into the cerebellum, the opposite event occurred: an anomalous migration of cerebellar tissue into the transplant. The invading cerebellar tissue consisted of neurons, glomeruli and glia. The results suggest that as yet unidentified "tactic factors" can influence the mobility and migratory direction of CNS neurons.

2) Orthogonal aggregates of small (5-7 nm) particles, called assemblies, characterize plasma membranes of astrocytes and have been examined in reactive, proliferating astrocytes. Within the cell membranes of glial scars, individual assemblies were generally larger than in normal astrocytes. Contrary to normal, parallel lamellae, the number of assemblies in the scars did not diminish in successively deeper layers. A striking feature of the cell membranes of the most superficial marginal astrocytes of a subtle gliosis was the orderly alignment of assemblies into long parallel trains of particles.

We have now determined that the assemblies are protein in nature. Primary cultures of astrocytes were established from dissociated cerebral cortex of 5-7 day old rats. Three hours after the cultures were exposed to 10^{-6} M cycloheximide, there was a total loss of assemblies although background particles and gap junctions persisted. This evidence implies that assemblies are a protein with a high turnover rate. The assembly protein will be isolated by differential ultracentrifugation and characterized by SDS gel electrophoresis.

3) The demonstration of ultra-axonal, anterograde transfer of exogenous protein is not well documented. Neurosecretory neurons of the supraoptic nucleus were selected because their metabolic activity can be modified during axonal transport of horseradish peroxidase (HRP). In dehydrated animals, these neurons increase their synthesis and packaging of certain hormones. HRP was presented to these neurons by way of the cerebral ventricles in mice. More HRP was taken up by the somata and dendrites of these neurons and transported orthogradely into their axons and neurohemal terminals in dehydrated mice than in hydrated animals.

The anterograde movement of HRP was accompanied by a transfer of acid hydrolases from secondary lysosomes and cisterns within the soma to like organelles in the axon terminals. These results reflect enhanced metabolic activities and orthograde transfer of both exogenous materials and degradative enzymes within the axon.

4) Three enolase isoenzymes can be detected immunocytochemically in mammalian brain: neuron-specific enolase (NSE), non-neuronal enolase (NNE), and hybrid enolase. In primary tissue culture, antibody to NSE consistently stains all neurons and the intensity of staining correlates with synaptic activity. Outside of the CNS, NSE is also a molecular marker for neuroendocrine cells including adrenal medullary chromaffin cells, C-cells of thyroid and pancreatic islet cells.

In developing rat and monkey brain, there is a marked shift from NNE to NSE. In cerebellum, for example, postmitotic young neurons generated from proliferative zones are NNE (+) during migration and acquire NSE only after arrival in their final location and after presumed synaptic and functional maturation. Present efforts are directed at this new dimension of NSE as a marker for other differentiating nerve cells.

5) Hyperosmotic saccharides, such as 1.4 M arabinose, when injected intravascularly, cause the escape of exogenous horseradish peroxidase (HRP; MW 40,000) from cerebral vessels. In order to better define the pathways of escape, the much smaller tracer, ionic lanthanum (La) has been used. In single, thin plastic sections, La did not extend continuously between endothelial cells from luminal to abluminal side. However, in serial sections the La penetrated into the interjunctional pools between successive tight junctions. Very few endothelial vesicles contained La. These experiments are being continued in order to determine whether the barrier opening is by way of junctions, vesicular transfer or damage to endothelium.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02282-03 LNNS																
PERIOD COVERED October 1, 1978 to September 30, 1979																		
TITLE OF PROJECT (80 characters or less) Effect of interference with CSF dynamics on the survival rate of Mongolian gerbils subjected to cerebral ischemia																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">M. Smialek</td> <td style="width: 35%;">Visiting Scientist</td> <td style="width: 15%;">LNNS NINCDS</td> </tr> <tr> <td>Other:</td> <td>I. Klatzo</td> <td>Chief, Lab. Neuropath. Neuroanat. Sci.</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>J. T. Walker, Jr.</td> <td>Biol. Lab. Tech.</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>M. Spatz</td> <td>Head, Section on Neurocytobiology</td> <td>LNNS NINCDS</td> </tr> </table>			PI:	M. Smialek	Visiting Scientist	LNNS NINCDS	Other:	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS		J. T. Walker, Jr.	Biol. Lab. Tech.	LNNS NINCDS		M. Spatz	Head, Section on Neurocytobiology	LNNS NINCDS
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	M. Spatz	Head, Section on Neurocytobiology	LNNS NINCDS															
COOPERATING UNITS (if any) None																		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences																		
SECTION Section on Cerebrovascular Pathology																		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205																		
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SUMMARY OF WORK (200 words or less - underline keywords) Tapping of small amounts of CSF from cisterna magna of Mongolian gerbils was carried out 2 hours before subjecting the animals to bilateral occlusion of the common carotid arteries for 15 minutes. Immediately before the clamping of the arteries, the gerbils were given 2 ml of distilled water intraperitoneally. Mortality of these animals during one month observation was compared with that in other groups consisting of a) gerbils subjected to bilateral carotid occlusion alone, b) gerbils subjected to occlusion and administration of distilled water and c) gerbils in which CSF tapping was performed 2 hours before occlusion, but H ₂ O was not given. The group of gerbils subjected to CSF, tapping and H ₂ O injection in addition to carotid occlusion showed 85% survival rate after 30 days of clip release. This compared with 60% survival rate of animals subjected to tapping before occlusion. The survival rate of gerbils subjected to occlusion alone was 33%, whereas in animals which in addition received water intraperitoneally the survival rate amounted only to 15%. This project is completed.																		

Project Description:

Objectives: The main rationale for this study is a search for measures which would significantly influence the clinical course and the outcome of an ischemic brain injury. In a serendipitous way a procedure has been discovered which dramatically reduces the mortality rate (from 67% to 15%) of Mongolian gerbils subjected to bilateral occlusion of the common carotid arteries for 15 minutes. The important objective in this study remains the elucidation of factors responsible for such improvement in survival of animals exposed to a severe cerebral ischemia.

Methods Employed: The following groups of animals were used in this investigation: 1) sham operated animals, 2) animals subjected to bilateral clipping of the common carotid arteries of the neck and followed for 30 days after the release of occlusion, 3) animals which at the time of occlusion received 2 ml of distilled water intraperitoneally, 4) animals which 2 hours prior to carotid occlusion were subjected to tapping of small amounts of the CSF from the cisterna magna and 5) gerbils in which bilateral carotid occlusion was combined with CSF tapping, and intraperitoneal injection of distilled water at the times specified in the previous groups. Mortality rate in all groups was determined during 30 days. To establish the duration of the effect of CSF tapping and distilled water, coefficient of survival was estimated in groups of animals which were tapped at 2 hours before occlusion and injected with H₂O at different periods and in groups of animals in which H₂O was injected always at the time of release of occlusion and the tapping was performed at different periods.

Major Findings: The survival rates of different groups of animals were as follows: a) bilateral 15 minute occlusion alone - 33%; b) carotid occlusion plus distilled water injection - 15%; c) CSF tapping prior to carotid occlusion - 60%; d) CSF tapping plus intraperitoneal water injection in bilaterally occluded gerbils - 85%. From the groups of animals in which the time of CSF tapping or injection of H₂O was modified it was evident that CSF tapping was effective even when performed 48 hours before carotid occlusion, whereas beneficial effect of H₂O lasted between 2 hours before occlusion and the time of occlusion.

Significance to Biomedical Research and the Program of the Institute: An effort to influence the clinical course of animals subjected to cerebral ischemia has been of great importance, since the experimental findings could lead to improvements in the clinical management of stroke patients. A dramatic difference in the survival rate (85% v. 33%) of animals which, in addition to being subjected to bilateral ischemic occlusion, received intraperitoneal distilled water and were subjected to CSF tapping warrants further studies on the pathomechanisms involved. Also, it will be of importance to reproduce a similar effect in a different species and in a different experimental model of ischemia.

Project No. Z01 NS 02282-03 LNNS

Proposed Course of the Project: This project has been completed. The results were presented at the International Symposium on Postresuscitation Pathology of the Brain in Moscow, Nov. 27-Dec. 1, 1978, the proceedings of which are in press.

Publications: See Project No. Z01 NS 02322-02 LNNS.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MS 02322-02 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Permeability of the blood-brain barrier (BBB) to norepinephrine (NE) in experimental cerebral ischemia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	H. Hervonen	Visiting Fellow LNNS NINCDS
Other:	O. Steinwall	Visiting Scientist LNNS NINCDS
	J. T. Walker, Jr.	Biol. Lab. Tech. LNNS NINCDS
	M. Spatz	Head, Section on Neurocytobiology LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci. LNNS NINCDS
COOPERATING UNITS (if any) K. Nishimoto, Tokyo Medical and Dental University, Tokyo, Japan		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.55	0.2
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS	<input type="checkbox"/> (b) HUMAN TISSUES	<input checked="" type="checkbox"/> (c) NEITHER
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p><u>Abnormal penetration of exogenous norepinephrine (NE) through the BBB</u> was evaluated in <u>cerebral ischemia</u> produced in gerbils by unilateral occlusion of the common carotid artery. The preliminary findings indicate that the peak of such penetration occurs 72 hours after release of 1 hour occlusion. This was demonstrated by quantitative assay using ^3H labeled tracer and by radioautography. Extravasation of exogenous NE into brain parenchyma induced also a formation of bizarre noradrenergic structures in the vicinity of ischemic lesions. This project has been completed.</p>		

Project Description:

Objectives: The main objective of this study is to evaluate an abnormal penetration of exogenous NE occurring in cerebral ischemia. This may elucidate further pathomechanisms of ischemic brain tissue injury. Also by concurrent application of other BBB tracers the differences in abnormal passages observed may provide new information in different regulatory mechanisms of BBB systems.

Methods Employed: The investigations are carried out on Mongolian gerbils subjected to 1 hour unilateral occlusion of the common carotid artery, selecting only the animals which show symptoms of infarction following release of occlusion. The abnormal passage of exogenous NE into the brain tissue is evaluated by systemic injections of ^3H labeled NE, and for comparison, ^{14}C sucrose. This is followed by selective counting of the radioactive tracers in hemispheres ipsilateral and contralateral to occlusion, sacrificing animals at various post-ischemic periods. Also, abnormal penetration of NE is evaluated by radioautography using ^{14}C NE injected systemically. Independently, histofluorescent study of noradrenergic structures is carried out in animals some of which before injection of exogenous NE are reserpinized to suppress the endogenous NE.

Major Findings: The abnormal penetration of ^3H NE was demonstrable in animals sacrificed 10 hours after release of occlusion. It reached its peak in the hemisphere ipsilateral to occlusion at 72 hours after release of clamping. The radioautography revealed dark areas of abnormal ^{14}C NE penetration in affected hemispheres, especially conspicuous at 72 hours after ischemic insult. The fluorescence microscopy showed formation of abnormal noradrenergic structures in the vicinity of ischemic lesions. These formations were especially conspicuous at the 72 hour post-ischemic period.

Significance to Biomedical Research and the Program of the Institute: The biogenic amines play an important role in pathophysiology of cerebral ischemia in view of their involvement in certain energy metabolic pathways and in neural regulation of cerebral blood flow. Study of abnormal penetration of NE from blood into the brain occurring in cerebral ischemia is unquestionably significant for better understanding of this condition.

Proposed Course of the Project: This project has been completed after supplementing observations on abnormal penetration of NE with those on passage of exogenous serotonin (5-HT) and dopamine (DA). These observations revealed that exogenous 5-HT and DA also pass abnormally at the 72 hour period after 1 hour occlusion but to a lesser degree than norepinephrine.

Publications:

Klatzo, I., Smialek, M., Hervonen, H., Steinwall, O., and Spatz, I.: Post-ischemic changes and application of some therapeutic measures to influence the clinical course of cerebral ischemia. In Proceedings of the International Symposium on Postresuscitation Pathology of the Brain, Moscow, Nov.-27-Dec. 1, 1978 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02323-02 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Effect of hypothermia on the survival rate of Mongolian gerbils subjected to bilateral carotid artery occlusion		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. Smialek Visiting Scientist LNNS NINCDS Other: I. Klatzo Chief, Lab. Neuropath. Neuroanat. Sci. LNNS NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Mongolian gerbils in which <u>bilateral occlusion of common carotid arteries for 15 minutes was carried out in conditions of hypothermia</u> (body temperature approx. 30°C) showed a remarkable improvement of survival rate when compared with control group subjected to carotid occlusions alone (98% v. 33%). This project has been completed.		

Project Description:

Objectives: The main objective of this study is to assess how much hypothermia by lowering the metabolic rate can affect the clinical course of cerebral ischemia in gerbils subjected to bilateral occlusion of common carotid artery for 15 minutes. It is also important to ascertain the length of time during which the application of hypothermia can still influence the ischemic brain damage.

Methods Employed: As the control group, gerbils were subjected to 15 minute bilateral occlusion of the common carotid artery and following release of the clips they were observed for 1 month. In experimental groups, the animals were subjected to hypothermia (30°C body temperature) for a period 20 minutes preceding and 30 minutes following 15 minute bilateral carotid occlusion. The hypothermia was induced by allowing the animals to swim in water at room temperature for 2 minutes and then transferring them to the cooling box.

Major Findings: In the control group, the survival rate of animals was 33% after 1 month following carotid occlusion. The hypothermic animals showed 98% survival rate. In the animals in which hypothermia was induced 1 hour following release of occlusion, the survival rate was 65%. Hypothermia had no visible effect when it was induced longer than 3 hours following release of occlusion.

Significance to Biomedical Research and the Program of the Institute: This remarkable improvement of survival rate in ischemic animals which were subjected to hypothermia raises the possibility that this finding might be of clinical significance for patients who can be cooled within a few hours of an ischemic insult. The evaluation of the effect of hypothermia on various biochemical parameters of ischemic injury may elucidate major pathomechanisms involved.

Proposed Course of the Project: This project has been completed. The results were presented at the International Symposium on Postresuscitation Pathology of the Brain in Moscow, Nov. 27-Dec. 1, 1978, the proceedings of which are in press.

Publications: See Project No. Z01 NS02322-02 LNNS

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02356-01 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Studies on resolution of the vasogenic brain edema (VBE)		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci. LNNS NINCDS
Other:	E. Chui	Visiting Fellow LNNS NINCDS
	K. Fujiwara	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 Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.2	PROFESSIONAL: 2.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Mechanisms of resolution of the vasogenic brain edema (VBE) were studied by application of <u>specific gravity</u> measurements correlated with <u>immunocytochemical</u> observations on <u>extravasated serum proteins</u> . The main mechanism of VBE resolution appears to be related to <u>intracellular uptake of serum proteins by the glial cells</u> .		

Project Description:

Objectives: The main objective of this study is to elucidate the mechanisms which are responsible for the resolution of the VBE. This could lead to designing effective therapeutic measures for the treatment of brain edema patients.

Methods Employed: Cortical cold injury in cats served as the model of VBE. As a marker for the spreading of edema fluid, Evans Blue dye was injected before the operation to produce the cold injury. The animals were sacrificed after various time intervals. The brains were sectioned into two coronal blocks, one of which was immediately submerged into kerosene and the other subjected to paraformaldehyde fixation. The block in kerosene was photographed with a Color Polaroid camera and the samples to be taken for specific gravity measurements were marked on the photograph. The specific gravity of the samples from areas of edema and control regions was measured in gradient columns and the values were marked on another color photograph of the same block. The coronal block of the brain fixed in paraformaldehyde was subjected to immunocytochemical procedures to demonstrate the localization of serum proteins in vibratome-cut sections.

Major Findings: The plotting of specific gravity measurements on the coronal sections of brains with cold lesions visualized progression and resolution of brain edema at various time intervals following cold injury. The edema was spreading preferentially through the white matter into the gyri adjacent to the injury. The resolution of edema was observed to take place from the periphery toward the site of the lesion. This coincided with the dramatic uptake of extravasated serum proteins in the extracellular spaces by the glial cells, and particularly by the astrocytes.

Significance to Biomedical Research and the Program of the Institute: The described findings allow proposal of the following hypothesis for the mechanism of resolution of VBE. The enhanced content of water in edematous areas is related to the presence of extracellular, extravasated serum proteins. The resulting shift in normal difference (25 mm Hg) between the colloidal osmotic pressure of the plasma and the interstitial fluid is responsible for retention of water in the edematous white matter. The vigorous intragial uptake of serum proteins reinstates the basic normal relationship of transcapillary flow according to Starling's law restoring differences in hydrostatic and osmotic colloidal pressures. The water unbound from the proteins diffuses away and this constitutes the main mechanism for the resolution of the VBE.

Proposed Course of the Project: The studies will be extended to application of immunocytochemical methods on the EM level. Also the shifts in the extracellular osmolarity will be assessed by the measurements of electrical impedance in edematous and control areas. The metabolic status of edematous and the surrounding regions will be evaluated by the application of 2D-glucose utilizing radioautographic method.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01999-07 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Transport studies in ischemic cerebral edema.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. Spatz Head, Section on Neurocytobiol. LNNS NINCDS Other: I. Klatzo Chief, Lab. Neuropath. LNNS NINCDS Neuroanat. Sci.		
COOPERATING UNITS (if any) T. Fujimoto, Department of Neurosurgery, Tokyo Medical and Dental University, Tokyo, Japan		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project has been temporarily discontinued.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02000-07 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Brain edema in cerebral ischemia of gerbils		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. Spatz Head, Section on Neurocytobiology LNNS NINCDS Other: K. Abe Visiting Fellow LNNS NINCDS I. Klatzo Chief, Lab. Neuroanatom. Sci. LNNS NINCDS		
COOPERATING UNITS (if any) H. Pappius, Montreal Neurological Institute, Montreal, Quebec, Canada		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Experimentally, cerebral <u>ischemic edema</u> can be produced easily in 30% of Mongolian gerbils by unilateral ligation of the common carotid artery. The cerebral water content was assessed by the determination of <u>wet and dry weight</u> and the <u>specific gravity</u> of the tissues. In short-term ischemia of 1 hour duration, the cerebral water content was increased, but did not show any progression until 10 hours after the release of arterial occlusion. At this time, the percent of water content was more pronounced coinciding with the increased permeability of blood-brain barrier to Evans blue tracer. A week later only half of the animals showed recovery. In long-term ischemia, progressive accumulation of water content was observed with prolonged duration of ischemia.		

Project Description:

Objectives: In human cerebral ischemia, brain edema is considered to be an important factor in causing mortality (Shaw, C., Alvord, E., and Berry, R., *Arch. Neurol.* 1: 161-177, 1959). Experimentally, cerebral ischemia can be easily produced in Mongolian gerbils by ligation of a single common carotid artery (Levine, S., Payan, H., *Exp. Neurol.* 16: 252-255, 1966; Kahn, K., *Arch. Pathol.* 69: 544-553, 1972; Ito et al., *Acta Neuropath.* 32: 209-223, 1975). In our recent studies of ischemic brain edema in gerbils, a progressive decrease in percent of dry weight (i.e., an increased water content) with a net loss of potassium and with a net gain of Na was observed in the affected hemisphere as compared to the unaffected and the control hemisphere in long-term ischemia. The present investigation has been a continuation of this study to determine the changes occurring in the brain after short periods of ischemia and various recovery periods as compared to the long period of ischemia.

Methods Employed: Several groups of adult gerbils were subjected to unilateral clipping and clip release of the left common carotid artery for various periods of time. Only the gerbils with definite cerebral symptoms were selected for this study. Two different methods were used for the determination of cerebral water content: (1) wet and dry weights, and (2) specific gravity, which allows the assay of small samples of brain tissue and therefore, regional alteration in the water content can be established (Nelson et al., *J. Appl. Physiol.* 30: 2680271, 1971).

Major Findings: An increase in water content of the brain tissue was observed already after 15 minutes of common carotid artery occlusion. In short-term ischemia of 1 hour duration, the cerebral water content (determined by both methods) was increased, but showed little variation until 10 hours following clip release. At this time, an increased BBB permeability to Evans blue albumin complex was seen in the brain. A week later, the examined animals could be divided into two groups of which one showed complete recovery only. In long-term ischemia, the reduction of the specific gravity in the cortex, basal ganglia and hippocampus progressed with the length of occlusion as was previously observed by the wet and dry weight determination of water content. The contributing factors in the recovery periods such as increased blood-brain barrier permeability and tissue necrosis, which most probably are responsible for secondary increased in cerebral water content, are under evaluation.

Significance to Biomedical Research and the Program of the Institute: Cerebral edema occurs as one of the major complications of many neurological disorders such as ischemia, trauma, tumors, chemical poisoning, and others. The basic understanding of the type of edema and its development is very crucial for the clinician who is faced not only with the diagnosis, but with the appropriate selection of treatment. Thus, various investigations of this problem are essential for finding the factor or factors responsible for the occurrence of cerebral edema and its treatment.

Project No. Z01 NS 02000-07 LNNS

Proposed Course of the Project: The study of brain edema has been extended to the model of complete ischemia in gerbils. Another manuscript is in preparation.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02084-06 LNNS									
PERIOD COVERED October 1, 1978 to September 30, 1979											
TITLE OF PROJECT (80 characters or less) Properties of cerebral capillaries in organotypic cultures											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 33%;">PI: M. Spatz</td> <td style="width: 33%;">Head, Section on Neurocytol.</td> <td style="width: 33%;">LNNS NINCDS</td> </tr> <tr> <td>Other: M. R. Murray</td> <td>Research Biologist</td> <td>LNNS NINCDS</td> </tr> <tr> <td>I. Klatzo</td> <td>Chief, Lab. Neuropath. Neuroanat. Sci.</td> <td>LNNS NINCDS</td> </tr> </table>			PI: M. Spatz	Head, Section on Neurocytol.	LNNS NINCDS	Other: M. R. Murray	Research Biologist	LNNS NINCDS	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS
PI: M. Spatz	Head, Section on Neurocytol.	LNNS NINCDS									
Other: M. R. Murray	Research Biologist	LNNS NINCDS									
I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS									
COOPERATING UNITS (if any) J. U. Bubis, The Chaim Sheba Medical Center, Tel Hashomer, Israel; J. Renkawek, Institute of Neuropathology, Polish Academy of Sciences, Warsaw, Poland											
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences											
SECTION Section on Neurocytobiology											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205											
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) This project has been discontinued for the present time.											

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02166-05 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Synaptosomal uptake of neutral amino acids		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. Spatz Head, Section on Neurocytobiology LNNS NINCDS Other: I. Klatzo Chief, Lab. Neuropath. Neuroanat. Sci. LNNS NINCDS		
COOPERATING UNITS (if any) D. Micic, Institute of Biochemistry, Faculty of Medicine, Belgrade, Yugoslavia		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project has been completed and the manuscript has been published. Micic, D., Swink, M. E., Klatzo, I., and Spatz, M.: Transient ischemic alteration of synaptosomal neutral amino acid uptake. <u>Experientia</u> 34: 1461, 1978.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02197-04 LNNS									
PERIOD COVERED October 1, 1978 to September 30, 1979											
TITLE OF PROJECT (80 characters or less) Demonstration of ATPase in cerebellar cultures											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 30%;">PI: M. Spatz</td> <td style="width: 40%;">Head, Section on Neurocytobiol.</td> <td style="width: 30%;">LNNS NINCDS</td> </tr> <tr> <td>Other: M. R. Murray</td> <td>Res. Bio.</td> <td>LNNS NINCDS</td> </tr> <tr> <td>I. Klatzo</td> <td>Chief, Lab. Neuropath. Neuroanat. Sci.</td> <td>LNNS NINCDS</td> </tr> </table>			PI: M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS	Other: M. R. Murray	Res. Bio.	LNNS NINCDS	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS
PI: M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS									
Other: M. R. Murray	Res. Bio.	LNNS NINCDS									
I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS									
COOPERATING UNITS (if any) K. Renkawek, Institute of Neuropathology, Polish Academy of Sciences, Warsaw, Poland											
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences											
SECTION Section on Neurocytobiology											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205											
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) This project has been discontinued for the present time.											

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02198-04 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Ischemic and postischemic effect on the uptake of neutral amino acids in isolated cerebral capillaries		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. Spatz Head, Section on Neurocytobiology LNNS NINCDS Other: I. Klatzo Chief, Lab. Neuropath. Neuroanat. Sci. 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 MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This part of the investigation is completed and two manuscripts have been published. Micic, D., Swink, M., Micic, J., Klatzo, I., and Spatz, M.: The ischemic and postischemic effect on the uptake of neutral amino acids in isolated cerebral capillaries. <u>Experientia</u> 35, 625-626, 1979. Spatz, M., Micic, D., Fujimoto, T., Mrsulja, B. B., and Klatzo, I.: Transport phenomena in cerebral ischemia. In Mrsulja, B. B., Rakic, Lj. M., Klatzo, I., and Spatz, M. (Eds.): <u>Pathophysiology of Cerebral Energy Metabolism</u> . New York, Plenum Press, 1979, pp. 148-153.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02275-03 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Cerebral capillary endothelial cultures		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. Spatz Head, Section on Neurocytobiology LNNS NINCDS Other: M. Murray Research Biologist LNNS NINCDS I. Klatzo Chief, Lab. Neuropath. Neuroanat. Sci LNNS NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIR, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.6	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Cerebral capillary endothelial cell cultures have been established from isolated cerebral capillaries of 2-day-old rats. The cells have the same characteristic enzymatic properties of capillary endothelial cells as ones seen in cerebral microvessels <u>in situ</u> .		

Project Description:

Objectives: The availability of a simple technique for isolation of cerebral capillaries (Mrsulja, B. B., Mrsulja, B. J., Klatzo, I., and Spatz, M., Brain Res. 110: 361-365, 1976) provided the impetus for establishment of cerebral capillaries as an organotypic and dissociated cell culture for morphological, histochemical and biochemical studies under normal and pathologic conditions.

Methods Employed: The cerebral capillaries were separated from the non-vascular tissue of 2-day-old rats by homogenization, centrifugation and sucrose gradient under sterile conditions. The cellular pellet was washed 4 times in Simms' balanced solution (BSS) containing antibiotics for a 30 minute period, centrifuged at 1000 rpm and resuspended in fresh BSS. Thereafter the capillaries were suspended in 25 ml Trypsin-Versene solution and dissociated for 30 minutes. The process was repeated after 10 minutes of recentrifugation at 1000 rpm. The cultures kept in T-60 flasks or in Petri dishes have been cultivated in a mixture of 199 medium containing 30% fetal calf serum, amino acids, MEM vitamin solution and antibiotics for 1 week. Thereafter the cultures are fed twice a week with the same medium but with a reduced content of fetal calf serum (20%).

Major Findings: We succeeded in establishing capillary endothelial cultures from dissociated cells of isolated cerebral microvessels. Cell cultures grow as sheets or as a network of elongated cells in which the activity of alkaline phosphatase, γ -glutamyl transpeptidase, butyryl cholinesterase and the uptake of L-dopa can be demonstrated by histochemical and fluorescent techniques, respectively. The cellular properties thus observed are characteristic of cerebral capillary endothelium. Therefore, these cultures provide a model for the study of normal and altered endothelial function. This study is to be presented at the 55th Annual Meeting of the American Association of Neuropathologists, July 7-10, 1979.

Significance for Biomedical Research and the Program of the Institute: The establishment of cerebral capillary endothelial cell cultures will provide a pure cell line which will be useful for the investigation of cerebral endothelial cells in the living state without the influence of any other cells. Thus, the function of cerebral capillary endothelium as compared to endothelium derived for capillaries not belonging to the blood-brain barrier (BBB) system can be characterized under normal and pathologic conditions. This approach will also add another dimension for the studies related to the BBB permeability.

Proposed Course of the Project: The primary objective of this project has been to obtain an easily reproducible endothelial cell line which will provide sufficient material for morphological and histochemical investigations of the cerebral capillary endothelial properties in the living state as compared to the one in situ. Thereafter various functional studies of these cells and the ones derived from capillaries of other organs will be studied using histochemical, immunological and radioautographic techniques.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02277-03 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Biochemistry of cerebral microvessels		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. Spatz Head, Section on Neurocytobiology LNNS NINCDS Other: I. Klatzo Chief, Lab. Neuropath. Neuroanat. Sci. LNNS NINCDS		
COOPERATING UNITS (if any) B. B. Mrsulja, B. M. Djuricic and B. Cjevic, Institute of Biochemistry, and B. J. Mrsulja, Institute of Biology, Faculty of Medicine, Belgrade, Yugoslavia		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project is completed.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02279-03 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Electrolyte changes in ischemic cerebral edema		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. Spatz Head, Section on Neurocytobiology LNNS NINCDS Other: I. Klatzo Chief, Lab. Neuropath. Neuroanat. Sci. LNNS NINCDS		
COOPERATING UNITS (if any) H. Pappius, Montreal Neurological Institute, Montreal, Canada; T. Fujimoto and K. Nishimoto, Tokyo Medical and Dental University, Tokyo, Japan		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project is completed and the resulting manuscript has been published. Pappius, H. M., Fujimoto, T., Nishimoto, K., Klatzo, I., and Spatz, M.: Cerebral water and electrolyte content following ischemia and blood brain barrier disturbances. In Mrsulja, B. B., Rakic, Lj, M., Klatzo, I., and Spatz, M. (Eds.): <u>Pathophysiology of Cerebral Energy Metabolism</u> . New York, Plenum Press, 1979, pp. 91-98.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02280-03 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) The effect of cerebral ischemia and postischemia on monoamine oxidase activity (MAO)		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. Spatz Head, Section on Neurocytobiology LNNS NINCDS Other: I. Klatzo Chief, Lab. Neuropath. Neuroanat. Sci. LNNS NINCDS		
COOPERATING UNITS (if any) D. Micic, Institute of Biochemistry, Faculty of Medicine, Belgrade, Yugoslavia		
LAS/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project has been completed and one of the resulting manuscripts has been published. Micic, D., Klatzo, I., and Spatz, M.: The effect of sodium pentobarbital on some mitochondrial enzymes. <u>J. Neurochem.</u> 30: 1627-1628, 1978.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02324-02 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) The ³ H-norepinephrine uptake and fate in the isolated cerebral capillaries		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: T. Abe Visiting Fellow LNNS NINCDS Other: K. Abe Visiting Fellow LNNS NINCDS I. Klatzo Chief, Lab. Neuroanat. Sci. 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 MANYEARS: 0.8	PROFESSIONAL: 0.6	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The investigations of ³ H-norepinephrine uptake in the isolated capillaries suggest that it is taken up by saturable carrier mediated process. However, the ³ H-norepinephrine is not retained as such but metabolized in the capillaries.		

Project Description:

Objectives: In vivo studies had shown that norepinephrine doesn't cross the blood brain barrier (Weil-Malherbe et al., *J. Neurochem.* 8: 55-64, 1961). In order to elucidate the mechanism responsible for the reported observations the uptake of ^3H norepinephrine was investigated in isolated capillaries which were previously proven to be metabolically active and suitable for such studies (Mrsulja et al., *Brain Res.* 110: 361-365, 1976).

Methods Employed: The isolated cerebral capillaries were incubated with ^3H norepinephrine in Ringer's solution containing .1% albumin (pH 7.4) alone or with various concentrations of unlabeled (cold) norepinephrine, L-dopa, dopamine, epinephrine, metaraminol, normetanephrine and metanephrine for various periods of time. The inhibitory effect of catechol-O-methyl transferase and MAO in the capillary uptake of ^3H norepinephrine was determined by adding pyragallo and pargyline to the incubating solution, respectively. Thin layer chromatography was used for the identification of the metabolites.

Major Findings: The isolated capillaries took up the ^3H norepinephrine and the labeled substance increased with the duration of incubation (2-60 minutes). The uptake of ^3H norepinephrine in the capillaries was found to be saturable since it was inhibited by increasing concentrations of unlabeled (cold) norepinephrine when it was added to the incubating media containing the labeled substrate. The capillary ^3H uptake of norepinephrine was also cross inhibited by addition of cold L-dopa, dopamine, epinephrine and metaraminol but not by normetanephrine or metanephrine in concentrations of 1-2 mole. Pyragallo, the known inhibitor of catechol-O-methyl transferase competitively inhibited the uptake of ^3H norepinephrine in the isolated capillaries. Moreover, capillary ^3H norepinephrine uptake was competitively inhibited by S-adenosylmethionine but not by S-adenosylhomocysteine.

The accumulated substances in the capillaries were found to be methylated and deaminated metabolites of norepinephrine.

Significance to Biomedical Research and the Program of the Institute: These results suggest that the uptake of norepinephrine takes place by carrier mediated process (which may be shared by other catecholamines) but the norepinephrine is not accumulated as such since it is metabolized by the catechol-O-methyl transferase and MAO present in the capillaries. These findings also indicate that the capillaries are probably unable to retain the norepinephrine after the inhibition of the enzymes since the inhibition of methyl transferase and MAO inhibited also the "uptake" of norepinephrine. Therefore the cerebral capillaries are the site of enzymatic barrier which prevents the intact norepinephrine from entering or leaving the brain.

Proposed Course of the Project: These investigations have been extended to other members of the catecholamine family and this study is being prepared for publication.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02326-02 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Changes in the capillary lactate and 2-deoxy-D-glucose uptake in developing brain		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. Spatz Head, Section on Neurocytobiology LNNS NINCDS		
COOPERATING UNITS (if any) D. Micic, B. B. Mrsulja, Institute of Biochemistry, Faculty of Medicine, Belgrade, Yugoslavia		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project has been completed and the manuscript published. Spatz, M., Micic, D., Mrsulja, B. B., Swink, M., and Micic, J.: Changes in the capillary lactate and 2-deoxy-D-glucose uptake in developing brain. <u>Brain Res.</u> 151: 619-632, 1978.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02327-02 LNNS									
PERIOD COVERED October 1, 1978 to September 30, 1979											
TITLE OF PROJECT (80 characters or less) The uptake of biogenic amines into the cells of pia arachnoid cultures. A histofluorescence study											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 33%;">PI: H. Hervonen</td> <td style="width: 33%;">Visiting Fellow</td> <td style="width: 33%;">LNNS NINCDS</td> </tr> <tr> <td>Other: M. Spatz</td> <td>Head, Section on Neurocytobiology</td> <td>LNNS NINCDS</td> </tr> <tr> <td>M. R. Murray</td> <td>Research Biologist</td> <td>LNNS NINCDS</td> </tr> </table>			PI: H. Hervonen	Visiting Fellow	LNNS NINCDS	Other: M. Spatz	Head, Section on Neurocytobiology	LNNS NINCDS	M. R. Murray	Research Biologist	LNNS NINCDS
PI: H. Hervonen	Visiting Fellow	LNNS NINCDS									
Other: M. Spatz	Head, Section on Neurocytobiology	LNNS NINCDS									
M. R. Murray	Research Biologist	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 MANYEARS: 1.0	PROFESSIONAL: 0.7	OTHER: 0.3									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) The uptake of biogenic amines and a precursor, <u>L-dopa</u> , into the cells of <u>pial cultures</u> has been studied by <u>histochemical techniques</u> with special attention to <u>capillary endothelial cells</u> , which took up L-dopa only. This work is completed and a manuscript is being prepared for publication.											

Project Description:

Objectives: The aim of this study is to explore the characteristics of pia-arachnoid cells toward exogenous catecholamines, with special reference to capillary endothelial cells, in order to evaluate the blood-brain barrier function.

Methods Employed: The pia-arachnoid membrane was prepared from newborn rats and cultured on glass in a Maximow double coverslip assembly for 2-3 weeks.

The incubations for the catecholamine uptake were performed in a Hepes-buffered (20 mM) Locke's salt solution, pH 7.4, at room temperature. The cultures were first briefly washed to remove the culture medium, then preincubated for 10 minutes with or without pargyline (a monoamine oxidase inhibitor) and pyrogallol (a catechol-O-methyl transferase inhibitor). The incubation time was 10 minutes, again with or without pargyline and pyrogallol according to the preincubation. The following biogenic amines and precursors were used in 10^{-5} - 10^{-6} M concentrations: L-dopa, dopamine, noradrenalin, adrenalin and serotonin. After incubation the cultures were washed in Hepes-Locke's solution for 5 seconds - 10 minutes before processing for either formaldehyde-induced fluorescence or glyoxylic acid-induced fluorescence.

Zeiss Axiomat microscope was used to observe the fluorescence operating either with transmitted light with EG 12 excitation filter, dark-field condensor and LP 500 barrier filter or with epi-illumination with BG 12 and BP 405 excitation filters, LP 470 barrier filter and a dichroic mirror. The same microscope was used for phase-contrast microscopy. Photography was performed using Zeiss automatic camera using Kodak Panatomic or Tri-x-pan film.

Major Findings: Norepinephrine uptake: The incubation with 10^{-2} M norepinephrine yielded an intracellular concentration high enough to be observed as bright fluorescence by histofluorescence method in all cell types in cultures, namely capillary endothelial cells, pericytes and pial cells. The 10^{-3} M concentration yielded no detectable fluorescence. When the intermediate concentrations were used lower histofluorescence intensity was observed with 10^{-4} M concentration than with 10^{-3} M concentration. Moreover, with these concentrations brighter fluorescence was observed in the endothelial cells and the pericytes than in the pial cells.

The fluorescence intensity declined with prolongation of the washing time after incubation. The use of the enzyme inhibitors in the incubations seems not to have an effect to increase the fluorescence intensity in any of the conditions.

Dopamine gives results parallel to those with norepinephrine while the use of adrenalin (and serotonin?) yielded lower levels of fluorophore in identical conditions.

L-dopa yields higher fluorophore concentrations than norepinephrine at the same concentrations, especially in the endothelial cells, suggesting a higher permeability of the endothelial cell membrane to the precursor than to the catecholamines.

Significance to Biomedical Research and the Program of the Institute:
The study will bring new knowledge on the blood brain barrier function of the pial vessels.

Proposed Course of the Project: This work is completed and the manuscript is being prepared for publication.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02328-02 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) The effect of cholinesterase inhibitors on nerve cells developing in cultures of spinal and sympathetic ganglia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: H. Hervonen Visiting Fellow LNNS NINCDS Other: M. R. Murray Research Biologist 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 MANYEARS: 0.9	PROFESSIONAL: 0.6	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The inhibition of <u>cholinesterases</u> , especially <u>acetylcholinesterase</u> , in the <u>developing neuroblast of the spinal and sympathetic ganglion</u> leads into a growth inhibition, abnormal changes in the morphology of the cells and degeneration of the neurons strongly supporting the hypothesis of an important <u>role of acetylcholinesterase in the maturation of the neurons.</u>		

Project Description:

Objectives: Acetylcholinesterase activity appears early during the development in the neurons of various parts of the central and peripheral nervous system, already before the onset of cholinergic neurotransmission as in sympathetic ganglia or in neurons, which are neither cholinergic nor cholinceptive as in the neurons of spinal ganglion. This has led to an idea that acetylcholinesterase might play a role in the maturation process of neurons (see e.g. Silver, 1974). The aim of this study was to test this hypothesis by studying the effect of cholinesterase inhibition on the development of the neurons in cultures of spinal and sympathetic neurons.

Methods Employed: The spinal and sympathetic ganglia were prepared from 8-day-old chick embryos and cultured in Maximow double coverslip assembly up to 4 weeks *in vitro*. The inhibitors were added to the culture medium for the whole culture period in concentrations 10^{-6} - 10^{-3} M. The following inhibitors were used: Eserine (physostigmine), iso-OMPA, BW 274 C 51, DFP and paraoxon.

The effect of the inhibitors was estimated by light microscopy of the living cultures and after fixation and staining with cresyl violet or Holmes' silver impregnation.

Major Findings: The inhibitors of cholinesterases in general have little effect *in vitro* on growth and maturation of embryonic sensory and sympathetic neurons, with the exception of the specific cholinesterase (AChE) inhibitors which when present in the medium bring about growth stoppage and gradual pathological changes in neuronal morphology.

Significance to Biomedical Research and the Program of the Institute: The significance of this study is to further explore the role(s) of an enzyme/a group of enzymes (acetylcholinesterase/cholinesterases) which have a widespread occurrence in the nervous system.

Proposed Course of the Project: This investigation has been extended to the electron microscopical level and the evaluation of the above changes is in progress.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02357-01 LNNS									
PERIOD COVERED October 1, 1978 to September 30, 1979											
TITLE OF PROJECT (80 characters or less) The therapeutic chemical effect on experimental cerebral ischemia in Mongolian gerbils											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 33%;">PI: M. Smialek</td> <td style="width: 33%;">Visiting Scientist</td> <td style="width: 33%;">LNNS NINCDS</td> </tr> <tr> <td>Other: I. Klatzo</td> <td>Chief, Lab. Neuropath. Neuroanat. Sci.</td> <td>LNNS NINCDS</td> </tr> <tr> <td>M. Spatz</td> <td>Head, Section on Neurocytobiology</td> <td>LNNS NINCDS</td> </tr> </table>			PI: M. Smialek	Visiting Scientist	LNNS NINCDS	Other: I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS	M. Spatz	Head, Section on Neurocytobiology	LNNS NINCDS
PI: M. Smialek	Visiting Scientist	LNNS NINCDS									
Other: I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	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 MANYEARS: 0.5	PROFESSIONAL: 0.3	OTHER: 0.2									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) The investigations of <u>endogenously occurring central nervous system depressants</u> γ -hydroxybutyrate (GHB) and its lactone γ -butyrolactone have proven to be more effective than the endogenous CNS depressant (pentobarbital) as <u>preventive and therapeutic agents</u> of cerebral ischemic induced in Mongolian gerbils.											

Project Description:

Objectives: The search for a therapeutic agent which could greatly influence the clinical and metabolic events of cerebral ischemia led to investigations of central nervous system depressants. Both the exogenous barbiturate and the endogenous depressants, such as γ -hydroxybutyrate (GHB) and its lactone γ -butyrolactone (GBL) were reported to modify the metabolism of hypoxic animals.

Methods Employed: Fifteen minutes of bilateral common carotid artery occlusion served as a model for the production of cerebral ischemia in gerbils. The treatment consisted of a single injection of either Na pentobarbital (20 or 55 mg/kg) or GHB or GBL (500 and 300 mg/kg, respectively). The preischemic group (50-65 gerbils/group) was injected 2 minutes (with the exception of pentobarbital which was also given 30 minutes) prior to the occlusion and the postischemic group (50-70 gerbils/group) received the injection 1 or 2 or 3 hours following clip release; sham operated, saline injected and untreated groups (except for Na pentobarbital anesthesia, 20 mg/kg) of gerbils served as respective controls. The clinical behavior and the survival rate were followed up for one month. In addition constituents of carbohydrate metabolism were determined in the brains frozen in situ of animals from some of the respective but separately prepared groups.

Major Findings: The outcome of cerebral ischemia was influenced by CNS depressants as was indicated by a greater survival rate of the treated than untreated gerbils subjected to the bilateral deprivation of blood supply. The effectiveness depended not only on the timing of such treatment but also on the type of the used chemical. The metabolic investigations suggested that the GBL and GHB pretreatments have a greater modifying effect on the carbohydrate and energy metabolites than that of pentobarbital. GBL, GHB and pentobarbital acted similarly in reducing the accumulation of lactate (except pentobarbital given 2 minutes prior to clipping) maintaining normal concentration of pyruvate without preventing the drop of cerebral glucose and glycogen level in ischemia. However, divergent results were seen with GBL and GHB as compared to the pentobarbital pretreatment which were manifested by lesser reduction of ATP and P-creatine in GBL and GHB as compared to the pentobarbital pretreated and untreated animals. The dissimilar activity continued into the recovery period when 5 minutes after clip release the glucose, glycogen and lactate levels recovered in GBL and GHB but not in pentobarbital injected gerbils.

Significance to Biomedical Research and the Program of the Institute: The beneficial therapeutic effect of the naturally occurring central nervous system depressants in the experimentally induced ischemia indicates that these substances might be useful clinically following a complete experimental evaluation of these agents.

Proposed Course of the Project: The effect of GBL and GHB on catecholamine and other metabolic pathways in the brain will be evaluated in the cerebral ischemia in order to elucidate the pathophysiological mechanism of their beneficial action.

Publications: See Project No. Z01 NS 02322-02 LNNS.

Smialek, M., Klatzo, I., and Spatz, M.: The therapeutic effect on experimental cerebral ischemia in Mongolian gerbils. In Proceedings of the 9th Salzburg Conference on Cerebral Vascular Diseases, September 27-30, 1978 (in press).

Klatzo, I. and Spatz, M.: Experimental cerebral edema. In Thompson, R. H. S. and Davidson, A. N. (Eds.): The Molecular Basis of Neuropathology. London, Edward Arnold Limited (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02358-01 LNNS																
PERIOD COVERED October 1, 1978 to September 30, 1979																		
TITLE OF PROJECT (80 characters or less) The postischemic effect on the uptake of monoamines in isolated cerebral capillaries																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>T. Abe</td> <td>Visiting Fellow</td> <td>LNNS NINCDS</td> </tr> <tr> <td>Other:</td> <td>K. Abe</td> <td>Visiting Fellow</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>I. Klatzo</td> <td>Chief, Lab. Neuropath. Neuroanat. Sci.</td> <td>LNNS NINCDS</td> </tr> <tr> <td></td> <td>M. Spatz</td> <td>Head, Section on Neurocytobiology</td> <td>LNNS NINCDS</td> </tr> </table>			PI:	T. Abe	Visiting Fellow	LNNS NINCDS	Other:	K. Abe	Visiting Fellow	LNNS NINCDS		I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS		M. Spatz	Head, Section on Neurocytobiology	LNNS NINCDS
PI:	T. Abe	Visiting Fellow	LNNS NINCDS															
Other:	K. Abe	Visiting Fellow	LNNS NINCDS															
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	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 MANYEARS: 0.75	PROFESSIONAL: 0.55	OTHER: 0.2																
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINDRS <input type="checkbox"/> (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) The <u>cerebral microvessels</u> isolated from brains of gerbils subjected to 15 minutes of <u>complete cerebral ischemia</u> and various periods of <u>recovery</u> had shown an increased <u>monoamine uptake</u> which mirrored the increased uptake of these substances across the <u>blood-brain barrier</u> observed in <u>in vivo</u> studies. Thus, the results suggest that the capillaries are the site responsible for the altered passage of the amine from blood to brain.																		

Project Description:

Objectives: The isolated cerebral microvessels have been useful in the investigation of transport processes occurring in the blood brain level (Spatz et al., in Pathophysiology of Cerebral Energy Metabolism, New York, Plenum Press 1979, pp. 143-153). Recently we have shown a postischemic increase in the brain uptake of monoamines which under normal conditions don't penetrate the blood brain barrier (BBB). In order to elucidate the mechanism responsible for these observations we undertook the evaluation of the monoamine uptake in isolated cerebral capillaries.

Methods Employed: The cerebral capillaries were isolated from brain of gerbils subjected to 15 minutes of bilateral carotid artery occlusion and various periods of release. The procedures for the determination of ^3H labeled norepinephrine (NE) or 5-hydroxytryptamine (5HT) or metaraminol (M) were similar to those used for the uptake of 2-deoxy-D-glucose (Spatz et al., Brain Res. 120, 141-145, 1977).

Major Findings: The cerebral microvessels isolated from brains of gerbils subjected to 15 minutes' deprivation of blood supply and various periods of recovery had shown an increase of monoamine uptake which completely reflected the state of BBB permeability observed in in vivo studies. Thus, a greater uptake of norepinephrine, metaraminol and 5-hydroxytryptamine was found in microvessels obtained from the experimental brain when compared to the monoamine uptake in capillaries separated from control brains. However, the capillary ^3H monoamine uptake of both (experimental and control) was equally susceptible to the inhibition with their respective unlabeled substrate. The similarity in the behavior of the microvessels in regard to the metabolizing (NE and 5HT) and nonmetabolizing (M) monoamines is not only due to altered capillary metabolism but also due to increased uptake of the monoamines.

Significance to Biomedical Research and the Program of the Institute: Based on our investigation, the cerebral capillaries are useful for the study of some parameters of brain transport phenomena occurring in both physiological and pathological conditions. The knowledge of the functional state of cerebral capillaries is extremely important, since it may either be responsible for many metabolic changes occurring in the brain and/or it may reflect the altered metabolic state of the brain in many disease processes.

Proposed Course of the Project: This project is incomplete as yet. A similar model will be used for evaluation of other catecholamines' capillary uptake as well as release studies in ischemic and postischemic gerbils. Moreover the capillary uptake of monoamines will be correlated with the activity of enzymes involved in the synthesis and metabolism of the respective substrate. This work will be presented at the Second International Belgrade Symposium on Pathophysiology of Cerebral Metabolism, September 1979.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02359-01 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) ³ H metaraminol uptake in isolated cerebral capillaries		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: T. Abe Visiting Fellow LNNS NINCDS Other: K. Abe 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 MANYEARS: 0.7	PROFESSIONAL: 0.5	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The elucidation of the mechanism involved in the monoamine uptake in the cerebral capillaries has been investigated by using ³ H metaraminol, a norepinephrine analogue which is neither metabolized by COMT or MAO. These studies have shown that the ³ H metaraminol is taken up by K ⁺ and Na ⁺ dependent carrier mediated process in the isolated cerebral microvessels.		

Project Description:

Objectives: Norepinephrine, which doesn't cross the blood brain barrier, can be taken up and metabolized in isolated cerebral microvessels showing features of both extraneuronal and neuronal uptake. In order to elucidate further the mogoamine's uptake in cerebral microvessels, we investigated the uptake of ^3H metaraminol, a norepinephrine analogue which is neither metabolized by MAO nor COMT.

Methods Employed: The isolated cerebral capillaries were incubated with ^3H metaraminol in Ringer's solution containing .1% albumin (pH 7.4) alone or with various concentrations of unlabeled (cold) metaraminol, L-dopa, dopamine, 5-hydroxydopamine, 6-hydroxydopamine, norepinephrine, 5-hydroxytryptamine, normetanephrine and metanephrine. The effect of metabolic inhibitors and adrenergic blocking agents on the uptake of ^3H metaraminol was also evaluated under physiological conditions.

Major Findings: The capillary uptake of ^3H metaraminol increased with the time of incubation (30 sec-15 min). The uptake was found to be saturable, because it could be inhibited by addition of unlabeled metaraminol in increasing concentrations to the incubation media containing the labeled substance. The accumulation of ^3H metaraminol in the capillaries was stimulated by K^+ and Na^+ and inhibited by ouabain, KCN, DPN, adrenergic blocking agents (imipramine, propranolol, dichloroisoproterenol and phentolamine). Moreover, the ^3H metaraminol capillary uptake was competitively inhibited by arterenol, 5-hydroxytryptamine and cross inhibited by dopamine, 6-hydroxydopamine, 5-hydroxydopamine, L-dopa but not by normetanephrine or metanephrine.

Significance to Biomedical Research and the Program of the Institute: These results indicate that ^3H metaraminol is taken up by K^+ and Na^+ dependent carrier-mediated mechanism (which may be shared by other monoamines) in the cerebral microvessels. This process appears to be similar to the one described for neuronal monoamine uptake especially since extraneuronal uptake of amines was reported to be insensitive to metaraminol but sensitive to normetanephrine and metanephrine.

Proposed Course of the Project: These investigations are still in progress and several kinetic parameters have to be established before part of this work will be presented at the Neuroscience meeting in the fall of 1979.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02360-01 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (90 characters or less) The effect of central nervous system depressants on ischemic cerebral edema of gerbils		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: K. Abe Visiting Fellow LNNS NINCDS Other: T. Abe Visiting Fellow LNNS NINCDS I. Klatzo Chief, Lab. Neuropath. Neuroanat. Sci. 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 MANYEARS: 0.75	PROFESSIONAL: 0.55	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The <u>endogenous central nervous system depressants</u> , γ -hydroxybutyrate (GBH) and its lactone γ -butyrolactone (GBL), have proven to be beneficial in the treatment of <u>ischemic cerebral edema</u> although they were ineffective in preventing the development of such edema.		

Project Description:

Objectives: Recently, we had shown that the outcome of cerebral ischemia can be modified by treatment with the naturally occurring central nervous system depressants [γ -hydroxybutyrate (GHB) or γ -butyrolactone (GBL)]. The effectiveness of these compounds was manifested by amelioration of cerebral carbohydrate metabolism and increased survival rate of Mongolian gerbils subjected to bilateral common carotid artery occlusion for 15 min (Smialek, Klatzo and Spatz, Proc. 9th Internat. Salzburg Conf., 1978). Since edema is one of the most conspicuous features of cerebral ischemia, the evaluation of GHB and GBL effect on the formation and treatment of this type of edema was undertaken in the bilateral ischemia.

Methods Employed: Fifteen minutes of bilateral common carotid artery occlusion served as a model for the production of cerebral ischemic edema in gerbils. The treatment consisted of a single intravenous injection of either GHB (500 mg/kg) or GBL (300 mg/kg) either 2 minutes prior to or 2 or 3 hours following the occlusion. The changes in the BBB permeability to radiolabeled substances representative of various transport systems were correlated with the changes in the specific gravity of cortex, hippocampus and basal ganglia in order to assess the extent of edema formation and its susceptibility to the action of CNS depressants.

Major Findings: A progressively increased selective permeability change of the BBB and regional decrease in the cerebral specific gravity were observed in gerbils recovering from bilateral carotid artery clipping of 15 min duration. In the first few hours following the arterial clip release, the altered BBB permeability and the specific gravity values in the GHB or GBL treated gerbils were not different from the ones observed in the untreated animals. However, within 24 hrs an improvement with almost complete restoration of normal BBB permeability and the values of the brain's specific gravity were found 7 days after the reestablishment of blood circulation in the treated animals. Both compounds acted similarly and its effect was independent of the time of administration whether given 2 min prior to or 2 or 3 hr after clip release. These findings suggest that GHB or GBL do not prevent the development of ischemic cerebral edema but are beneficial for its treatment.

Significance to Biomedical Research and the Program of the Institute: The evaluation of chemicals which may modify cerebral ischemic sequelae such as cerebral edema is of utmost importance clinically. Further elucidation of the therapeutic pathomechanism involved in the beneficial effect of central nervous system depressants in experimental cerebral ischemia and edema will be useful in considering the safety of such compounds for human treatment. A part of this project will be presented at the Berlin Symposium on Brain Edema in the fall of 1979.

Proposed Course of the Project: This investigation is still in progress. This beneficial therapeutic effect of GBL and GHB will be compared with the effect of exogenous central nervous system depressants such as pentobarbital on ischemic edema.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02361-01 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) The effect of bilateral ischemia on the permeability of the blood brain barrier (BBB)		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: K. Abe Visiting Fellow LNNS NINCDS Other: I. Klatzo Chief, Lab. Neuropath. Neuroanat. Sci. 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, NIR, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The permeability of the blood-brain barrier has been evaluated in gerbils subjected to bilateral cerebral ischemia and postischemia. The BBB permeability was found to be selectively altered in the postischemic period but not during cerebral ischemia. This project is completed and a manuscript is being prepared for publication.		

Project Description:

Objectives: The aim of this study has been to investigate the permeability of BBB in bilateral cerebral ischemia, since unilateral ischemia produced selective and diverse effects of BBB function in the affected cerebral hemisphere (Spatz, Fujimoto, Go, in Dynamics of Brain Edema, Berlin-Heidelberg, Springer Verlag 1976, pp. 181-186).

Methods Employed: Several groups of adult gerbils were subjected to bilateral common carotid artery clipping for 3, 6 and 15 minutes with and without clip release. The following tracers have been used so far for the evaluation of the BBB: NaFl, Evans blue, ^{14}C sucrose, ^3H inulin, ^3H nor-epinephrine and ^3H 5-hydroxytryptamine.

Major Findings: The BBB permeability was found to be intact to NaFl and Evans blue during the 3, 6 and 15 minutes of bilateral common carotid artery occlusion. However, 30-50% of gerbils showed an increased BBB permeability to NaFl after 30 minutes of reestablished cerebral circulation. The incidence of increased BBB permeability to NaFl depended on the duration of ischemia not seen in animals with the released clip for 3 and 5 hrs following occlusion for 3 and 6 minutes, respectively. The incidence of the altered permeability increased up to 3 days following the release of 15 minutes occlusion, at which time the BBB permeability was also increased to neurotransmitters.

Significance to Biomedical Research and the Program of the Institute: The basic comprehension of the blood-brain barrier behavior and function concerned with the passage of nutrient and non-nutrient substances from blood to brain following cerebral ischemia is of major importance (1) for the understanding of the mechanism responsible for the development of ischemic edema, as well as elucidating other pathophysiological processes in cerebrovascular disease and many other neurological disorders, and (2) for selecting the best therapeutic approach to a given disease.

Proposed Course of the Project: This project is completed and a manuscript is being prepared for publication.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01995-07 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Morphological studies of myelin formation, breakdown, and regeneration		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: H. deF. Webster Associate Chief LNNS NINCDS Other: N. Sternberger Research Associate LNNS NINCDS B. Trapp Staff Fellow LNNS NINCDS Y. Itoyama Visiting Fellow LNNS NINCDS R. Quarles Chief, Myelin and Brain Development Section LNNS NINCDS		
COOPERATING UNITS (if any) Developmental and Metabolic Neurology Branch, NINCDS; Department of Neurology, Johns Hopkins Medical School, Baltimore, Md.; Departments of Neuropathology and Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, Mass.		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cellular Neuropathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 6.2	PROFESSIONAL: 4.2	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The long range goal of this project is to use <u>immunocytochemical staining</u> along with <u>light and electron microscopy</u> to study the <u>cellular mechanisms of myelin formation, breakdown and regeneration</u> . Nervous tissues from experimental animals and humans have been studied in the following current projects: 1) Localization of myelin-associated glycoprotein (MAG) in developing and mature nervous tissue, 2) Distribution of MAG and myelin basic protein (BP or P ₁) in multiple sclerosis (MS) lesions, 3) Comparison of the distribution of BP, small myelin basic protein (P ₂) and the major peripheral glycoprotein (P ₃) in peripheral myelin sheaths, and 4) Use of ethanolic phosphotungstic acid (E-PTA) to study the distribution of basic proteins in the cytoplasm of myelin forming oligodendrocytes..		

Project Description:

Objectives: 1) To use our modification of the unlabelled antibody enzyme (peroxidase-antiperoxidase, or PAP) method to study the distribution of MAG in newborn, developing, and mature rat nervous tissue. 2) Using specific antisera, to modify the PAP method so it can be used on paraffin sections of human nervous tissue to study the distribution of MAG and BP in multiple sclerosis lesions. 3) To prepare antisera to peripheral myelin proteins, P_2 and P_0 , and, using the PAP method, to compare the distribution of BP, P_2 and P_0 in peripheral myelin sheaths. 4) To define the distribution of E-PTA stained organelles and membranes in electron micrographs of myelin-forming oligodendroglia in tadpole optic nerves.

Methods Employed: 1) Groups of newborn and developing rats were fixed by perfusion with a mixture of formalin and mercuric chloride. Vibratome sections of CNS and PNS were immunostained with a specific antiserum to MAG and were mounted in glycerin on glass slides for examination with a differential interference contrast microscope. Semiquantitative estimates of oligodendroglial staining intensities were measured with an Optomax image analyzer and were used to calculate optical densities of the stained cells. 2) Paraffin sections of CNS and PNS from control and MS patients were immunostained with BP and MAG antisera according to the PAP method with various pretreatments and incubation intervals. The effects of postmortem autolysis were studied in rat CNS which also was used to compare BP and MAG distribution after immersion and perfusion fixation, after several intervals of formalin fixation, and after vibratome vs. paraffin sectioning. 3) P_0 and P_2 myelin proteins were isolated from bovine spinal roots, purified by polyacrylamide gel electrophoresis, mixed with complete Freund's adjuvant, and injected intradermally into rabbits. The specificity of the antisera was established by immunodiffusion; the P_0 and P_2 antisera were used in the PAP technique to immunostain developing and mature PNS tissue. 4) Myelinating optic nerves of *Xenopus* tadpoles were fixed in aldehyde, dehydrated, stained with an ethanolic solution of phosphotungstic acid (E-PTA) and embedded in epon. The distribution of staining was studied in thin sections that were examined electron microscopically.

Major Findings: 1) Myelin-associated glycoprotein was found in oligodendroglia before myelin formation began in the anterior commissure and was present in brainstem oligodendroglia and myelin sheaths in newborn rats. The staining intensity of oligodendroglia increased during early development and slowly declined during the period of rapid myelination. Myelin staining was confined to periaxonal regions of myelin sheaths and did not increase as large compact sheaths were formed. MAG antiserum also stained Schwann cells in developing trigeminal ganglia and periaxonal regions of peripheral myelin sheaths. 2) In postmortem human nervous tissue, better localization of BP and MAG was obtained by immunostaining paraffin blocks than by using stored frozen or formalin fixed tissue. The distribution of BP and MAG in the human CNS and PNS was the same as we found in the developing and mature rat nervous system. Both were found in myelin-forming oligodendroglia and immature sheaths.

Compact sheaths of the CNS and PNS contained BP but MAG was limited to periaxonal regions of myelinated fibers. In MS lesions, the most striking finding was the extension of decreased MAG immunostaining into white matter that appeared normal when treated with BP antiserum or luxol fast blue, a histological stain for myelin. In acute early MS lesions, the decrease in MAG immunostaining extended far beyond the margin of acute demyelination where the BP staining of degenerating sheaths often was increased. In chronic inactive plaques, the decreased MAG immunostaining was limited to relatively few fibers in a thin rim around each lesion. In shadow plaques, BP antiserum also stained some oligodendroglia. 3) In the rat, both PNS and CNS myelin sheaths contained BP (also called P_1 protein). P_0 and P_2 proteins were found exclusively in PNS sheaths. Antisera to BP and P_0 stained all PNS myelin sheaths uniformly. P_2 protein was not present in all myelin sheaths; when present, it was concentrated in Schmidt-Lanterman clefts. 4) During rapid optic nerve myelination, intense E-PTA staining was observed on cytoplasmic faces of paranodal terminal loops and on loosely wrapped oligodendroglial membranes found along inner and outer surfaces of compact myelin sheaths. Oligodendroglial microtubules also were heavily stained.

Significance to Biomedical Research and the Program of the Institute:

1) Our morphological study that used a specific antiserum and a sensitive immunocytochemical method has convincingly established the localization of MAG in developing and mature nervous tissue. Since it is found in oligodendroglia and Schwann cells early in development and subsequently becomes localized in periaxonal regions of myelinated fibers, it probably plays an important role in glial-myelin-axon interactions. 2) Additional evidence for the role of MAG in myelin sheath maintenance is the decreased periaxonal staining by MAG antiserum in otherwise normal myelinated fibers in margins of MS plaques. The decreased staining could be due to loss or breakdown of MAG, to altered MAG immunoreactivity or it could reflect changes in oligodendroglial processes and membranes not detected by routine histological methods. Since oligodendroglia are only stained by BP antiserum during myelin formation, BP stained oligodendroglia in MS lesions probably are remyelinating previously demyelinated axons. Finally, our results demonstrate the advantages in using specific antisera and immunocytochemical methods to study disease mechanisms. 3) Selective localization of a myelin protein such as P_2 in some sheaths and not in others is a new finding of major importance. Generally, larger sheaths contained P_2 , but the proportion of sheaths containing P_2 depends on the nerve examined, the species, and the developmental stage studied. When normal distributions have been established, alterations may be extremely useful in assessing abnormalities in myelin formation and patterns of demyelination observed in human neuropathies. 4) The results suggest that E-PTA stains myelin BP as it is being transported along microtubules to insertion sites on oligodendroglial surface membranes. While loosely wrapped, these surface membranes remain heavily stained. Much fainter staining is found in compact lamellae. Even though E-PTA is not a specific stain for BP, the results add to our understanding of intracellular synthesis and transport of basic proteins in myelin-forming oligodendroglia.

Proposed Course of the Project: To be continued. The above findings were presented at annual meetings of the Society for Neuroscience, the American Society for Neurochemistry, the American Neurological Association, the Winter Conference on Brain Research, and the 1979 Workshop on Immunocytochemistry.

Publications:

Tabira, T., Cullen, M. J., Reier, P. J., and Webster, H. deF.: An experimental analysis of interlamellar tight junctions in amphibian and mammalian CNS myelin. J. Neurocytol. 7: 489-583, 1978.

Trapp, B. D., Honegger, P., Richelson, E., and Webster, H. deF.: Morphological differentiation of mechanically dissociated fetal rat brain in aggregating cell cultures. Brain Res. 160: 117-130, 1979.

Cullen, M. J. and Webster, H. deF.: Remodelling of optic nerve sheaths and axons during metamorphosis in Xenopus Laevis. J. Comp. Neurol. 184: 353-362, 1979.

Sternberger, N. H., Quarles, R. H., Itoyama, Y., and Webster, H. deF.: Myelin-associated glycoprotein demonstrated immunocytochemically in myelin and myelin-forming cells of developing rat. Proc. Natl. Acad. Sci. USA 76: 1510-1514, 1979.

Trapp, B. D., McIntyre, L. J., Quarles, R. H., Sternberger, N. H., and Webster, H. deF.: Immunocytochemical localization of rat PNS myelin proteins: P₂ protein is not a component of all PNS myelin sheaths. Proc. Natl. Acad. Sci. USA 1979 (in press).

Itoyama, Y., Sternberger, N. H., Kies, M. W., Cohen, S. R., Richardson, E. P., Jr., and Webster, H. deF.: Immunocytochemical method to identify myelin basic protein in oligodendroglia and myelin sheaths of the human nervous system. Ann. Neurol. 1979 (in press).

Itoyama, Y., Sternberger, N. H., Quarles, R. H., Cohen, S. R., Richardson, E. P., Jr., and Webster, H. deF.: Immunocytochemical observations on the distribution of myelin-associated glycoprotein and myelin basic protein in multiple sclerosis lesions. Ann. Neurol. 1979 (in press).

Tabira, T. and Webster, H. deF.: E-PTA stains oligodendroglial surface membranes and microtubules in optic nerves during myelination. J. Neurol. Sci. 1979 (in press).

Bray, G. M., Cullen, M. J., Aguayo, A. J., and Rasminsky, M.: Node-like areas of intramembranous particles in the unensheathed axons of dystrophic mice. Neuroscience Letters 1979 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01996-07 LNNS
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Membrane structure in CNS tissue and subcellular brain fractions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. deF. Webster	Associate Chief	LNNS NINCDS
Other:	B. D. Trapp	Staff Fellow	LNNS NINCDS
	R. H. Quarles	Chief, Myelin and Brain Development Section	LNNS NINCDS

COOPERATING UNITS (if any) Developmental and Metabolic Neurology Branch, NINCDS;
J. M. Matthieu, University of Lausanne School of Medicine, Lausanne, Switzerland;
Department of Neurology, Johns Hopkins Medical School, Baltimore, Maryland

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Cellular Neuropathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.5	OTHER: 0.3
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CHECK APPROPRIATE BOX(ES)
 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINDRS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
 The long range goal of this project is to use electron microscopy and freeze fracture techniques to study the structure of myelin and cell membranes in CNS tissue and in subcellular fractions. Current projects are concerned with the biochemical characterization of myelin isolated from Xenopus tadpoles, and the distribution of PNS myelin proteins and membrane enzymes in fractions isolated by continuous gradient zonal centrifugation.

Project Description:

Objectives: 1) To isolate myelin from the CNS of Xenopus tadpoles, characterize its protein, lipid, and enzyme composition, and compare its composition with Xenopus frog and mammalian myelin. 2) To isolate and purify myelin from adult rabbit sciatic nerve by discontinuous gradient centrifugation and by continuous zonal centrifugation in order to compare the results obtained by the two procedures.

Methods Employed: 1) CNS myelin from stage 54-56 Xenopus tadpoles, Xenopus frogs, Osborn Mendel rats, and humans without neurological disease was isolated by the method of Norton and Poduslo. Proteins were separated on polyacrylamide slab gels and lipids were analyzed by thin layer and high performance liquid chromatography. Aliquots of isolated myelin were processed for electron microscopic study by fixation in aldehyde, post fixation in osmium, dehydration and epoxy embedding. 2) Adult rabbit sciatic nerve myelin was isolated by 2 sucrose gradient centrifugation methods. The discontinuous gradient method of Norton and Poduslo which has been used primarily for CNS myelin isolation was compared to a continuous zonal gradient method.

Major Findings: 1) The biochemical results show that myelin isolated from tadpoles is an immature form of Xenopus frog myelin. Isolated tadpole myelin contains high levels of the enzyme 2',3' cyclic nucleotide 3' phosphohydrolase (CNP) and a higher proportion of high molecular weight proteins than Xenopus frog or mature mammalian myelin. Tadpole myelin lipids contain a higher proportion of phospholipids and less galactolipid than mammalian myelin. 2) Two fractions were obtained from the discontinuous gradient. Both showed typical myelin membranes by electron microscopy and typical myelin proteins by gel electrophoresis. The continuous sucrose gradient provided 3 peaks. All showed typical myelin proteins but there were significant quantitative differences.

Significance to Biomedical Research and the Program of the Institute:

1) Optic nerves of Xenopus tadpoles have been used as a test system for myelinotoxic agents important in the pathogenesis of human demyelinating diseases. Since the biochemical composition of tadpole, rat and human CNS myelin is similar, the use of tadpole CNS as an in vivo test system seems justified. 2) Comparison of the two methods showed that the discontinuous gradient used for CNS myelin isolated can also be used to isolate PNS myelin. By using a continuous gradient, 3 fractions were isolated. The first two had high CNP activity and the third, at 0.57 M sucrose, yielded 92% of the material applied. Quantitative differences in the myelin protein content in the 3 fractions suggest that PNS myelin shows considerable heterogeneity.

Proposed Course of the Project: To be continued.

Publications:

Quarles, R. H., Webster, H. deF., Sakuragawa, N., Everly, J. L., Trapp, B. D., and Pasnak, C. F.: A biochemical comparison of Xenopus Laevis and mammalian myelin from the central and peripheral nervous systems. J. Neurobiol. 9: 217-228, 1978.

Matthieu, J.-M., Webster, H. deF., DeVries, G. H.: Glial versus neuronal origin of myelin proteins and glycoproteins studied by combined intraocular and intracranial labelling. J. Neurochem. 31: 93-102, 1978.

Matthieu, J.-M., Waehneltdt, T. V., Webster, H. deF., Bény, M., and Fagg, G.: Distribution of PNS myelin proteins and membrane enzymes in fractions isolated by continuous gradient zonal centrifugation. Brain Res. 1979 (in press).

Trapp, B. D., McIntyre, L. J., Quarles, R. H., Nonaka, G., Moser, A., Moser, H., and Webster, H. deF.: Biochemical comparison of myelin isolated from the central nervous system of Xenopus tadpoles. J. Neurochem. 1979 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE	PROJECT NUMBER Z01 NS 01805-11 LNNS
	NOTICE OF INTRAMURAL RESEARCH PROJECT	

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Membrane Structure and Cytosol Enzymes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. J. Anders	Guest Worker	LNNS NINCDS
Other:	D. Schmechel	Research Associate	LCS NIMH
	M. W. Brightman	Head, Section on Neurocytology	LNNS NINCDS
	P. Marangos	Chief, Unit on Neurochemistry	LCS NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	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 (200 words or less - underline keywords)
Distinct aggregates of small particles, or assemblies, characterize the plasma membranes of astrocytes. Having established the baseline of the number, shape and area of assemblies in developing and adult rat astrocytes, plasma membranes of reactive astrocytes are being examined for any morphological changes. The most striking difference in the cell membrane of reactive astrocytes is a change in the shape and an increase in the size of assemblies, especially within the plasma membranes of excrescences jutting into the subarachnoid space from the most superficial, marginal astrocytes. We are also examining assembly properties by adding various chemicals and drugs to primary cultures of astrocytes from 5-7 day old rats. In such astrocytes exposed to the protein inhibitor cycloheximide, assemblies are lost from the plasma membranes by 3 hours, although background particles and gap junctions persist. This evidence implies that assemblies are a protein with a high turnover rate. The immunocytochemical localization of neuron-specific enolase (NSE) has enabled us to distinguish, in vitro, small neurons from glial cells, which contain non-neuronal enolase (NNE). During development, migrating neurons either contain NNE only, or no detectable enolases. Only when the neurons become differentiated do they acquire NSE.

Project Description:

Objectives: To determine the nature and function of aggregates of small particles within plasma membranes of astrocytes by examining reactive astrocytes, and primary cultures. To follow the changes in neuron-specific enolase in developing neurons.

Methods Employed: Reactive astrocytes are produced by placing autonomic neurons on the surface of the medulla or by freezing a 2 mm area of dorsal cortex with a brass rod cooled by dry ice. Primary cultures of astrocytes are established from dissociated cerebral cortex of 5 to 7 day old rats. Protein inhibitors such as cycloheximide and other chemicals and drugs are added to the 1-2 week old cultures. The scarred brains and the cultures are briefly fixed with aldehydes and freeze-cleaved. For the demonstration of neuron-specific enolase (NSE) and non-neuronal enolase, the immunocytochemical method of indirect labeling is used.

Major Findings: There is an increase in the number of assemblies and a change in their size and shape within plasma membranes of all parallel sheets of reactive astrocytes. Assemblies from astrocytic cultures exposed to 10^{-6} M cycloheximide are totally lost from the fracture faces after 3 hours although background particles and gap junctions persist. This rapid loss implies that assemblies are a protein with a high turnover rate.

Significance to Biomedical Research and the Program of the Institute: By defining the nature and function of these particle arrays in normal and abnormal astrocytic cell membranes, the role of assemblies at epileptic foci and in neoplastic cells may be gleaned and, ultimately, modified. Neuronal enolase is a new marker for the differentiated state of developing neurons and for neuroendocrine cells, both normal and, probably, neoplastic.

Proposed Course of the Project: By means of the primary astrocytic cultures, to examine effects on assemblies of pH changes, chemicals and drugs such as ammonium salts and acetazolamide. Work on reactive astrocytes is also continuing with specific emphasis on early changes in astrocytic cell membranes related to scar formation.

Publications: Schmechel, D., Marangos, P.J., and Brightman, M.W.: Neurone-specific enolase is a molecular marker for peripheral and central neuroendocrine cells. Nature 276: 834-836, 1978.

Brightman, M.W., Anders, J.J., Schmechel, D., and Rosenstein, J.M.: The Lability of the Shape and Content of Glial Cells. In Schoffeniels, E. et al (Ed.): Dynamic Properties of Glial Cells. Oxford and New York, Pergamon Press, 1978, pp. 21-44.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02086-06 LNNS
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Regeneration in Vertebrate Nerves

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. M. Rosenstein Guest Worker LNNS NINCDS
Other: M. W. Brightman Head, Section on Neurocytology LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.1	OTHER: 0.2
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Allografts of autonomic ganglion transplanted to the intact developing brain can survive for at least 14 months. Normal appearing ganglion cells, neurites and synaptic contacts, initially numerous, gradually diminished over time. Schwann cell processes which surrounded neurites and growth cones and myelinated many larger axons became replete with filaments and formed gap junctions. The Schwann cells had become astrocyte-like. The SCG graft on the developing cerebellar cortical surface caused the arrest and anomalous migration of the multipotential external granule cells (EGL). Bridges of intact cerebellar tissue, some as long as 1 mm in length invaded the graft. Neurons and synaptic beds within the bridge implied that EGL cell can differentiate in a foreign environment. Surprisingly, mossy fibre afferents from the spinal cord also entered the graft alongside misplaced granule cells. Other tissues, such as muscle, gland, and nerve transplanted to the developing cerebellar surface indicated heterotopia is caused infrequently. Similarly transplanted non-biological materials, caused inflammation and mechanical deformation but did not alter cerebellar development. Thus, certain "tactic factors" in regenerating nervous tissue could influence brain development.

Project Description:

Objectives: To induce regenerating neurites of peripheral ganglia to grow into selected regions of the CNS and to determine the nature of the stimulus from the ganglia which induce CNS tissue to migrate anomalously.

Methods Employed: Pieces of superior cervical ganglion (SCG) from weanling rats are gently inserted upon the floor of the fourth ventricle beneath the cerebellum of 6-10 day old recipient rats. Non-biological substances: amberlite beads, and pieces of dacron and silastic are also so placed in the ventricle.

Major Findings: Allografted autonomic ganglia can survive for at least 14 months in the recipient brain. Schwann cells which envelope neurites and growth processes may take on astrocyte-like characteristics over time. Other Schwann cells myelinate many more axons than in normal sympathetic trunks. The placement of the graft on the cerebellar surface profoundly alters, locally, cerebellar development. External granule cells and elements of cerebellar neuropil invade the SCG and differentiate within it.

Significance to Biomedical Research and the Program of the Institute: These results provide the first demonstration of central nervous tissue growing into transplanted tissue of peripheral, autonomic ganglia. It is likely that factors other than nerve growth factor, which does not act upon central neurons, are involved.

Proposed Course of the Project: To determine what the factors are that "coax" neurons and axons from the developing brain into the graft and what is required for neurites from the graft to penetrate the brain. To detect, cytochemically, nor-adrenalin and acetylcholine and thereby to identify those neurites which might invade the brain.

Publications: Rosenstein, J.M., and Brightman, M.W.: Intact fourth ventricle as a site for tissue transplantation. Nature 276: 83-85, 1978.

Rosenstein, J.M., and Brightman, M.W.: Regeneration and myelination in autonomic ganglia transplanted to intact brain surfaces. J. Neurocyt. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02144-05 LNNS
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Effects of Hypertension on the Permeability of Cerebral Endothelium to Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. W. Brightman	Head, Section on Neurocytology	LNNS NINCDS
Other:	K. Dorovini-Zis	Visiting Fellow	LNNS NINCDS
	S. I. Rapoport	Chief, Lab. of Neurosciences	LNS NIA

COOPERATING UNITS (if any)
J. Robinson, Evanston Hospital, Evanston, Illinois
Laboratory of Neurosciences, NIA

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.5	0.2

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lanthanum (La) chloride, a tracer smaller than colloidal La, is used here to follow the routes across cerebral vessels rendered permeable to ions and salts by hyperosmotic arabinose. Since La is invisible in the light microscope, horseradish peroxidase (HRP) is used together with the La. It has been correctly assumed that La has escaped within the region of visible HRP exudates. Serial sections have so far failed to reveal how the HRP crosses the endothelium, but the ionic La does penetrate successive pools of extracellular space between tight junctions in arterioles. No complete penetration of these junctions, from vessel lumen to basal lamina has been found in single thin sections, however. Very few endothelial vesicles contain the La. The distribution of ionic La, the smallest tracer available, must be examined in many serial sections in order to follow its route of passage. In this way, possible differences in routes taken by protein and by salts or some ions may be uncovered.

Position Description:

Objectives: To study the mechanisms involved in the reversible opening of the blood-brain barrier following administration of hyperosmotic agents and the possible differences in the passage of tracers of different sizes through the barrier after such treatment.

Methods Employed: To infuse into one carotid artery a threshold concentration of 1.4 M Arabinose and immediately after to inject 3-5 mM lanthanum chloride through the same carotid artery; 30 sec. later to inject HRP systemically, fix by perfusion and examine ultrastructurally the blood vessels in areas of exudates. The order of injecting the two tracers and the arabinose and the interval between the infusions is varied.

Major Findings: Although HRP escapes into the basement membrane and enters cytoplasmic vesicles of the capillary, arteriolar and venular endothelium, vesicular transport of the peroxidase cannot be demonstrated in serial sections so far examined. Passage of HRP through successive tight junctions into interjunctional pools of extracellular space is not seen either. Clefts between endothelial cells contain HRP for a limited distance. However, lanthanum does penetrate successive interjunctional pools between successive tight junctions from the lumen toward the basement membrane in serial sections of the same capillary. Lanthanum does not enter cytoplasmic vesicles or the basement membrane.

Significance to Biomedical Research and the Program of the Institute: Reversible opening of the blood-brain barrier without permanent damage to the elements contributing to its integrity may be of use in considering therapeutic substances that are normally excluded from the brain by its barriers and should provide information on the transient effects of hyperosmolarity on vessel permeability.

Proposed Course of the Project: To further differentiate the purported roles of vesicular transfer, passage through actively formed hypothetical parajunctional channels, migration through junctions or across a damaged endothelium in the escape of tracers from cerebral vessels exposed to hyperosmotic saccharides.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02145-05 LNNS
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Anterograde Movement of Exogenous Protein and Hydrolases Within Neurosecretory Axons (Previously titled: "Identification of Neurons Having Terminals in the Median Eminence and Area Postrema")

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R. D. Broadwell Staff Fellow LNNS NINCDS
Other: M. W. Brightman Head, Section on Neurocytology LNNS NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
In neurosecretory neurons of the hypothalamo-neurohypophysial system, the anterograde axonal transport of exogenous horseradish peroxidase taken into the cell body coincides with the anterograde movement of acid hydrolases. HRP laden dense bodies in neuronal perikarya are secondary lysosomes since HRP and acid hydrolase activity occur in the same dense body. When mice are given 2% NaCl to drink for 5 days, the number of acid hydrolase-positive lysosomes and cisterns in the neurosecretory cell body and axons is greater under hyperosmotic stress than in unstressed controls. The anterograde transport of HRP likewise increases in these stressed neurons. The HRP appears in structures similar to those with acid hydrolase activity. These cisterns closely resemble the agranular reticulum. In the soma, cisterns are confluent with secondary lysosomes. The confluence can provide the route through which acid hydrolases can leave perikaryal secondary lysosomes for transport down the axon. Acid hydrolase activity in the axon and terminals of the neurosecretory neuron would be elevated in response to hyperosmotic stress for degradative functions. The anterograde transport of substances slated for enzymatic degradation in perikaryal secondary lysosomes, would, therefore, be associated with the efflux of acid hydrolases from the secondary lysosome.

Project Description:

Objectives: To draw a parallel between the anterograde transport of peroxidase and that of acid hydrolases and to define the lysosomal system of organelles within the neuron.

Methods Employed: Peroxidase is injected intravenously or by ventriculo-cisternal perfusion in normal mice and in those given 2% sodium chloride to drink for 5-8 days. The mice are fixed from one to 12 hours later. Acid phosphatase, thiamine pyrophosphatase and HRP activity are localized at light and electron microscope levels.

Major Findings: The lysosomal system pervades the entire neuron and is a dynamic conduit subject to alterations in morphology and enzymatic activities as dictated by functional demands placed upon the cell. The transport of acid hydrolases out of the cell body involves a special compartment of the agranular reticulum concerned with catabolic activities in the neuron. Peroxidase, normally sequestered in perikaryal lysosomes for degradation, leaves the lysosomes together with acid hydrolases, when the latter are required to help maintain the internal milieu of the cell during osmotic stress.

Significance to Biomedical Research and the Program of the Institute: The lysosomal system of organelles is involved with hydrolytic activity in the neuron. It is not a static or rigidly defined system but manifests itself only when required to do so, as in hyperosmotically stressed neuro-secretory cells or in the injured neuron.

Proposed Course of the Project: The work is being prepared for publication.

Publications: Broadwell, R.D. and Brightman, M.W.: Cytochemistry of undamaged neurons transporting exogenous protein in vivo. J. Comp. Neurol. 185: 31-79.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02200-04 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Freeze-Fracture of Cell Membranes Intercalated with Lipids		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. W. Brightman Head, Section on Neurocytology LNNS NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project is held in abeyance.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 201 NS 01442-13 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Permeability of Cellular Layers in the Vertebrate Nervous System		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: T. S. Reese Head, Section on Functional Neuroanatomy LNNS NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Functional Neuroanatomy		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project is held in abeyance.		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01881-09 LNNS
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PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Structural Basis of Synaptic Transmission

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. S. Reese	Head, Section on Functional Neuroanatomy	LNNS NINCDS
Other:	C. P. Ko	IPA Physiologist	LNNS NINCDS
	K. J. Lynch	Guest Worker	LNNS NINCDS
	R. L. Ornberg	Staff Fellow	LNNS NINCDS
	D. W. Pumplin	Guest Worker	LNNS NINCDS
	R. P. Rees	Guest Worker	LNNS NINCDS

COOPERATING UNITS (if any)

S. A. Cohen, Albert Einstein School of Medicine, Bronx, NY
M. Henkart, Armed Forces Radiobiology Research Institute, Bethesda, MD
J. E. Heuser, University of California Medical Center, San Francisco, CA (cont'd)

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Functional Neuroanatomy

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

5.5

PROFESSIONAL:

4.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

 (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project seeks to determine the location and mechanism of neurotransmitter secretion and reception. Rapid freezing and subsequent freeze-fracture of synapses exposes fleeting structural changes in the cell membrane accompanying discharge of synaptic vesicles. This approach has shown that each quantum of transmitter is released by one synaptic vesicle. Structural details which may be specific for different pharmacological types of synapses are being investigated by examining postsynaptic membrane structure. New methods have been developed to use rapid freezing to localize calcium in neural tissue in different states of activity, in order to define its role in controlling these states. This work is significant in that it defines the normal structure of synapses and relates normal variations in structure to different functional states. Thus, it becomes possible to distinguish pathological changes in structure, an issue of great importance in studying the etiology of epilepsy or myasthenia gravis. The current program also includes freeze-fracture of developing synapses, which will aid in understanding of both normal development and developmental failures in the brain and peripheral nervous system.

Cooperating Units (continued):

- B. J. McLaughlin, University of Tennessee, Memphis, TN
- S. Nakajima, Purdue University, West Lafayette, IN
- E. Raviola, Harvard University Medical School, Boston, MA
- M. B. Rheuben, Pennsylvania State University, University Park, PA
- A. P. Somlyo, Pennsylvania Muscle Institute, Philadelphia, PA
- A. V. Somlyo, Pennsylvania Muscle Institute, Philadelphia, PA

Project Description:

Objectives: Synapses are sites where electrical signals pass between neurons or between neurons and muscle cells. This project seeks to find the exact location and mechanism of synaptic transmission in the central and peripheral nervous systems in both adult and immature animals, and in tissue cultured neurons and muscles.

Methods Employed: Tissues are prepared for freeze-fracturing or for freeze-substitution by a new technique which rapidly freezes surfaces, up to 15 μ m deep, in one msec. Thus, tissue prepared for freeze-fracturing experiences no chemical treatment, while tissue prepared for sectioning is fixed at low temperatures in non-aqueous solvents. The vertebrate nerve-muscle has been the main object of study in the last year. Our main purpose is to visualize the events which accompany and immediately follow transmitter secretion. A typical experiment consists of giving a nerve-muscle preparation a single shock and freezing it from 3 to 1000 msec later in preparation for either freeze-fracturing or freeze-substitution. The initial stages in secretion have also been studied in *Limulus* amoebocytes (blood cells), a preparation chosen because the secretory granules are very large and the secretion proceeds precipitously after these cells contact endotoxin. In order to localize calcium, muscle and other tissues are rapid-frozen and then cryofixed in acetone containing oxalic acid. This method was developed by measuring the loss of Ca^{++} from tissues during preparation to minimize loss, and by localizing calcium in frog muscles where its natural distribution is already known.

Other studies of synapses still depend on conventional freeze-fracture techniques using chemical fixatives. Nerve-muscle synapses from insects have been compared to those in frogs because they use glutamate rather than acetylcholine as a neurotransmitter, and the giant synapse from the squid has been examined because its physiological condition can be defined so precisely. Isolated tissues are exposed to a variety of different ionic environments in conjunction with different schedules of electrical nerve stimulation, black widow spider venom, and botulinum toxin. They are then put in an aldehyde fixative, frozen, freeze-fractured, and the resulting replicas of split membranes examined in a high resolution electron microscope.

For studies of developing synapses, developing cerebellum or retina is also fixed and freeze-fractured by conventional methods. For studies of synapse formation in culture, cultured neurons are fixed and stained with special methods to display the nature of the initial contacts which lead to synapse formation. Antibody to cholinergic receptors is applied to test its effect on synaptic development and maintenance. Alternatively, the development of postsynaptic receptors in cultured muscle cells is followed by freeze-fracturing areas of acetylcholine sensitivity previously identified with fluorescent bungarotoxin. A special method had to be developed in our laboratory to freeze-fracture identified regions of cultured muscle fibers.

Major Findings: By freeze-fracturing rapid frozen neuromuscular synapses, it has been possible to see, and count, synaptic vesicles fusing with the plasmalemma of synaptic terminals at several different levels of transmitter secretion. It turns out that each quantal secretory event results from the fusion of one synaptic vesicle with the plasmalemma. Since the temporal resolution of rapid freezing in the machine used by this section is less than 2 msec, as measured by a capacitance method developed here, the fate of synaptic vesicle membrane could be followed after vesicles fused with the synaptic plasmalemma. In less than 0.1 sec, the vesicle membrane is completely flattened out into the plasmalemma. Components of the vesicle membrane, appearing as particles after freeze-fracturing, then spread out randomly, finally to be collected a second later in little particle islands which are parts of the coated vesicle system. The final fate of these components of the vesicle membrane is reincorporation into synaptic vesicles. This finding of particle recycling extends earlier work of the section showing that local recycling of synaptic vesicles replaces those lost during synaptic activity.

The initial stages in membrane interaction which lead to membrane fusion and exocytosis are so rapid at the frog neuromuscular junction that we turned to a preparation in which we could examine much larger secretory granules and where we hoped the initial stages of secretion would be more long-lived. Limulus amoebocytes secrete precipitously within seconds after exposure to endotoxin, so the initiation of this process can be studied by freezing at different short intervals after application of endotoxin. The first change is a small perforation in the plasmalemma which rapidly widens, suggesting that exocytosis begins at a point rather than along a wide front of intermembrane contact.

The rapid freezing technique is also applicable to localizing calcium in tissues, if the frozen tissue is subsequently cryofixed in the presence of oxalic acid. In muscle treated in this manner, we could detect no washout of Ca^{++} , and the calcium was localized with an electron probe at its expected positions in terminal cisterns of sarcoplasmic reticulum. We are applying this approach to stimulated synapses, where the calcium which enters from the outside appears to be ultimately sequestered in endoplasmic reticulum.

Comparative studies of other types of synapses were made using either rapid-freezing or conventional fixation to prepare them for freeze-fracturing. The giant synapse in the squid was freeze-fractured for the first time and it was shown that this synapse has well defined synaptic vesicle release areas. Thus, it can be used for studies of synaptic activity in which the state of the synaptic terminal at the time of fixation can be defined precisely with micro-electrodes. Synapses in the insect which use glutamate as a transmitter show patterns of synaptic vesicle release and recovery similar to those at the frog neuromuscular junction. This year a study was published which applied the freeze-fracture technique to neuromuscular synapses to see why such medically important biological substances as botulinum toxin and black widow spider venom have such profound effects on synapses. Results indicate that the toxin specifically blocks exocytosis and that the venom lets sodium and calcium ions in to excite exocytosis.

The extent of local recycling at the frog neuromuscular junction was measured by stimulating isolated synapses for up to 48 hours and then looking for depletion of synaptic vesicles or surface membrane. So far, no depletion of membrane has been found, even in preparations where axoplasmic stores of membrane were reduced, either by blocking axoplasmic transport with colchicine or by ligating the nerve near the muscle.

The success of the rapid freezing and freeze substitution techniques in producing realistic views of labile membrane structures has led to exploration of several nonsynaptic systems in order to explore the uses of this new technique. In the toad retina, changes in spacing of the photoreceptor membranes in light and dark were resolved. Changes in the T-system of muscle exposed to hypertonic solutions were also resolved.

The freeze-fracture technique has also yielded new information about the structure of the postsynaptic membrane. Particulate structures, thought to be receptor molecules within the postsynaptic membrane, appear to be different at each chemical type of synapse. This year, manuscripts concerning the squid giant synapse, the insect neuromuscular junction, and synapses at the termination of auditory fibers in the brain stem were prepared and submitted for publication. It is of interest that the postsynaptic membrane at the squid giant synapse, the insect neuromuscular junction, the auditory terminals in the brain stem, and the sensory terminals at inner hair cells resembled each other but differed from both central nervous system inhibitory terminals in the cerebellum and olfactory bulb, and from excitatory cholinergic terminals in muscle and ganglia. While the transmitter in the squid synapse and mammalian inner ear are not known, the transmitters at the other two examples of this type of synaptic junction are thought to be amino acids. It is also of interest that the postsynaptic membrane at the inhibitory terminals in the middle ear, which are thought to be cholinergic, resembles that at central nervous system inhibitory synapses rather than at excitatory cholinergic synapses, because these observations suggest that different chemical types of synapses may be recognized with the freeze-fracture technique.

Several other current studies with the freeze-fracture technique have begun to yield new data on changes in the structure of synaptic membranes during development. At the developing presynaptic membrane, studied in the chick retina, components of the presynaptic specialization appear first in small islands which subsequently fuse to form the large adult specialization. In developing chick muscle in culture, particle aggregates of the type associated with receptors in the adult appear at spots of acetylcholine sensitivity (inferred from binding of fluorescent bungarotoxin) prior to the arrival of the nerve terminals. The changes in synaptic membranes which accompany initial contact between nerve terminals and target cells were studied in cultures of sympathetic ganglia, using a technique for staining cell coats in thin sections. This revealed a new form of junction which forms and then disappears as synaptic junctions form, suggesting that these initial contact junctions could have a role in the induction of synapse formation. However, careful study with the freeze-fracture technique of similar junctions at sites

of future synapses in the developing cerebellum failed to reveal any special structures inside membranes at these initial contact junctions. Thus, it is possible that these structures are confined to the surface coat of developing synaptic membranes. The effects of antibodies to receptors on synaptic development in the sympathetic ganglion cultures was also studied.

A scanning electron microscope was acquired in the last year and this instrument was used to reveal the structural organization of the true outer surfaces of the postsynaptic membrane at greater levels of detail than has previously been possible. We have developed a technique for chemically separating solid tissues in order to make them amenable to this form of examination, and have submitted a paper on our method. While this technique has succeeded in showing new structural details of the patterns of postsynaptic folds at neuromuscular junctions, we have not yet had the resolution to hope to see individual receptors.

Finally, freeze substitution is being performed on squid axons to look for rapid changes in structure subsequent to nerve impulses.

Significance to Biomedical Research and the Program of the Institute:

One of the most immediately practical aspects of the studies on synapses is that they define the normal structure of various types of synapses in a variety of functional states. This knowledge will permit distinction between normal and pathological, as well as between resting and active synapses, with the electron microscope. In structural studies of epileptic brains, it should now be possible to distinguish normally active from resting or damaged synapses. Similarly, in diseases involving peripheral nerve-muscle synapses at neuromuscular junctions, it becomes possible to distinguish pathological states from changes resulting from increased or decreased activity. The finding that different chemical types of synapses are distinguishable by the freeze-fracture technique may contribute to the task of determining the chemical organization of synapses in the central nervous system. Knowledge about the locations and pharmacological types of various central nervous system synapses will make it possible to understand the action of drugs on the brain on a cellular level. Our new studies on the development of synapses may reveal reasons why development or repair of synaptic systems is sometimes unsuccessful. Finally, our new directions in understanding how cells handle calcium will make it possible to study how these systems are affected by the wide variety of drugs and diseases which affect our nervous system.

Our program of developing and adapting the freeze-fracture technique to study neural structure has been helpful to other program areas of NINCDS, as evidenced by the fact that major programs in neuroviruses, otolaryngology, and multiple sclerosis have found it important to make, with our assistance, major commitments to setting up facilities to perform research with this technique. In every instance, their primary investigators were trained in this technique in the Section on Functional Neuroanatomy.

Proposed Course of the Project: Much of the work outlined above is currently being prepared for publication, or has been submitted. The major work on rapid changes in frog neuromuscular synapses will be finished and manuscripts submitted next year.

A major new direction is to extend the rapid freezing and freeze substitution techniques to new areas of synaptic and membrane physiology. In particular, we will take advantage of a new method we have developed to localize calcium to see how calcium is normally stored and released in a variety of neural tissues. A second direction is to use the scanning electron microscope and the freeze-fracture technique to study developing synapses. How are areas of pharmacological sensitivity formed? What interactions between the pre- and postsynaptic processes control their formation? We hope that the analytical work on calcium distribution and the high resolution imaging of synaptic surfaces will be greatly aided by our new high resolution analytical scanning transmission electron microscope.

- Publications: Rees, R.P.: Structure of cell coats during initial stages of synapse formation on isolated cultured sympathetic neurons. J. Neurocytol. 7: 679-691, 1978.
- Pumplin, D.W. and Reese, T.S.: Membrane ultrastructure of the giant synapse of the squid Loligo pealei. Neurosci. 3: 685-696, 1978.
- Rheuben, M.B. and Reese, T.S.: Three dimensional structure and membrane specializations of moth excitatory neuromuscular synapse. J. Ultrastr. Res. 65: 95-111, 1978.
- Franzini-Armstrong, C., Heuser, J.E., Reese, T.S., Somlyo, A.P. and Somlyo, A.V.: T-tubule swelling in hypertonic solutions: a freeze substitution study. J. Physiol. 283: 133-140, 1978.
- Heuser, J.E., Reese, T.S., Dennis, M.J., Jan, Y., Jan, L. and Evans, L.: Synaptic vesicle exocytosis captured by quick freezing and correlated with quantal transmitter release. J. Cell Biol. 81: 275-300, 1979.
- Henkart, M.P., Reese, T.S. and Brinley, Jr., F.J.: Endoplasmic reticulum sequesters calcium in the squid giant axon. Science 202: 1300-1303, 1978.
- Shotton, D.M., Heuser, J.E., Reese, B.F. and Reese, T.S.: Postsynaptic membrane folds of the frog neuromuscular junction visualized by scanning electron microscopy. Neurosci. 4: 427-435, 1979.

Project No. Z01 NS 01881-09 LNNS

Reese, T.S. and Heuser, J.E.: Changes in the structure of presynaptic membranes during transmitter secretion. In Hall, Z.W. and Otsuka, M. (Eds.): Neurobiology of Chemical Transmission. John Wiley, New York. In press.

Cohen, S.A. and Pumplin, D.W.: Clusters of intramembrane particles associated with binding sites for α -bungarotoxin in cultured chick myotubes. J. Cell Biol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02284-03 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Improvement of current methods of fixation by perfusion for preservation of glycogen		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. Cammermeyer Head, Section on Exp. Neuropath. LNNS NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Experimental Neuropathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.1	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The <u>histochemical demonstration of glycogen</u> was intensified after utilization of a <u>perfusion procedure</u> in which various recommendations were incorporated.		

Project Description:

Objectives: To obtain consistent glycogen reaction in neurons and astrocytes for electron microscopic studies.

Methods Employed: The neuronal glycogen reaction is tested in animals which in narcosis were subjected to artificial respiration and fixation by perfusion with solutions containing inhibitors of glycolysis. Paraffin sections treated with dimedone are stained with periodic acid Schiff. Plastic embedded material is prepared for electron microscopic studies.

Major Findings: Glycogen in neurons varies greatly and may be absent when perfusion is delayed three to five minutes. Use of a glycogenolytic inhibitor results in a more intense staining. When paratoluene sulphonic acid is used instead of Bouin's picric acid solution, glycogen is preserved in neurons but not in astrocytes.

Significance to Biomedical Research and the Program of the Institute: A method which may reduce the speed of glycogen depletion is needed in order to estimate correctly the involvement of this substance in neurons and astrocytes under different experimental conditions. Also it may provide a basis to determine whether by changing the content of glycogen the vulnerability of various elements can be modified.

Proposed Course of the Project: To adopt the principles of enhanced glycogen preservation in material fixed for electron microscopy.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02285-03 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Cliniconeuropathologic study of brains from Guam		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. Cammermeyer Head, Section on Exp. Neuropath. LNNS NINCDS Other: D. C. Gajdusek Chief, Lab. CNS Studies CNSS NINCDS C. J. Gibbs, Jr. Assoc. Chief, Lab. CNS Studies CNSS NINCDS		
COOPERATING UNITS (if any) Laboratory of Central Nervous System Studies, NINCDS		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Experimental Neuropathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Brains</u> from <u>patients</u> observed in <u>Guam</u> are being studied histologically in order to verify the clinical diagnosis.		

Project Description:

Objectives: To determine the nature of cerebral changes in a heterogeneous neurologic material from Guam, as part of the Chronic Diseases Studies: Slow, Latent and Temperate Virus Infections of Drs. Gajdusek and Gibbs (Project Nos. Z01 NS 00969-15 CNSS; Z01 NS 01282-15CNSS).

Methods Employed: Gross examination and routine histologic techniques.

Major Findings: Scrutiny of the microscopic sections has revealed a variety of anatomical changes, such as hemorrhages, metastatic adenocarcinoma, and senile neuronal changes with cerebral atrophy of differing intensity.

Significance to Biomedical Research and the Program of the Institute: The material is from patients who were examined clinically over long periods of time. Verification of the clinical material is essential for genetic studies on diseases peculiar to this region.

Proposed Course of the Project: To determine the type of anatomical changes demonstrable in 26 brains which have been studied grossly, photographed and dissected. A total of approximately 260 representative pieces of the central nervous system have been embedded into approximately 160 paraffin blocks; microscopic sections cut from these blocks have been stained with Einarson's gallocyanin, Bodian's silver method, Mallory's phosphotungstic acid hematoxylin, and Weil-Weigert's hematoxylin.

This study will be completed as soon as the results of an experimental study on cerebral hemorrhages is completed.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02286-03 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Mechanism of cerebral hemorrhages		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. Cammermeyer Head, Section on Exp. Neuropath. LNNS NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Experimental Neuropathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.2	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Petechial cerebral hemorrhages induced by oil embolism in material fixed by perfusion are compared with those in material fixed by immersion.		

Project Description:

Objectives: To assess factors contributing to development of petechial hemorrhages and hemorrhagic infarction.

Methods Employed: Injection of fat in systemic circulation of cats. Fixation by perfusion or by immersion after varying postinjection intervals. Intracardial injection of India ink during the perfusion. Embedding in paraffin or plastic. Histologic techniques for staining of erythrocytes and vascular walls.

Major Findings: Petechial hemorrhages and larger hemorrhagic infarctions composed of fresh erythrocytes aggregated near sites of vascular ruptures. They are of different appearance after use of the two types of fixation.

Significance to Biomedical Research and the Program of the Institute: An assessment of the factors contributing to hemorrhages may help to determine whether these hemorrhages occur during life or whether they can be the cause of death. Formulation of therapeutic measures as well as interpretation of hemorrhages as the cause of death will be dictated by the results of morphologic studies. Can interpretation based on material fixed by perfusion be applied to material fixed by immersion, as is the case for experimental and human material, respectively.

Proposed Course of the Project:

- (1) Compare appearance of petechial hemorrhages in material fixed by perfusion and immersion, respectively.
- (2) Determine time of occurrence of the two types of hemorrhages after use of the two modes of fixation.
- (3) Determine mode of escape of erythrocytes in material fixed by perfusion and embedded in plastic.
- (4) Determine time of escape in perfusion fixed material by introducing India ink.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02333-02 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (50 characters or less) The significance of neuronal argentophilia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. Cammermeyer Head, Section on Exp. Neuropath. LNNS NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Experimental Neuropathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project has been completed and the results were reported at the VIIIth International Congress of the International Society of Neuropathology, Washington, D. C., September 1978. The manuscript has been accepted for publication. Cammermeyer, J.: Argentophil neuronal perikarya and neurofibrils induced by postmortem trauma and hypertonic perfusates. <u>Acta Anat.</u> (in press).		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02362-01 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Effect of dimethyl sulfoxide on the histochemical demonstration of glycogen in the perfusion fixed brain		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. Cammermeyer Head, Section on Exp. Neuropath. LNNS NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Experimental Neuropathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.3	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) When normal <u>Netherlands dwarf rabbits</u> were perfused with dimethyl sulfoxide (DMSO)-containing solutions, the brains exhibited pericapillary foci with acute tissue destruction and perivenous areas in which <u>neurons</u> were filled with <u>glycogen</u> . Glycogen was also discernible in <u>microglial cells</u> and <u>oligodendrocytes</u> . Because of the irregular distribution of glycogen-filled cells this method of fixation is not recommended for systematic studies on the distribution of glycogen in normal and experimental animals.		

Project Description:

Objectives: To prevent polarization of glycogen in Purkinje cells by adding a drug to the fixative which will enhance infiltration of tissues.

Methods Employed: Glycogen was stained by the dimedone periodic acid Schiff technique in paraffin sections from brain fixed by perfusion with a modified Bouin's solution mixed with dimethyl sulfoxide in varying concentrations.

Major Findings: Irregular perivenous areas contain neurons filled with glycogen.

Purkinje cells do not display polarization of glycogen.

Glycogen is demonstrated in cerebellar granule cells, oligodendrocytes and microglial cells as well as astrocytes.

Significance to Biomedical Research and the Program of the Institute: The adoption of a special fixative in which DMSO is added made it possible to demonstrate glycogen to a degree not previously seen except in extraordinary conditions of hibernation, recovery from narcosis, X-irradiation and seasonal variations. These observations were typical of the Netherlands dwarf rabbit but they were not reproducible in the conventional rabbit or other animals. The method is instructive for investigations on qualitative characteristics of glycogen in animals but not for systematic studies in normal or experimental animals.

Proposed Course of the Project: Publication of results.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02363-01 LNNS
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (30 characters or less) Segmental dendritic shrinkage and argentophilia in pericapillary lesions induced by dimethyl sulfoxide (DMSO) included in perfusates		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. Cammermeyer Head, Section on Exp. Neuropath. LNNS NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Experimental Neuropathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.2	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) When animals of different species were <u>perfused</u> with <u>DMSO-containing solutions</u> , the <u>dendrons</u> at sites of acute <u>pericapillary lesions</u> displayed <u>segmental shrinkage</u> and <u>segmental argentophilia</u> . In these foci the neuronal <u>perikarya</u> are shrunken and there is a striking <u>argentophilia of perikaryal neurofibrils</u> , regarded as an artifactual reaction induced by the postmortem flow of DMSO.		

Project Description:

Objectives: To ascertain the specificity of segmental dendritic argentophilia demonstrated in material fixed by perfusion with DMSO-containing solutions.

Methods Employed: Bodian silver impregnated sections are acquired from animals fixed by perfusion with or without DMSO-containing solutions.

Major Findings:

(1) Within perivascular foci neuronal perikarya and dendrons manifest shrinkage concomittantly with argentophilia.

(2) The perikaryal neurofibrils exhibit argentophilia.

(3) Similar observations were not made with other fixatives.

Significance to Biomedical Research and the Program of the Institute:

(1) DMSO, which is considered to be innocuous, has proved to have a toxic effect on the brain when used for fixation by perfusion.

(2) The segmental dendritic alteration must not be confused with a pathologic reaction.

(3) DMSO is not recommended for histologic studies with silver methods.

Proposed Course of the Project: Publication of the observations.

Publications: None

ANNUAL REPORT

October 1, 1978 through September 30, 1979

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

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ANNUAL REPORT
October 1, 1978 through September 30, 1979
Laboratory of Neural Control, Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

Robert E. Burke, M.D., Chief

Introduction

The research work in the Laboratory of Neural Control (LNLC) is concentrated primarily on the central and peripheral nervous system mechanisms that are involved in the control of movement in mammals. Primary emphasis is given to those central nervous system (CNS) structures that contain the neural organizations of motor output - i.e., the spinal cord and those regions of the brain stem and cerebral cortex which project directly to the cord. Several projects deal also with the characteristics and organization of the motor units, which are the functional quantum elements of motor output, and with afferent neurons that deliver sensory input and proprioceptive feedback information into the spinal cord.

A variety of technical approaches have been used in LNLC projects, ranging from conventional electrophysiological methods applied to anesthetized or otherwise neurologically reduced preparations, to methods of neuroanatomical pathway tracing using exogenous proteins, to studies of muscle morphology and histochemistry, and finally, to quite novel techniques, many developed within LNLC, for recording neural and mechanical data from awake, intact animals which are comfortable and either free to move or moving with minimal restraint. Both cats and monkeys have been used in this research.

Many of the newer techniques for recording neural activity in intact animals have derived from the long-standing interest of LNLC staff members in the problem of developing workable neural prostheses to aid the neurologically handicapped patient. A brisk interchange of ideas and information in this area exists between present staff and former LNLC staff members now with the Fundamental Neurosciences Program of NINCDS, as well as with other groups in this country and abroad.

Present Organization

During FY 1979, the staff of the Laboratory of Neural Control (LNLC) has consisted of ten professional scientists, including four permanent senior scientists (two M.D. and two Ph.D.), one Staff Fellow (Ph.D.), one Visiting Fellow (Ph.D.) and three Post-doctoral Fellows with other outside support (one M.D. - Ph.D. and two Ph.D.) One scientist (Ph.D.) from the Department of Neurology, University of Maryland, has worked in LNLC full time on an Intergovernment Personnel Act Assignment. The permanent staff also includes three senior support personnel (two engineers and one physiologist), and the laboratory secretary; the non-permanent, part time staff includes a Junior Fellow (in Electronic Engineering) and a Laboratory Aide. One scientist from

the Department of Neurology at the University of Maryland has collaborated with LNLC members as a Guest Worker on a part-time basis.

The staff members of LNLC have, in various combinations, backgrounds in clinical medicine and neurology and in biomedical engineering and computer sciences, as well as in a variety of aspects of physiological research on the nervous system. Several staff members also have considerable expertise with biomaterials and techniques for fabrication of devices designed for chronic implantation. There is a great deal of interaction and interchange of ideas within the LNLC group and staff members frequently collaborate with one another across formal "project" lines. In order to facilitate this interchange, LNLC has never been divided into formal Sections. Nevertheless, the research effort of LNLC can be described under four general headings, with divisions based on methodological approach:

1. Research involving more or less conventional electrophysiological techniques and directed toward clarifying aspects of the cellular physiology and neuronal circuitry operating in the control of movement at the spinal cord level. This work is done largely using acute reduced preparations (both cats and monkeys), usually anesthetized or decerebrated under anesthesia. Some phases of this work also involve neuroanatomical techniques of cell labeling and pathway tracing with exogenous protein tracers such as horseradish peroxidase (HRP), while other aspects involve study of muscles with conventional methods for muscle fiber histochemistry.

2. Research projects that utilize novel methods for recording the activity of individual neural elements in the central or peripheral nervous system of awake, intact animals (both cat and monkey) that are either free to move or able to perform motor tasks with minimal restraint. Some phases of this work also use techniques for recording kinesiological data, including limb position, joint angles, muscle lengths and the forces produced by individual muscles. Many of the methods and techniques involved in this work have been developed within LNLC and the necessary devices are designed and constructed by LNLC staff.

3. Theoretical and computer modeling studies of: (a) information processing in neural networks; and (b) ways in which data recorded from ensembles of neural elements can be analyzed and interpreted. This work is closely related to aspects of recording data from moving animals, since this involves analysis of multiple channels of neural records during non-stereotyped (i.e., imperfectly repeatable) movements.

4. Activities concerned directly with the development of additional techniques, and the further refinement of existing methods, for recording and analyzing neurally-relevant data from intact, freely moving animals.

Project Summaries:

Virtually all of the in-house research work of LNLC deals with studies of the mechanisms by which the central nervous system controls movement. The functional output elements of the motor system - the quanta of movement - are

the motor units, each of which includes a spinal motoneuron plus the set of muscle fibers (or "muscle unit") innervated by it. Work carried out under the project entitled "Intrinsic Properties of Motor Units" is designed to produce a comprehensive description of the electrophysiological, mechanical, morphological and histochemical characteristics of motor unit populations in particular muscles of the cat hindlimb. Cats are used because of the extensive background information available about the spinal cord anatomy and physiology of this animal, and because they are suitable for a variety of experimental approaches, including investigations of motor behaviors such as locomotion and postural maintenance. Heterogeneous muscles in the cat hindlimb, such as medial gastrocnemius (MG) and flexor digitorum longus (FDL) have been shown to contain four recognizable types of motor units (including 3 varieties of fast twitch and one variety of slow twitch units), each with a distinctive set of mechanical and histochemical properties. In contrast, the soleus (SOL) of the cat is virtually homogeneous and consists almost entirely of slow twitch (type S) motor units.

The mechanisms underlying the differentiation and subsequent maintenance of the different motor unit types remain unknown. Previous work in LNLC has suggested that the unit types present in the normal, fully mature MG motor pool are resistant to alteration with respect to the characteristics that serve to distinguish unit "types". Experimental manipulations such as removal of synergists (to produce compensatory hypertrophy) or limb immobilization (to produce atrophy) do not produce recognizable shifts between unit types, although features such as maximum force output per unit do change appropriately. During FY 1979, we have completed a study of the effect of chronic spinal cord lesions (hemisection or complete spinal section) on the MG unit population. As with the other models, there was no evidence for significant shifts between unit types, although the experimental sample contained a larger than expected proportion of fast twitch units with intermediate fatigue resistance (type F(int)) after chronic (6 months) of complete spinal section with spastic paraplegia, perhaps as the result of increased reflex activity. This can be regarded as a minor alteration in unit organization and does not militate against the overall conclusion that, when there has been no direct injury or interference with motor unit populations, the organization of their constituent motor units is robust and resistant to qualitative change.

A different picture emerges from current studies of the effect of surgical interruption of motor axon connections, with reinnervation of muscle by either its own or foreign motoneurons. We are re-evaluating the model of nerve cross-union between motoneurons of a nominally fast, heterogeneous pool (FDL) with muscle fibers in a homogeneous muscle (SOL), and *vice versa*, and we are also assessing the characteristics of motor units after self-reinnervation. The results to date in part confirm earlier data from other laboratories and are partly at variance with them. In the case of self-reinnervation, there is considerable evidence to indicate that the innervating motoneuron can specify completely the mechanical, histochemical and morphological characteristics of the muscle fibers innervated by it, irrespective of their former type. In the model of cross-reinnervation, the situation is much more complex. Both cross-reinnervation models tested (FDL

to SOL and SOL to FDL) result in slow twitch, type S motor unit by physiological criteria. The type S motoneurons of the SOL nucleus, in the "best" cases, appear to produce virtually homogeneous populations of histochemical type I muscle fibers, normally associated with type S motor units, and the whole muscles contract more slowly than normal. In other cases, "conversion" is less complete and in some cases, the histochemistry of cross-innervated fibers is not interpretable. In contrast to these variable results, innervation of the homogeneous SOL muscle by the heterogeneous FDL motoneuron pool regularly produces virtually no change from the normal SOL histochemistry (fibers remain type I) but does produce some decrease in twitch contraction times. Individual motor units are nevertheless all type S by the usual criteria. In this case, there is an apparent failure of the expected conversion of slow twitch muscle fibers into fast twitch types. The results of the two crosses are asymmetrical and this has so far not been explained. Work on this model is continuing with further data collection, evaluation of muscle histochemistry by recently developed powerful methods of immunocytochemistry, and with study of the activity patterns of cross-innervated muscles with chronic EMG and force recording techniques.

Work done under the project entitled "Motor Control Systems in the Spinal Cord" is in many respects closely allied to the above, in that considerable effort has been devoted to examination of the spinal segmental systems that project to alpha motoneurons identified as innervating one or another of the defined motor unit types. Previous work has assessed the qualitative and quantitative organization of synaptic inputs from muscle stretch receptors (group Ia) and from a particular set of low threshold skin afferents from distal skin regions on the hindlimb. Work in these areas was suspended for much of FY 1979, but will resume by the start of FY 1980 on two aspects: 1) evaluation of the convergence of other afferent and of supraspinal descending input systems on the interneurons that carry excitatory information from distal skin to ankle extensor motoneurons; and 2) on the detailed anatomy of the synaptic contacts established by group Ia afferents on homonymous and heteronymous alpha motoneurons belonging to defined motor unit types, studied by intracellular iontophoresis of horseradish peroxidase. During FY 1979, work has continued on defining the electrical and mechanical activity of the FDL muscle in intact cats during normal locomotion, posture and jumping, since the motor unit composition of this muscle is now characterized (including group Ia synaptic organization). This data will be compared with those from the MG and SOL muscles, obtained earlier, in order to evaluate predictions about recruitment order and utilization of different motor unit types in various kinds of movements.

The work described above largely involves studies of motor mechanisms in anesthetized, immobile animals. The information and conceptual models obtained from such neurologically reduced preparations must be tested and supplemented by examination of neural activity in the intact, freely moving animal that can exhibit purposive behaviors. One approach to this now rapidly-developing area is represented by the project entitled "Neuron Activity in Locomotion". Much of this effort has been devoted to the design and application of methods that permit recording of activity in individual neural elements in freely moving cats, using chronically implanted electrodes

in conjunction with other devices (length and force transducers, and videotape equipment) that permit monitoring the details of the studied movements. For the past several years, the main focus of the project has been on studies of the activity in single, functionally-identified afferent neurons using semi-microelectrodes implanted in dorsal root ganglia. During FY 1979, the method has been extended to examine activity patterns in individual motor axons in the ventral roots. Major results to date indicate that the gamma motor system, which controls the sensitivity of muscle stretch receptor afferents, operates in much more complex ways during normal movement than had been envisioned from conventional reflex physiology. There is evidence that gamma motoneurons are co-activated with the alpha motoneurons when their muscle is used as a prime mover but this co-activation is much less evident when muscles act as stabilizers of joints. Some work currently is devoted to examination of the reflex behavior of spindle afferents during perturbed step cycles or in response to cutaneous or muscle stimuli during stepping. Recent work has shown that the normal electrical activity patterns of muscles can be perturbed by electrical stimuli delivered during stepping, and that the effects produced vary in relation to the phase of the step cycle in which they are delivered. Preliminary experience with recording motor axons chronically in ventral roots gives great promise in that it provides a way to record reliably the discharge patterns of individual motoneurons during normal locomotion and jumping, not hitherto possible, and in addition offers the promise of being able to identify the recorded motor units as to unit type, using criteria developed in other work in LNLC.

The project entitled "Cortical Mechanisms of Voluntary Motor Control" is also concerned mainly with studies of neural activity during voluntary motor behavior in awake intact animals, in this case Rhesus monkeys. Most of the work involves examination of activity patterns of individual neurons recorded in the hand-arm area of the motor cortex, or in the supplementary motor area, during free or goal-directed movements (target-following with a manipulum). Some experiments have utilized a novel electrode system, developed in LNLC, that permits stable, long-term (days or weeks) recording from single neurons, with consequent capability to study the activity patterns under a variety of conditions, including operant conditioning of cell firing. With careful monitoring of resulting movements, and additional data on the sensory receptive fields and effects of intracortical microstimulation through the recording electrode, a detailed picture of the specificity of association between particular cortical cell groups and particular muscles or muscle groups can be assessed. One important result of the long-term recording experiments is that the association between cell discharge properties and "best" movements appears to be stable over prolonged periods of time. Ongoing work continues to explore the issue of spatial specificity for cortical cell "colonies", using conventional (movable) microelectrodes to search for cortical cell groups that are temporally associated with activity in specific wrist muscles. The latter are implanted with EMG wires and, sometimes, with tendon force transducers. The main point of this study is to examine the spatial extent of cell distribution that can be associated with specific muscles by criteria that include: 1) temporal association between cell firing and muscle activity; 2) production of muscle activity during microstimulation of the same region; 3) detection of presumed monosynaptic

projection to the same motor nucleus using spike-triggered averaging from cortical spike to EMG; and 4) sensory receptive fields in related regions. Results to date indicate that cortical "colonies" related to a given muscle can be very extensive and perhaps multiple, and that within a given "colony", areas that produce excitation or inhibition of the target muscle can be located close together. A subproject has begun, using the chronic floating microelectrode method, to investigate the activity patterns of individual neurons in the forelimb area of the sensorimotor cortex of cats during various postural and locomotor behaviors. A main issue in this work concerns the possibility that sensory information to cortical neurons may be "gated" on or off, depending on the phase of stepping or other aspects of the movement.

Work under the project entitled "Models of Neural Interaction" has concerned the problem of extracting information about the activity pattern of individual neurons from multichannel data recorded from an ensemble of active neurons. This is a general problem throughout neurophysiology but is especially applicable to analysis of data streams obtained from intact, moving animals. Of particular interest is the problem of determining the degree to which independent channels of information input determine the firing behavior of individual neurons or groups of neurons. Functionally "similar" groups have been defined in many ways (e.g., common destination of axons, similar spatial position of cell bodies, assumptions about input information species, etc.), but there has been no quantitative definition of this issue. One approach is to examine the question of commonality of functional behavior under a given set of conditions, with examination of the number of independent input processes that control this behavior. This project attempts to study this issue according to current mathematical models of factor and cluster analysis, and to test predictions on the well-defined set of neurons that innervate a given muscle, which can be assumed a priori to be functionally similar. The model system used to test the model has been that of records obtained from active muscle via multiple EMG electrodes. This is a well studied system having all the necessary properties for an adequate test of the model (individual motoneuron firing patterns are reasonably well constrained and EMG records characteristically contain potentials generated by many different motoneurons). Preliminary results have been encouraging as to the extraction of single unit firing patterns from multichannel data, which is a necessary prerequisite for testing the underlying hypotheses, but much remains to be done to refine the biological test system.

LNLIC has always maintained a separate identification for the project entitled "Techniques for Making Contact with the Nervous System". Work done under this project very largely results from the needs and demands generated by other projects in LNLIC, although some input is received from outside groups in terms of questions or specific fabrication needs. Many of the methods developed in LNLIC have been of considerable general interest and staff members identified with this project serve as sources of information for inquiries from around the world. An informal newsletter about matters relevant to biomaterials and chronic implantation of various devices has been circulated for several years and now reaches over 90 individuals regularly,

by their request. Evaluation of the state of the art in biomaterials, electrode wires, insulations and electronic and electromechanical devices applicable to motor systems research and/or motor prosthesis applications continues constantly in LNLC and this information is made available to other groups at NIH and elsewhere.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02079-06 LNLC
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Models of Neural Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: William B. Marks, Ph.D. Research Physiologist LNLC NINCDS
Other: Michael J. O'Donovan, M.D., Guest Worker LNLC NINCDS
Ph.D.

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this project is to examine certain principles of organization among populations of neurons and to devise means of recording the activity of populations of neurons. One such principle is that the independent components of the pattern of activity of a population of similar neurons are related to the physiological function of those neurons. Thus, among neurons of sensory systems, the strength of the connections from the afferents onto neurons at a given level would tend to be such that these neurons detect the independent component patterns among these afferent fibers. In motor systems, the independent components in the activity of a population of neurons would be an estimate of the degrees of freedom in the motor command signals and/or sensory information feeding onto them.

Project Description:

Objectives: This project is concerned with the question, 'Why does a particular neuron have certain inputs and not others, and how is the strength of each input determined?' It answers it with a hypothesis: 'Each neuron specializes in a particular aspect of the whole, and accepts inputs in proportion to the amount of information about its specialty carried by that input, with the constraint that its activity be relatively independent of the activity of other neurons.' The terms are defined quantitatively, and methods are proposed for testing the hypothesis. The hypothesis is considered to be only one of many principles operating simultaneously to influence neuronal connectivities. The detection of complex objects, for example, might require groupings of cells whose connections are determined by other requirements. In the visual system, however, single cells appear to detect individual features. This report discusses the interconnections required to accomplish this in the visual system. The principles are then advocated as also appropriate to other sensory systems and motor systems.

Consider the visual cortex, a system with relatively well characterized patterned inputs and responses. The time varying pattern of parallel activity from the LGN caused by a sequence of natural stimuli no doubt carries natural correlations not removed by the retinal ganglion cells. Let us hypothesize that these correlations can be reproduced by allowing the activity of each fiber to be a weighted linear sum of a number of underlying independent variables. The correlations could certainly be accounted for in terms of a set of non-linear relations to some underlying independent variables. This more general situation will be considered below, but as usual in mathematics, the linear case is very instructive, and also appears in this case to be a realistic approximation.

In a forthcoming publication we demonstrate that if the activity among the afferents to a group of neurons can be considered as linear combinations of independent sources whose amplitudes depart significantly from a gaussian probability distribution (e.g., bursty sources), then each neuron among those receiving these afferents can become a detector of one of these sources, and not be influenced by the others. To achieve this, each neuron should accept input in proportion to the correlation of that input with its own activity, but be constrained by similar neurons detecting the other sources to fire independently of them, by inhibitory interconnections, for example. We can test whether these neurons actually do this by computing the correlations in an ensemble of natural patterns that fall on the retina of a cat, and computing from them the independent components of the patterns. In a forthcoming publication we show that this is soluble by the methods of factor analysis, augmented to utilize correlations present only in non-gaussian signals. These independent components can then be compared to the published features that visual cortical neurons actually detect.

For all sensory systems, at a given level the independent components of the immediately afferent fibers are the natural features that might be expected to be detected, because they are a property of the patterns themselves, and are the efficient descriptors of the patterns. Any other

descriptors would be combinations of them whose occurrence would not be independent, and their use would require more neuronal activity. Also, the number of independent features would usually be smaller than the number of fibers carrying the patterns, often much smaller. Thus the coding of pattern information in terms of its long term independent features saves energy for the organism, and increases the information capacity of a given amount of neural tissue. In perception the detection of constant aspects of objects in spite of distortions in their presentation depends on correlations in sensory channels due to the constant properties of the objects. Thus internal models which could account for the correlations would contain independent variables which would correspond to the objective constant features in the environment. These constraints may be non-linear and have time delays. These and the large number of channels would necessitate a stage by stage approach to deeper underlying variables, as is seen in the visual system.

If visual cortical neurons detect features characteristic of the correlations in the visual environment, they might be expected to detect different features when the environment of visual patterns changes, which is the case. These cells do have a plastic period during development, and the changed features they detect do appear to be appropriate to the various visual regimes experienced. Modelling by the P.I. shows that reasonable rules of synaptic plasticity can lead towards connections for which neurons detect independent features. In this model, if the patterns were made of bars, the features detected were bars.

In motor systems similar ideas apply. It would be efficient for the nervous system to contain representations of the relatively independent components of the patterns of muscle use required by anatomy and environment. These 'units of movement' are probably not used one at a time but are simultaneously combined in various proportions. Thus initially the analysis of natural movements should resemble the analysis of sensory patterns: the search for combinations of muscles that are used together must also require that these combinations occur relatively independently, and our algorithm for calculating the independent components of correlated channels might be applied directly to several simultaneously recorded EMG records made during a variety of movements. Each component would presumably reflect some underlying anatomical specialization. For example during locomotion the fluctuations that occur in normal stepping may appear as profiles of activation or inhibition of several muscles in various combinations, which are added and subtracted from the 'standard' profile of the EMG time course that occurs in a step. In movements of the arm it is not known whether all combinations of muscles are used, whether the number of independent components ('degrees of freedom') of the arm is much smaller than the number of muscles, or whether, as seems most likely, there is a continuous gradation of the importance of a moderately long list of degrees of freedom. Finally, questions of the control of the pool of motor neurons of one muscle should also be susceptible to an analysis of degrees of freedom. If all the motor neurons of a pool are controlled by one degree of freedom, their activity could be modelled by a single controlling variable which determines their firing rate through a coupling constant, a threshold, and perhaps a time function unique to each

neuron. Concerted departures from activity consistent with a single driving function would suggest a time course for an additional driving function. Thus motor units would provide a first opportunity to derive underlying relatively independent sources to account for correlated activity in a case having important nonlinearities.

To test the major hypothesis we wanted simultaneous multi-unit records of a population of related neurons, from which to compute the independent components. An array of contacts with overlapping recording fields was adopted in order to record from most of the neurons in a local volume. Motor units were chosen after previous unsuccessful attempts with nerve fiber bundles and neurons because of technical factors and because motoneurons of a given pool can a priori be considered as a single functional class of cells.

Methods Employed:

Multichannel EMG Methods

The problems to be overcome in order to detect the firing of individual motor units in a muscle have always been 1) to prevent excessive movement of the muscle fibers with respect to the electrodes, and 2) to limit the number of different unit potentials detected so that they do not obscure each other when they occur simultaneously. Our objective is 3) to record several units at once. The use of several leads contributes to all three requirements. It is less sensitive to movement than bipolar recording because it does not rely on maintaining close proximity to one muscle fiber, but allows each contact to record from several fibers at a greater distance. Since each unit appears in several leads, units can be distinguished by their multichannel waveforms. Though more units can be distinguished, the number of units must be held below the number of leads. This is done by limiting the muscle activation (speed of locomotion) and by surrounding the contact array by an electrical shield. Units are recognized even when obscured by other units by a multichannel filter algorithm (Roberts & Hartline, Brain Res. 94: 141, 1975). This filter depends on the fact that if there are as many or more channels than waveforms, it is possible to form linear combinations of the voltages at all sample times and channels, which are zero for all but a corresponding unit waveform. The algorithm computes the filter constants from isolated multichannel waveforms acquired by a waveform recognition program in the laboratory computer which then uses the constants to simulate the filter.

Major Findings

1) Unit potentials were observed in 12 wires inserted into a 1 cubic mm volume of the dorsal margin of the MG muscle of two cats. Good unit isolation occurred in both preparations. In one cat 5 units were reliably identified by the waveform recognition program in the laboratory computer. Where changes occurred in some channels the potentials were still recognizable because they were unchanged in other channels. The cage surrounding the electrodes performed as hoped, reducing the number of units, simplifying the waveforms

and reducing temporal overlap. The waveforms were stable for several days. The five units were successively recruited as the hindlimb began to push off, and ceased firing in the sequence: last on, first off. Some unit waveforms were obscured by coincident firing of larger ones. The average waveform of each unit was saved and will be transported to the NIH PDP-10 computer where the overlap filter constants are computed. The multichannel filter successfully sorted some simulated waveforms.

2) In designing the cage surrounding the EMG electrodes, a method was developed for computing the change in field potential distribution in electrically active tissue caused by the introduction of a grounding (or stimulating) electrode of any shape.

3) The theory of signal factor analysis was extended to include cases where there are delays between sources and receivers which are different for each source-receiver pair, and also cases in which pulsatile source waveforms are distorted in transit in ways that are different for each source receiver pair. This makes the theory relevant to the EMG problem of this project, in which a unit EMG wave (a 'source') appears in various electrodes with different shapes. EMG waveforms which never occur except in the presence of masking activity of other units (i.e. synchronized firings) could in theory be uncovered by this technique. Finally, the algorithm for estimating the number of data samples required to recover signals from mixtures of signals was extended to cover cases where the signals are measured with added noise and background activity.

Significance to Biomedical Research and the Program of the Institute:

Motor unit discharge patterns have only rarely been observed in the past during normal movements in animal muscle, especially when actual muscle shortening and lengthening occurs. Such records of one motor unit at a time have recently been seen by others in our laboratory. The success reported above for several motor units simultaneously is probably the first for non-isometric normal movements in animals. Since the output of the nervous system is largely through parallel motor neuron firings, direct knowledge of these multi-unit patterns would help us understand the circuits that generate motoneuron activity and movements, especially if differences other than threshold and excitability are inferred from the activity of individual neurons. The EMG is a clinical tool; the use of multiple leads could extend the range of lengths and forces compatible with the observation of motor units and perhaps allow clinical observation of multiple motor unit patterns during such movements, thereby strengthening the EMG as a diagnostic tool.

The notion that independent functions of patterns of variables are candidates for important underlying variables in the system under study may be relevant wherever multivariate signals or data are encountered. The nervous system especially is concerned with detecting such underlying variables; the principle appears to be useful in understanding both sensory and motor organization.

Proposed Course of Project

After simulating the multichannel filter on our new computer, if the filter is sufficiently promising, we will build it as hardware. It should then be capable of recognizing about 10 units even when overlapping, in real time.

The multi-unit data produced by this technique will first be fitted to the simplest model for the generation of motoneuron activity, in which each motoneuron has its own threshold and coupling strength to a common excitation signal. More complicated models using one driving signal will be tested if necessary before including any other excitation signals.

The theory of signal factor analysis will be applied for the first time on some of the following problems. 1) Derivation of the 9 constants in the 3 physiological color mechanisms of the retina, which form 3 linear combinations of the 3 color receptor outputs. The correlations among the photoreceptors can be computed from their known sensitivity spectra, and from them we can compute those linear combinations of the receptor responses that vary independently for a sequence of normal colored lights. These can be compared to the known color mechanisms computed by the retinal ganglion cells. 2) The computation of those mixtures of the EMG signals from a number of muscles of one limb that vary independently, as candidates for muscle activation patterns controlled as a unit to generate standard movements. 3) The uncovering of multichannel EMG units that never occur in isolation. 4) The computation of the independent features in a sequence of natural visual patterns for comparison with the published features detected by visual ganglion cells and cortical cells.

Publications.

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01686-11 LNLC
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Motor Control Systems in the Spinal Cord		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
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Other:	Richard P. Dum, Ph.D.	Guest Worker LNLC NINCDS
	Michael J. O'Donovan, M.D., Ph.D.	Guest Worker LNLC NINCDS
	Martin J. Pinter, Ph.D.	Guest Worker LNLC NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.0	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project is designed to provide information on the mechanisms operating within <u>reflex</u> systems which include <u>alpha motoneurons</u> as the output link, as well as on the interconnection and interaction of different reflex systems. Particular consideration is given to correlations between <u>synaptic organization</u> , intrinsic neuronal properties, dynamic behavior and the <u>physiological</u> characteristics of the <u>muscle fibers</u> innervated by the motoneurons studies.		

Project Description:

Objectives: This project is designed to provide information about the organization of neuronal systems in the spinal cord of mammals which ultimately control the activity patterns of motor units (motoneurons and the muscle fibers they innervate), including the interaction between primary afferent and supraspinal descending systems in the control of information flow in spinal segmental motor mechanisms. Of particular interest is the organization of synaptic input to motor units of different types within a particular motor pool.

Methods Employed: A variety of experimental approaches have been used in this project but all are applied to study the lumbosacral spinal cord of the adult cat. Much of the work has been done on animals anesthetized with barbiturate or inhalation (Halothane) anesthesia, or on unanesthetized animals following destruction of the supratentorial brain under rapidly-reversible inhalation anesthesia (decerebrate preparations). Most of such experiments have been devoted to intracellular recording from type-identified alpha motoneurons using conventional micropipette electrodes, with analysis of synaptic potentials produced by electrical stimulation of peripheral nerves and/or of selected CNS sites (e.g., red nucleus, reticular formation, motor cortex, etc.) using stereotaxically placed electrodes. We have recently begun to use the method of intracellular iontophoresis of the tracer protein, horseradish peroxidase (HRP), to permit neuroanatomical study of functionally-identified neuronal elements in the spinal cord. Thus far, this method has been used mainly to study the intraspinal anatomy of identified muscle afferents (mainly group Ia fibers) in relation to their terminations on alpha motoneurons belonging to identified types of motor units. Within 8 to 15 hours after the first HRP injection, the animals are perfused transcardially with fixative under deep anesthesia, and spinal cords are then dissected, photographed and processed for demonstration of HRP (diaminobenzidine - cobalt method) in frozen section material.

Another series of experiments under this project is quite different, in that they involve study of the electrical and mechanical activity patterns of selected muscles in the cat hindlimb during normal motor activity (e.g., locomotion, jumping, posture, etc.) in intact cats that are essentially free to move. This work uses chronic implantation of recording devices such as electromyographic (EMG) electrodes, tendon force transducers developed in LNLc (see previous Annual Reports) and muscle length transducers, in conjunction with videotape recording of the animal's movements on a treadmill or overground (cf. report of Project Z01 NS 02080-06 LNLc, "Neuron Activity During Locomotion").

Major Findings:

A. Supraspinal Control of Cutaneous Excitation in Extensor Motoneurons.

Work on this subproject resumed in late FY 1979, primarily with regard to a re-evaluation of the role of the rubrospinal and corticospinal tracts in the excitatory control of transmission of information in the interneuronal pathway carrying input from low threshold cutaneous afferents from distal hindlimb to ankle extensor motoneurons. This pathway has been of interest to us for several years because the distribution of its effects differs from most other known pathways in that it tends to excite motoneurons of fast twitch (FF and FR) motor units much more than the cells of slow twitch (type S). Initial work is being devoted to an evaluation of anesthetic agents and experimental paradigms that can be most effectively used to study this complex system. Later, the work will involve study of individual interneurons in the pathway, using extracellular and intracellular recording methods, with simultaneous recording (through a separate micropipette) from ankle extensor motoneurons, to assess activity in the whole pathway and to identify interneurons as projecting directly to motoneurons ("last-order" interneurons).

B. Anatomy of Muscle Afferent - Motoneuron Relations.

In the Annual Report for FY 1978, we reported preliminary experiments involving injection of HRP into functionally-identified group Ia afferents and into alpha motoneurons postsynaptic to them (identified as to motor unit type). At that time it was decided not to continue this study because of interest in it by other laboratories around the world. In the past 6 months, contacts with other interested investigators have led us to conclude that our approach will not duplicate ongoing efforts elsewhere, except for the group of Dr. J.-O. Kellerth, at the Dept. of Anatomy, Karolinska Institutet, Stockholm. Dr. Kellerth has agreed that we shall both proceed according to the same experimental protocol, so that the results from both groups can be pooled.

C. Recruitment Models for Motor Unit Populations.

Based on previous data on the organization of synaptic input systems to medial gastrocnemius (MG) motor units of known type, and on other evidence from this and other laboratories, we have suggested several "recruitment models" by which the output of a motor unit population can be predicted in terms of motor unit types active at particular levels of force generation. Testing of such models demands development of methods for assessing muscle and motor unit pool output in intact, freely moving animals (cf. Project Z01 NS 02080-06 LNLc). Of particular relevance to the present project is the comparison of whole muscle EMG and force output with the characteristics of the motor unit populations making up the same muscles. Previous work had been with the MG and soleus muscles. Currently, we are exploring a similar vein, using the flexor digitorum longus muscle (FDL), which was studied in

detail in connection with nerve cross-union experiments reported in Project Z01 NS 02160-05 LNLG. Muscle force and length transducers have been adapted for the FDL in order to characterize its mechanical activity during locomotion, jumping, etc., along with its EMG activity (as well as that in other limb muscles). The FDL is primarily a claw protruder and digit plantar-flexor. During the step cycle, it produces moderate (0.4 - 0.5 kg. peak force during the mid-third of stance and produces another EMG burst at the end of stance, at which time FDL force is low because the muscle is shortening rapidly. Activity is more pronounced during vertical jumps, when FDL can produce as much as 1 kg. in a short burst just at foot takeoff (the fully tetanized FDL can produce about 1.5 to 2.0 kg. isometric force). The data provide a useful contrast to those from the ankle extensors, which have quite different function, especially in view of the fact that the motor unit composition of FDL exhibits some specific differences from that found, for example, in MG (see previous Annual Reports).

Significance to Biomedical Research and the Program of the Institute:

Active movement of mammals in space is accomplished by motor units with motoneurons located in the spinal cord. Analysis of the central nervous system control of movement requires a detailed understanding of the organization and interaction of input systems to the spinal cord segments, both from peripheral afferent sources and from supraspinal structures. There is now considerable evidence for the existence of functional specializations among the muscle fibers of different motor unit types, indicating rather precise patterns of motor unit "usage" during movements of various sorts. The long-range goal of the present project is to analyze the patterns of neuronal organization present in the spinal cord as they relate to motor unit type in order to further our understanding of how motor units, and therefore movements, are controlled. Such studies are of clear relevance to analyses of both normal and abnormal movement patterns in man and bear importantly on the interpretation of results of clinical neurophysiological investigations in normal human subjects and in patients with neurological diseases.

Proposed Course of the Project:

It is anticipated that present work on the interaction of primary afferent and descending control of transmission in the excitatory cutaneous pathway from distal skin to ankle extensor motoneurons will continue well into FY 1980. Depending on the results from this work, we hope to have a catalog of convergent inputs onto interneurons in this pathway that will enable us to identify candidate interneurons in the pathway when they are encountered individually by microelectrodes. Further work will involve attempts to further characterize input patterns as they converge on individual last-order excitatory interneurons, demonstrated by spike-triggered averaging methods to project directly to ankle extensor motoneurons. Eventually, we intend to inject some of these cells with HRP in order to visualize their axonal terminal distribution, including their projection to motoneurons. Work on the injection of HRP into identified

group Ia afferents and alpha motoneurons will continue through FY 1980. Similarly, the analysis of the mechanical activity of the FDL muscle in free movement will continue into FY 1980 but should be completed during that fiscal year.

Publications:

Walmsley, B., Hodgson, J. A. and Burke, R. E. Forces produced by medial gastrocnemius and soleus muscles during locomotion in freely moving cats. J. Neurophysiol. 41:1203-1216, 1978.

Burke, R. E., Walmsley, B. and Hodgson, J. A. HRP anatomy of group Ia afferents contacts on alpha motoneurons. Brain Res. 160: 347-352, 1979.

Burke, R. E., Walmsley, B. and Hodgson, J. A. Structural - functional relations in monosynaptic action on spinal motoneurons. In Wilson, V. J. and Asanuma, H. (Eds.): Integration in the Nervous System. Tokyo: Igaku-Shoin, 1979. pp. 27 - 45.

Burke, R. E. Motor unit recruitment: What are the critical factors? In Desmedt, J. E. (Ed.): Recruitment Patterns of Motor Units and the Gradation of Muscle Force. Progress in Clinical Neurophysiology, Vol. 9 Basel: Karger (in press).

Burke, R. E. "Command" as functional concept rather than cellular label. Brain and Behav. Sci. (in press).

Burke, R. E. The role of synaptic organization in the control of motor unit activity during movement. In Pompeiano, O. (Ed.) Reflex Control of Posture and Movement. Progress in Brain Research, Vol. 50. Amsterdam: Elsevier. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01687-11 LNLC
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Techniques for Making Connections with the Nervous System		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	Martin J. Bak	Electronics Engineer LNLC NINCDS
Other:	George M. Dold	Engineering Technician LNLC NINCDS
	Richard P. Dum, Ph.D.	Guest Worker LNLC NINCDS
	Juaquin A. Hoffer, Ph.D.	Staff Fellow LNLC NINCDS
	Gerald E. Loeb, M.D.	Medical Officer LNLC NINCDS
	William B. Marks, Ph.D.	Research Physiologist LNLC NINCDS
	Joan S. McIntosh	Physiologist LNLC NINCDS
	Michael J. O'Donovan, M.D.	Guest Worker LNLC NINCDS
	Claude I. Palmer, Ph.D.	Visiting Fellow LNLC NINCDS
	Edward M. Schmidt, Ph.D.	Research Physiologist LNLC NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.8	PROFESSIONAL: 1.1	OTHER: 1.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project is intended to develop techniques for the acquisition and processing of <u>neuroelectric signals</u> from the central and peripheral nervous system in <u>acute and chronic neurophysiological preparations</u> .		

Project Description:

Objectives: Successful monitoring of neural activity from the peripheral or central nervous system requires the development of techniques as they apply to unique recording situations. The latter include recording from both acute and chronic preparations in which stable and discriminable single unit activity is required, but the problems are particularly critical in recording from animals that are awake, comfortable and either free to move or moving with minimal restraint. In addition to recording, delivery of effective stimuli through metal microelectrodes without damaging the electrode or causing pathological changes to nearby neurons has now become essential for ongoing laboratory experiments, which requires different considerations. This project is designed to evaluate methods, materials and designs to solve particular research problems.

Methods Employed:

A. Material suited for biological implantation.

The evaluation of the physical properties and biocompatibility of Parylene-C, iridium and certain medical grade silastic rubbers has continued, primarily through examination of implanted materials and implant sites.

B. Designs for chronic recording intracortical microelectrodes.

Fabrication techniques for making "map-pin" electrodes continue to be refined and modified for the production of "hair brush" electrodes in which two or more electrodes are combined so that the inner electrode tip spacing is 100 microns or less. The design of the "hair brush" electrode is similar to the "map-pin" design in that the electrode shaft which is pure iridium is microwelded to a fine gold lead wire, this assembly is then parylene coated and the weld joints are reinforced with a small amount of epoxy. Two or more electrodes are bonded together with epoxy so that the shafts remain parallel to one another and then coated with parylene again, the tips are then re-arc'd to expose the iridium recording surface.

C. Microelectrode introducer capable of tracking the moving brain.

The introducer, a device developed previously and described in the 1975 Annual Report, is now being considered for use at several other institutions outside of the NIH.

D. Chronic single unit recording from dorsal root ganglion and spinal cord.

The technique of recording as described in the 1977 Annual Report is now being routinely employed in kinesiological studies in cats on a treadmill. This technique has been successfully adopted to recordings made from spinal cord and ventral root filaments and is now routinely used in cats in kinesiological studies.

E. 12-Channel high impedance amplifier system.

Three miniature 12-channel hybrid amplifiers are now being routinely employed in several studies in the laboratory and have become an integral part where multichannel recordings are made from chronically implanted cats and monkeys. Two specially designed printed circuit boards have been developed to "house" the amplifier, one of which contains 74 input and output connections to bring other information to and from the animal. A unique connector assembly is now being used for mounting on the cat's back, which utilizes a specially designed printed circuit board into which a 37 pin connector attaches.

F. Implantable strain gauge for monitoring muscle tension from associated tendon.

The semiconductor strain gauge has been routinely implanted in cats and has worked successfully; it has also been implanted in one monkey and has successfully worked for 3 months. Recent modification for 2 active elements has greatly improved temperature drift characteristics.

G. Chronic EMG recording from muscle.

EMG electrode designs are continuing to be assessed and modified in the hope of producing an electrode which will permit detection of single motor unit activity in the hindlimb muscles of cats performing a range of activities. At present a design which consists of 12 or more fine wires (.001" to .003" in diameter), the ends of which are cut off, are implanted so that the recording tips are arranged within a one square millimeter area. These electrodes are surrounded by an array of uninsulated wires which act to shield the recording electrodes from activity outside the array and also provide an indifferent recording potential.

H. Length gauge and associated A.C. bridge amplifier.

The length gauge as described in the 1977 Annual Report is being successfully used in kinesiological studies in cats. The A.C. bridge amplifier which was originally designed to operate with the length gauge has been adapted to fulfill a most needed function in detecting foot fall during locomotion by detecting a high frequency modulated carrier signal. The carrier signal is transmitted through the capacitively coupled treadmill belt and recorded via already implanted EMG electrodes. The signal amplitude is modulated by the cat's paw making contact with the treadmill belt.

I. Digitally controlled biphasic stimulator and recording amplifier for metal microelectrodes.

This instrument which was described in the 1978 Annual Report has been used to determine "safe stimulus parameters" for micro-stimulation of acute and chronic intracortical metal microelectrodes.

J. Resettable EMG integrator with sample and hold.

Several of these units (described in 1978 Annual Report) have been built and used in the laboratory for quantitative evaluation of brief reflex events and for smoothing EMG activity to produce voltage envelopes proportional to the integrated value of the activity and maintaining excellent signal dynamics.

K. Interspike interval computer.

An instrument has been designed and tested which utilizes a 12-bit digital to analog converter to accurately transform the interspike interval time to a voltage level which is linearly proportional to the firing rate of the neural spike activity.

L. Pressure sensitive force plates for determination of weight distribution and center of gravity in standing cat.

A device has been developed which utilizes strain gauges that are geometrically arranged so that static and dynamic forces upon a round disc will give absolute weight, relative position of all four paws, pitch and yaw. This device is to be used in several different studies including some where chronically implanted cats are used in kinesiological experiments.

M. Digitally controlled stimulator and isolator.

A microprocessor controlled biphasic stimulator is currently under development. This unit will provide precise control of amplitude, duration, and delay between pulses. An optically isolated biphasic constant current stimulator is also under development and will be used either with the microprocessor or a digital logic box for parameter generation. Precise control of stimulation parameters is essential for determining the most effective and safest stimulator parameters.

N. Other laboratory instruments.

The following list includes several of the instruments designed, built and put into operation in the laboratory within the last year; these are: a raster adder for displaying multiple sweeps on an oscilloscope screen, an EMG 3-pole filter, an analog delay with a much improved dynamic range, and an EMG amplifier with remote gating controls for stimulating through the same electrode which is being used for recording.

Major Findings:

Parylene-C insulation over pure iridium wires are very well tolerated by tissue. At least three electrodes have recorded single unit activity for over thirty eight months. Histological slides of brain sections from around

these electrodes show no reaction and viable neurons are evident within 100 microns of the electrode. Approximately thirty percent of the electrodes implanted for over thirty eight months showed no breaks in the insulation although a definite drop in tip impedance was evident in most electrodes.

"Map-pin" electrodes, some of which include up to three recording shafts within 100 microns of each other, have recorded single unit activity from cat motor cortex for over fifty days, five out of eleven electrodes are still recording activity. Several modifications in the surgical protocols for implantation have helped to increase the yield and longevity of the chronically implanted cats.

Although "map-pin" electrodes have been found to record single unit activity successfully from the spinal cord of freely walking cats, fine wires implanted into ventral roots and dorsal root ganglia have proven to be quite reliable and offer a simpler alternative for current research purposes.

EMG Electrodes:

5 motor units in the MG muscle have been simultaneously monitored during walking using 8 electrodes inserted just beneath the surface in the dorsal margin of the muscle. The method seems capable of monitoring perhaps twice as many units. In designing the shielding cage around the array, a method was devised for calculating the change in tissue field potentials caused by an arbitrarily shaped grounding electrode.

Significance to Biomedical Research and the Program of the Institute:

The successful development of techniques for recording signals from the nervous system and delivering safe current levels and waveform parameters for microstimulation is essential to the success of ongoing experiments in the laboratory. These newly developed techniques are also beneficial to other laboratories involved in neurophysiological research and may have an impact in the development of prosthetic devices for the neurologically handicapped.

Proposed Course of Project:

Continued use of the "map-pin" electrode in the superficial layers of the central nervous system is anticipated. Appropriate modifications to the electrode will be made in order to adapt it to a particular recording situation, with special emphasis on increasing the number of electrode recording tips with a sub-one hundred micron separation; i.e. "hair brush" design. Cats implanted with "map-pin" electrodes will be fitted with various EMG recording electrodes, strain and length gauges to permit detailed correlation of normal neural activity with various voluntary and reflex locomotory movements.

A programmable multichannel filter for resolving overlapping multichannel waveforms is being computer simulated, and a hardware version for real time use will be designed.

The pressure sensitive force plates will be utilized in conjunction with chronically implanted cats fitted with various EMG, strain and length gauges to analyze normal neural activity with respect to postural and dynamic weight distributions as they relate to the relative positioning of all four limbs.

Further cooperation with the Neural Prosthesis Program is anticipated.

Publications:

Bak, M.J. and Loeb, G.E.: A pulsed integrator for EMG analysis.
Electroenceph. and Clin. Neurophys. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01688-11 LNLG
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Cortical Mechanisms of Voluntary Motor Control		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	Edward M. Schmidt, Ph.D.	Biological Engineer LNLG NINCDS
Other:	Martin J. Bak	Electronics Engineer LNLG NINCDS
	George M. Dold	Engineering Technician LNLG NINCDS
	John A. Hodgson, Ph.D.	Visiting Fellow LNLG NINCDS
	Joan S. McIntosh	Physiologist LNLG NINCDS
	Claude Palmer, Ph.D.	Visiting Fellow LNLG NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.5	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project is investigating the size and spatial distribution of cortical "colonies" that are associated with individual muscles, as well as the function of neurons in such colonies in the motor cortex and supplementary motor area during voluntary motor behavior. Also under investigation is the possible gating of sensory input to neurons in the motor-sensory cortex of cats. Intracortical microstimulation can activate a forearm muscle over a wide region of cortex and possibly from multiple isolated regions. Excitation or inhibition of the same muscle can be produced from closely spaced positions in the cortex. Long-term chronic recording from the same neuron has now reached 109 days. During this period the relationship between the cell discharge properties and the "best" movements appears to be stable.		

Project Description:

Objectives: The major goals of this project are 1) an examination of the spatial organization of motor cortex outflow to particular muscle or muscle groups during free or goal directed movement in awake animals; 2) whether the firing patterns of small sets of cortical neurons contain sufficient information to specify the details of motor performance; 3) to investigate if gating of sensory information to neurons in motor-sensory cortex occurs during different phases of locomotion; and 4) to determine the safest, most effective stimulation parameters for intracortical microstimulation.

Methods Employed: Monkeys are initially trained to move a handle that positions a cursor light. The monkey is required to match the position of this cursor light to one of eight target lights. When a match occurs for a prescribed length of time, a juice reward is delivered to the monkey. When the animal is well trained, a chamber to support the microelectrode drive is implanted over the hand-arm area of the motor cortex. In addition a pyramidal tract stimulating electrode, EMG electrodes, and tendon strain gauge are implanted. After recovery from surgery, the monkey is required to move the handle between 3 pairs of targets at 4 different load conditions. Task correlated cells are sought, with a moveable microelectrode. After recording the task related parameters, EMGs, and the activity of a cortical neuron, microstimulation is applied through the electrode to determine which muscles respond. Sufficient cell firing data is collected to utilize spike triggered averaging to determine if the cell under investigation facilitates or inhibits the EMG activity of the implanted forearm muscles. For the locomotion studies cats are implanted with "map-pin" electrodes in the forelimb area of the motor-sensory cortex along with pyramidal tract electrodes, EMG electrodes and a muscle length transducer. Neural discharge patterns are recorded during locomotion, falling from various heights and sensory field mapping.

Major Findings:

1. Single unit recordings can be obtained from cortical neurons for extremely long periods of time with the "map-pin" electrode that have been described in the 1977 and 1978 Annual Reports. Recordings have been obtained for over three years from the motor cortex of a monkey implanted with these electrodes. The activity of individual cells can be observed for days or weeks. The longest recording from a single cell has been 109 days. Long term observations of single cells indicate that the relationship between firing pattern and movement is stable over time.

2. The vapor deposited insulation Parylene-C has proved to be exceptionally biocompatible as indicated by the ability of the "map-pin" electrodes to record from neurons for over three years. Histological examination of the tissue around the electrodes shows minimal reaction. However, problems exist with the insulation in that seven of ten implanted electrodes had holes in the insulation which was indicated by a drop in the

electrode impedance and visualized by bubble testing. All of the electrodes will be examined with a scanning electron microscope to try to determine the cause of insulation failure.

3. Intracortical microstimulation (ICMS) through a microelectrode while recording from chronically implanted EMG electrodes has provided a means of determining how muscles are affected by stimulation of specific cortical sites. Both proximal and distal muscles can be activated by trains of stimulation at current levels below 20 μ a. Excitatory or inhibitory EMG responses to ICMS occur as short as 11 ms after the start of the stimulus train and end 11 ms after the end of the train. Prompt termination after the end of the stimulus train suggests that the latency is due to conduction and synaptic delays rather than reverberating cortical circuits that might be expected to remain active after the end of stimulation. EMG patterns produced by ICMS have ranged from inhibition of a single muscle, inhibition and excitation of synergists, inhibition and excitation of antagonists, to excitation of single muscles. Areas that produce inhibition of a given muscle have been observed as close as 100 μ m to areas producing excitation of the same muscle. Cortical "colonies" related to a given muscle can be very extensive and perhaps multiple.

4. When the monkey is performing its trained task ICMS can be introduced at selected times, which produces repeatable EMG responses. To minimize tissue damage biphasic stimuli are required so that no net DC currents flow through the electrode. The pulse sequence of cathodal followed by anodal is a more effective stimulus than anodal followed by cathodal. Also a delay of approximately 100 μ s between the cathodal and anodal pulse produces a larger EMG response than zero delay, or less current can be used in conjunction with the delay to produce the same response.

5. Map-pin electrodes are chronically implanted in the forepaw area of the motor-sensory cortex of cats. In addition a pyramidal tract electrode, and EMG recording electrodes are implanted in appropriate muscles. Stimulating electrodes are also implanted in the forepaw to provide a controlled afferent input. Unit activity is recorded during locomotion at different speeds, during falling and landing from drops of various heights and during withdrawal from mild cutaneous stimulation. Preliminary studies of pyramidal tract neurons indicate that gating of sensory information to these neurons appears to occur during locomotion.

Proposed Course of Project: Work will continue in the area of obtaining long-term chronic recordings from neurons in the motor-sensory cortex and supplementary motor area (SMA). Initially, studies will be conducted with a microelectrode controlled with a microdrive so that a large number of cells that are related to movement can be obtained from the supplementary motor area SMA. This same technique will also be used in the motor cortex to map the areas of selected arm muscles. Strain gauges will be implanted on the tendon of selected muscles to measure force with voluntary movements and with intracortical microstimulation (ICMS). Muscle length will be measured by the position of the wrist while it moves the handle. These measures, combined with chronic EMG recordings, will provide information on how specific muscles are utilized in a movement. This data will be correlated with firing patterns of neurons

from cortical regions that produce either excitation or inhibition with ICMS. The parameters of ICMS will also be investigated in these animals to help determine the most efficient and safest waveform for long-term intracortical stimulation. Pyramidal tract stimulation will be employed to identify neurons projecting into the pyramidal tract, the majority of which are corticospinal. To further detect cortical projection to motor nuclei spike-triggered averaging from cortical spike to EMG will be employed. The studies with chronic "map-pin" electrodes in cat motor-sensory cortex investigating gating of sensory information during different movements and locomotion will continue.

Significance to Biomedical Research and the Program of the Institute:

The motor cortex and possibly the supplementary motor area are intimately involved in the production of distal, exploratory movements with hands and digits in primates. These functions are disturbed by stroke in many human patients. The mechanisms of compensation for motor deficits caused by cerebral lesions are unknown but information about normal cortical mechanisms and their stability (or instability) with time is important to increase our basic understanding which then can be applied to lesion problems. Studies on the dynamic activity of cortical neurons and their relationship to movement along with the spatial organization of neurons related to a specific muscle or movement will provide valuable basic information. With our newer microstimulation and microelectrode recording techniques we can now better attack the question of whether the cortex has a representation of muscles or movements. Because all movements involve the contraction (or relaxation) of one or more muscles, the basic representation must be in terms of muscles. However, the large extent of cortical "colonies" to a specific muscle and the overlap of these colonies to different muscles tends to indicate that a specific cortical region can be involved in specifying combinations of muscles that produce a movement.

Utilizing EMG recordings along with intracortical microstimulation at specific times during a trained movement task allows evaluation of stimulation parameters. Determination of the safest stimulation values will be directly applicable to neuroprosthesis applications.

The ability of the chronically implanted "map-pin" electrodes to record the activity of the same cortical neurons for many days, weeks and even months should prove useful for evaluating drugs. This work may also have significance for the development of cortically-controlled prosthetic devices, although the electrodes are not yet satisfactory for obtaining prosthetic control signals where recordings for a number of years are required.

Publications:

Schmidt, E.M. and Thomas, J.S.: Motor unit recruitment order: Modification under volitional control. Prog. Clin. Neurophysiol. 9: (in press).

Project No. Z01 NS 01688-11 LNLC

Schmidt, E.M., McIntosh, J.S., Durelli, L., and Bak, M.J.: Fine control of operantly conditioned firing patterns of cortical neurons. Exp. Neurol. 61: 349-369, 1978.

Schmidt, E.M. and McIntosh, J.S.: Microstimulation of precentral cortex with chronically implanted microelectrodes. Exp. Neurol. 63: 485-503, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02080-06 LNLG																																								
PERIOD COVERED October 1, 1978 to September 30, 1979																																										
TITLE OF PROJECT (80 characters or less) Neuron Activity During Locomotion																																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																										
<table border="0"> <tr> <td>PI:</td> <td>Gerald E. Loeb, M.D.</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Others:</td> <td>Martin J. Bak</td> <td>Electronics Engineer</td> <td>LNLG</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>Robert E. Burke, M.D.</td> <td>Chief</td> <td>LNLG</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>Juaquin A. Hoffer, Ph.D.</td> <td>Staff Fellow</td> <td>LNLG</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>William B. Marks, Ph.D.</td> <td>Research Physiologist</td> <td>LNLG</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>Michael J. O'Donovan, M.D., Ph.D.</td> <td>Guest Worker</td> <td>LNLG</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>Claude I. Palmer, Ph.D.</td> <td>Visiting Fellow</td> <td>LNLG</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>Martin J. Pinter, Ph.D.</td> <td>Guest Worker</td> <td>LNLG</td> <td>NINCDS</td> </tr> </table>			PI:	Gerald E. Loeb, M.D.				Others:	Martin J. Bak	Electronics Engineer	LNLG	NINCDS		Robert E. Burke, M.D.	Chief	LNLG	NINCDS		Juaquin A. Hoffer, Ph.D.	Staff Fellow	LNLG	NINCDS		William B. Marks, Ph.D.	Research Physiologist	LNLG	NINCDS		Michael J. O'Donovan, M.D., Ph.D.	Guest Worker	LNLG	NINCDS		Claude I. Palmer, Ph.D.	Visiting Fellow	LNLG	NINCDS		Martin J. Pinter, Ph.D.	Guest Worker	LNLG	NINCDS
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SUMMARY OF WORK (200 words or less - underline keywords) A variety of new techniques are being used to monitor the afferent and efferent neural activity in the spinal cord and motor cortex of intact cats during normal and perturbed locomotion. Flexible wire electrodes in the cerebral cortex lumbar dorsal root ganglia (DRG) and ventral roots record stable, identifiable unit activity which is correlated with kinesiological data from chronically implanted gauges of muscle force, length, and EMG activity developed for this project. Neurons are characterized by conduction velocity, anatomical origin, and modality using spike-triggered averaging of EMG signals and neurograms obtained from specially designed nerve cuff electrodes implanted around peripheral nerves. The reflex effects of various electrical stimuli to motor and cutaneous nerves are systematically examined as they vary through the step cycle.																																										

Project Description:

Objectives: The major goal of this project is to examine directly the roles of spinal neurons and primary afferent fibers in normal movement which up till now have been inferred from paralyzed or decerebrate acute preparations. The principal current emphasis is on natural patterns of afferent and efferent activity in the spinal cord and the role of afferents as part of a system of servo-mechanisms. Spinal reflexes elicited by both cutaneous and proprioceptive afferent activity are now known to have a profound effect on locomotory patterns.

Methods Employed:

1. The present method for obtaining stable afferent unit records during normal walking consists of inserting insulated 50 micron diameter wires into the dorsal root ganglion (DRG) via a small laminotomy. The cut ends of the wire constitute the recording surface and the only fixation is by a flexible Silastic carrying sleeve sutured to the dorsal spinous process.
2. A similar technique has been successfully employed in the ventral roots of the 5th lumbar spinal segment. Efferent unit activity in the axons of motor neurons is identified as such by spike-triggered averaging of the records obtained from a cuff electrode chronically implanted around the femoral nerve and from EMG electrodes designed to sample each of the five anterior thigh muscles to which this nerve projects.
3. Supporting kinesiological measurements include continuous read-out of joint angle at ankle, knee, and hip via implanted length gauges, force generated by individual muscles via chronically implanted tendon strain gauges, and videotape gait analysis.
4. Techniques have been developed to implant large numbers of bipolar recording and stimulating electrodes at a variety of sites in both hindlimbs of a freely walking cat, permitting analysis of reflexes elicitable by electrical stimulation of various afferent classes during stepping.
5. A new technique is being developed for microstimulating motor axons being recorded through their microelectrodes. It is now possible to characterize their muscle fiber types by recording the resultant tension profiles through the implanted strain gauge and to link such stimulation to the time of natural spike occurrence to study the refractory period of the muscle and to provide unusual but physiological patterns of activation to motor units during normal behavior.
6. An extensive hardware and software system has been devised to facilitate interactive reduction and analysis of neurophysiological and kinesiological data produced by the various projects using the neurokinesiology facility.

Major Findings:

1. Records from muscle spindle primary and spindle secondary afferents during walking and various perturbations have revealed a great variety of activity patterns in relation to parent muscle electromechanical activity.

During walking, the activity of a given spindle primary was usually consistent in similar step cycles but was usually poorly correlated with absolute muscle length. It could change markedly for similar movements performed under different conditions. Secondaries appeared to be predominantly passive indicators of muscle length during walking but could demonstrate apparently strong and rapid fusimotor modulation during other motor activities such as postural changes and paw shaking.

Spindle activity modulation not relatable to muscle length changes was assumed to be influenced by fusimotor activity. In certain muscles (particularly those not mechanically responsible for an ongoing movement), this presumption leads to the conclusion that gamma motoneurons may be activated out of phase with homonymous alpha motoneurons as well as by more conventional alpha-gamma motoneuron coactivation. Simultaneous recordings of two spindle primary afferents from extensor digitorum longus indicated that spindles within the same muscle may differ considerably with respect to this presumed gamma motoneuron drive.

On the basis of this preliminary survey, we would propose that the various "servo-control" hypotheses regarding tightly linked pathways for the simultaneous, proportional activation of alpha and gamma motoneurons may require considerable qualification. Our data suggests that even within a given motoneuron pool, voluntary or reflex activity may significantly and independently alter the alpha-gamma relationships for a given movement. Simultaneous activation of alpha and gamma motoneurons could often be inferred under particular circumstances, but the data as a whole indicate the ability to control independently the spindles as sense organs within their parent muscles. Activity in the alpha and gamma systems generally appears correlated during use of muscles as prime movers in a task and uncorrelated when the muscles are used as stabilizers or passive sensors of limb position for a given movement.

2. During normal walking, the firing patterns of motor neurons appear to be related in an orderly way to the overall muscle utilization. From an integrated record of overall muscle EMG, one may observe a threshold of effort at which motor units begin to fire and a frequency of firing which generally follows the amplitude of this integral. Slow twitch motor neurons may fire at rates up to 50 pps during slow walking. Some motor neurons show a high frequency doublet pattern at the beginning of their recruitment period, but this is variable and not well correlated with subsequent effort.

Significance to Biomedical Research and the Program of the Institute:

Much current work on the study of mammalian locomotion has been concentrated on the cat hindlimb, where considerable knowledge is already available concerning the physiological and anatomical properties of the muscles, motor neurons, afferents, and spinal reflexes. However, the details of the functioning of this system during normal locomotion under cerebral control can at present only be inferred, giving rise to a number of competing control theory hypotheses. The new methods employed in this project should provide data needed for testing such hypotheses and formulating new ones. An understanding of the normal control of movement is essential to understanding a number of degenerative diseases of the spinal cord (e.g. ALS, transverse myelitis, etc.) affecting locomotion. A longer range application of the technique of chronic transducers and afferent monitoring is in the field of functional neuromuscular stimulation (FNS). Sophisticated devices designed to restore motor function by bypassing CNS lesions (e.g. paraplegics) will probably require some form of closed loop servo-control utilizing transducers of muscle length and tension and of skin pressure. If it proves possible to obtain stable afferent activity rather than using implanted transducer signals over long periods of time, afferent recording electrodes could improve the function and simplify the design and implantation of complete FNS systems.

Proposed Course of Project:

A major objective of this project is the determination of the functional spinal organization underlying the generation and regulation of locomotion. The most promising sources of data appear to be the single unit activity of muscle afferents (particularly spindle endings), the EMG and force outputs of selected muscles, and unitary activity from identified spinal cord motoneurons. The experimental paradigms to be investigated include the various "voluntary" modes of locomotion, the effects of singular perturbations such as electrical and mechanical stimuli during locomotion, and the effects of various deprivations such as deafferentation, local anesthesia, mechanical and neurological deafferentations, and loss of environmental (e.g. visual) cues.

A new project is being initiated to combine these chronic studies with acute neurophysiological techniques for intra- and extra-cellular recording from spinal cord interneurons. When a satisfactory number of well-identified units are simultaneously present in a chronic preparation, the animal will be anesthetized and decerebrated and controlled locomotion using stimulation of the mid-brain locomotory centers will be established. The relevant spinal cord segment will be exposed and probed with classical microelectrode techniques with spike-triggered averaging to and from the chronically studied units.

A long-term technological goal is to determine the limits of recording device stability and tissue compatibility in both time and numbers of information channels with a view to assessing the feasibility of deriving somatosensory information for the feedback control of Functional Neuro-muscular Stimulation Prostheses.

Publications:

Duysens, J. and Loeb, G.E.: Precortical processing of somatosensory information. Behav. and Brain Sci. 1: 149-150, 1978.

Loeb, G.E., Walmsley, B., and Duysens, J.: Obtaining Proprioceptive Information from Natural Limbs: Implantable Transducers vs Somatosensory Neuron Recordings. In Fleming, D.G. (Ed.): Solid State Physical Sensors for Biomedical Application. CRC Publishing Company (in press).

Loeb, G.E., and Duysens, J.: Activity patterns in individual hindlimb primary and secondary muscle spindle afferents during normal movements in unrestrained cats. J. Neurophysiol. 42: 420-440, 1979.

Bak, M.J. and Loeb, G.E.: A pulsed integrator for EMG analysis. EEG Clin. Neurophysiol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02160-05 LNLC
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (60 characters or less) Intrinsic Properties of Motor Units		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
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SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.4	PROFESSIONAL: 3.0	OTHER: 0.4
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SUMMARY OF WORK (200 words or less - underline keywords) 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 <u>muscle fibers innervated by them</u> (i.e., the muscle unit). The <u>motor unit</u> populations in normal animals are compared with those in animals after various conditioning treatments.		

Project Description:

Objective: This project is designed to provide information about the populations of motor units that make up large limb muscle in mammals. The work involves study of the electrophysiological and morphological characteristics of spinal cord motoneurons in relation to the mechanical, histochemical and anatomical properties of the muscle fibers (termed "muscle units") innervated by them. The neural and muscular elements are functionally inseparable; the motor unit is the quantum element in all motor behavior. The characteristics of normal motor unit populations are studied as well as alterations in motor units that are produced by various experimental manipulations, including changes in mechanical demand, central nervous system lesions, or by re-innervation by foreign motoneurons.

Methods Employed: For the most part, analysis of motor unit properties is carried out in anesthetized cats using methods of intracellular recording and stimulation of individual spinal motoneurons to ensure functional isolation of motor units one at a time. Electrophysiological properties intrinsic to the motoneuron, and the quantitative and qualitative characteristics of synaptic inputs to the cell, are evaluated with conventional techniques. The mechanical properties of the muscle unit innervated by each cell is then assessed during stimulation of the motoneuron through the intracellular pipette electrode, with the innervated muscle attached to a force-measuring device under isometric conditions. Muscle fibers of individual motor units can be labeled by depleting intrafiber glycogen during prolonged stimulation of the motoneuron, permitting histochemical and morphological study of the muscle units belonging to physiologically-characterized individual units. During FY 1979, the Laboratory has implemented an in-house facility for routine muscle histochemistry. Classification of motor units by type is done using methods and criteria described in earlier Annual Reports. Analysis of the numbers and spatial organization of motoneurons innervating particular muscles has been done using intramuscular injection of the tracer protein, horseradish peroxidase, to label cells in the target motor nucleus by retrograde transport.

Major Findings:

A. Motor Units in Cat Medial Gastrocnemius after Spinal Lesions.

This subproject began in FY 1977 with study of the medial gastrocnemius (MG) motor unit population in cats that had had low thoracic spinal cord hemisection, with resultant spastic hemiparesis, 6 months before sampling the MG unit pool in a terminal experiment. Work continued into FY 1978 with study of 3 cats with complete transection of the spinal cord at segment T13 six months prior to evaluation. A total of 72 MG units have been studied completely and results have been pooled from the 3 cats. MG motor units were all classified into the same 4 categories as found in normal animals. Given the technical factors that limit the number of units that can be sampled from

any one animal, the results from the 3 cats so far studied suggest that the MG motor unit population is essentially unaltered by chronic spinal cord section, with severe spastic paraplegia. Comparing the data with a much larger sample from normal cats, there was an increased proportion of type F(int) units in the paraplegic animals (19.4% versus 5% in normal cats). Histochemical study of the MG muscles in the paraplegic cats showed that the usual fiber type profiles were unaltered except for some increase in the proportion of fibers with the profile equivalent to that found for normal type F(int) motor units. It thus appears that chronic spastic paraplegia may produce a small increase in the number of fast twitch motor units with intermediate fatigue resistance (i.e., type F(int)), but otherwise leaves the MG motor pool qualitatively unchanged. Hindlimb muscles in paraplegic cats (including MG) all showed moderate atrophy and there was a clear decrease in tetanic force output per unit among all of the MG motor unit groups, together with a small but statistically significant decrease in twitch time to peak for all groups, compared with data from normal cats. Twitch to tetanus ratios were more nearly normal than in earlier studies of compensatory hypertrophy and immobilization atrophy.

Our overall assessment of these results is that the MG motor unit population is resistant to qualitative change during chronic spastic paraplegia, at least as regards motor unit type composition, but that some features of the motor unit properties (e.g., twitch contraction speed) can be altered. This result parallels earlier evidence, described in previous Annual Reports, from studies of the effects on the MG motor unit population produced by removal of MG synergists (producing compensatory hypertrophy) and by chronic limb immobilization (producing immobilization atrophy). This motor unit population appears to be quite robust in terms of resisting qualitative change under the tested conditions. This conclusion is in marked contrast to the effects produced by experimental denervation and reinnervation, discussed in the next Section.

B. Properties of Muscles and Motor Units after Innervation by Foreign Motoneurons.

Cross-innervation of nominally "fast-twitch" muscles by motoneurons of normally "slow-twitch" pools, and vice versa, has been a much used model for studying the "trophic" interaction between motoneurons and muscle fibers. However, there is no comprehensive data on the effect of cross-innervation on individual motor units, nor on the detailed interrelations between whole muscle mechanical properties, histochemical fiber composition and myosin histo- and biochemistry, when the degree of homogeneity or heterogeneity of the altered muscles has been assessed. In addition, the characteristics of terminations of motor axons, neuromuscular junctions and acetylcholinesterase-positive sole plates is being compared in normal and reinnervated muscles, using methods applicable at the light microscopic level. Our current effort in this subproject is designed to fill this gap in existing information, using surgical cross-union between the nerves of the

heterogeneous flexor digitorum longus (FDL) and homogeneous soleus (SOL) muscles in young adult cats as the test model. The data from altered muscles and motor unit populations are compared with results from studies of normal FDL and SOL motor unit populations in this Laboratory, described in previous Annual Reports. Several series of young adult female cats have been prepared with various combinations of surgical section and reanastomosis of FDL or SOL nerves and muscles, including self-reinnervation (FDL-FDL or SOL-SOL) or cross-innervation (FDL-SOL or SOL-FDL). After post-operative survival of 8 to 10 months minimum (during which time the cats live together in large enclosures permitting free movement), each animal is studied in a terminal experiment involving sampling of one or more motor units in the altered population (routine micropipette methods) and then target and unoperated FDL and SOL muscles are removed and frozen in toto in isopentane cooled with liquid nitrogen. Muscles are stored in a liquid nitrogen freezer for routine frozen section histochemistry, after which blocks are being sent to Dr. G. F. Gauthier at Wellesley College, for study using recently developed methods for evaluating the molecular nature of the myosins in different fibers with immunofluorescent histochemistry. As a last stage, the residual material in the blocks will be studied for myosin composition by conventional gel electrophoresis.

Cross-innervation (SOL-FDL) of the heterogeneous FDL muscle (containing normally about 90% fast twitch (types FF, F(int) and FR) motor units and their corresponding histochemical muscle fiber types) by the SOL motoneuron pool (virtually 100% type S motor units) produces results that vary considerably from one cat to the next without evident relation to the mechanical success of surgical nerve anastomosis. SOL-FDL muscles vary from very small (albeit innervated), with tiny fibers exhibiting bizarre histochemistry, to muscles about 2/3 normal in size and weight, with few evidently denervated fibers remaining by histochemistry. The latter examples include several with very mixed histochemistry (including large proportions of type II and type I fibers, all mostly with high oxidative activity), to muscles that were almost exclusively composed of type I, high oxidative fibers resembling normal SOL fibers except for smaller average fiber area. We have thus far studied 32 individual motor units in 11 SOL-FDL muscles and all have been classed as type S by our usual criteria (normal FDL contains only about 10% type S). The total sample is small because we have attempted to use glycogen depletion to identify fibers of physiologically-studied units in a large percentage. Despite considerable effort, these depletions have not been very successful, mainly because many fibers in SOL-FDL muscles evidently have very low intrinsic glycogen to begin with; evaluation of this material is continuing with additional methods (see below). The mechanical twitch contraction times of SOL-FDL motor units cover the same range as found for type S units in the normal FDL and the whole muscle contraction times are longer (average 76 msec) than normal FDL (average 33 msec). Almost half of the SOL-FDL units exhibited post-tetanic depression of twitch tension, as do normal SOL muscle units, in contrast to normal S units in FDL, which rarely show this property. Each SOL-FDL motor unit studied by the intracellular

method can be definitely identified as innervated by a SOL motoneuron, on the basis of cell body location and group Ia synaptic input pattern, which remains unchanged despite chronic cross-innervation. Cross-innervation of the heterogeneous FDL muscle by homogeneous SOL motoneurons thus appears to produce slow twitch (type S) muscle units, although the explanation for the interanimal variations and examples of histochemical heterogeneity in some muscles remains to be explained.

In contrast to the above results, cross-innervation (FDL-SOL) of the homogeneous SOL muscle (95 - 100% type I fibers, and virtually 100% type S motor units) by the heterogeneous FDL motoneuron population produces predictable and uniform results. When the FDL to SOL nerve anastomosis is intact, the FDL-SOL muscle is essentially normal in size, color and weight. All of the 4 FDL-SOL thus far studied histochemically have exhibited virtually unchanged histochemical characteristics (all greater than 90% type I fibers), and would be difficult to distinguish from normal SOL without other information. However, all have had whole muscle twitch contraction times about half (mean 61 msec) those found for normal (mean 140 msec) or self-innervated SOL (mean 119). One case of dual innervation by FDL and some SOL motor axons was found, in which fibers innervated by the foreign FDL axons had an aggregate twitch contraction time of 61 msec while those self-innervated by SOL axons had a contraction time of 108 msec under the same mechanical conditions. This particular muscle showed no histochemical mosaic in stains for myosin properties at any pH, but fibers innervated by the FDL axons appeared to have much less neutral fat than SOL-innervated fibers. All 17 FDL-SOL motor units studied thus far in these muscles were typical type S, with all characteristics essentially the same as normal SOL units, even though all were definitely innervated by FDL motoneurons, except that twitch contraction times were faster (mean 53 msec) than normal (mean 97 msec).

Both cross-innervations studied in this work (FDL - SOL and SOL - FDL) appear to produce slow twitch (type S) motor units as identified by criteria developed for normal populations, although the heterogeneous FDL motoneurons do produce muscle units with faster contraction times than in SOL muscle fibers, and the SOL motoneurons produce slower average contraction speed than normal among FDL muscle fibers. There is some evident dissociation between observed contraction speed and myosin histochemistry which remains to be evaluated by immunofluorescence histochemistry and by gel electrophoresis of myosin subfragments.

C. Evaluation of ^{14}C -2-deoxyglucose Autoradiography as a Marker for Active Muscle Fibers in Individual Muscle Units.

For about 10 years, depletion of intrafiber glycogen has been used in this and other laboratories as a marker to identify the muscle fibers innervated by a single motoneuron stimulated repetitively for prolonged periods. This method is not completely satisfactory for identification of

fatigue-resistant fiber types that are very resistant to depletion of their already low glycogen levels, or in abnormal muscles (e.g., after cross-innervation) where glycogen levels can be quite low in many fibers. We have thus been evaluating an alternative method for fiber labeling, using ^{14}C -2-deoxyglucose (2-DG) autoradiography as developed for metabolic studies of brain nuclei by Dr. Louis Sokoloff and his group in NIMH. In collaboration with Dr. C. Smith of Dr. Sokoloff's group, we have injected 2-DG intravenously while stimulating an individual motoneuron with repetitive trains. After 30 - 90 minutes of stimulation, the target muscle is removed and frozen in toto and later processed by cryostat sectioning to obtain serial sections for conventional glycogen staining (PAS method) and for autoradiography by several different techniques. Preliminary results have been encouraging in that glycogen-depleted fibers do show up also in autoradiograms, but the latter have not given sufficient cellular resolution yet. We are currently evaluating different methods for film application to sections, and for image processing of resulting autoradiograms, in order to improve the method. If successful, this study will not only provide a possible method for evaluating metabolic activity in different fiber types and in identified motor units, but will also be useful more generally in extending resolution of the 2-DG technique to the single cell level, which has thus far not been possible.

D. Review of Motor Unit Populations and Their Relation to Movement Control.

During FY 1979, Dr. Burke has been preparing a large scale review of motor unit physiology, histochemistry, morphology and functional correlations for a forthcoming volume in the Handbook of Physiology series, by invitation of the American Physiological Society. This should be completed by the end of the fiscal year.

Significance to Biomedical Research and the Program of the Institute:

Analysis of the control of movement by the central nervous system requires consideration of the properties and functional specialization of motor units as these are the quantal elements from which all skeletal movements are composed. Studies of the interrelation between the intrinsic properties of motor units, including both the motoneuron and muscle unit portions, and the organization of synaptic input to the same units have aided our understanding of the control problem and have suggested new avenues for research. In addition, elucidation of the detailed interrelation between the physiological, morphological and histochemical characteristics of muscle units in animal muscle has immediate relevance to investigations of human neuromuscular disease, in which electromyography and muscle histochemistry play important diagnostic and research roles. There is growing evidence that the basic pattern of motor unit organization in animals and man is similar in principle. In particular, the histochemistry of limb muscles in the cat and in man is remarkably similar. Studies of the effects on motor unit

populations of altered usage, CNS lesions, and denervation - re-innervation in the cat have important relations to the interpretation of clinical investigations in patients with neuromuscular disorders, peripheral neuropathies and CNS lesions. This is particularly true of current studies of the effect of foreign re-innervation on well-characterized motor unit populations.

Proposed Course of the Project:

Experiments on cross-reinnervation between the FDL and SOL muscles will continue through early FY 1980, to be completed by January 1980. Evaluation of muscle histochemistry will continue beyond that date but should be completed in FY 1980. Collaborative work on this material using immunofluorescent histochemistry of myosin subfragments, with Dr. G. F. Gauthier, has begun and will continue through the same period. When these studies are complete, the remaining muscle blocks will be sent to the laboratory of Dr. Susan Lowey, of Brandeis University, for biochemical analysis of myosin subfragments in normal, self-reinnervated and cross-reinnervated muscles. We are hopeful that all of this work will be complete by the end of FY 1980. Evaluation of the 2-DG method will also continue into FY 1980.

Publications

Burke, R. E. Motor units: Physiological/histochemical profiles, neural connectivity and functional specializations. Am. Zoologist. 18: 127-134, 1978.

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Burke, R. E. Motor units in mammalian muscle. In: Sumner, A. J. (Ed.): The Physiology of Peripheral Nerve Disease. New York: W. B. Saunders (in press).

ANNUAL REPORT

October 1, 1978 through September 30, 1979

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

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ANNUAL REPORT
October 1, 1978 through September 30, 1979
Laboratory of Neurophysiology
National Institute of Neurological and Communicative Disorders and Stroke
Henry G. Wagner, M.D., Acting Chief

The research of the laboratory may be conveniently divided into several work objectives.

One of these is to gain insight on excitable membrane receptors. In particular, it is a study of the histochemical specificity of the receptor and the role of the many ligands that alter or modify the receptor in neurone excitation. Our studies utilize dissociated cultured neurons as model systems as these appear to have the attributes of excitable membranes, provide long term stability, permit easy accessibility for electrophysiological recording and application of various endogenous substances such as amino acids and peptides or exogenous drugs like benzodiazepine and barbiturates to specific sites on the membrane. Dissociated cultures of the mammalian central nervous system are proving to be an extremely useful preparation to study the physiology and pharmacology of central neurones. We have begun by focussing on several aspects of receptor pharmacology, examining the membrane effect of amino acids, peptides, purines, pyrimidines, benzodiazepines and barbiturates. We have been able to resolve some details of the cellular pharmacology to these ligands. It is clear from these studies that a variety of chemically important drugs affect receptor coupled changes in excitability which could be the basis for the pharmacological effects in the CNS. We have determined for example that all spinal cord cells grown in culture respond to glycine, β -alanine, γ -aminobutyric acid (GABA) and amino acid glutamate. These substances, when applied to the membrane change conductance mechanisms; some like glutamate appear to activate Na and K conductances while others like glycine activate chloride conductance mechanisms. We have also applied clinically important drugs like benzodiazepine and various members of the barbiturate family. These induce complex changes in conductances, involving particularly the chloride conductance. Our analysis of the response suggests that drugs may be acting by engaging receptors normally occupied by endogenous ligands. We have, thus far, eliminated GABA, inosine and thyrimine as possible candidates of endogenous ligands capable of interacting with the barbiturate receptors.

In May, we sponsored a three day symposium on the role of peptides in neuronal function. Approximately 700 scientists from America and Europe attended to consider 28 invited presentations covering a wide range of topics, from methodological strategies to investigate the role of peptides in the nervous system, to papers dealing with the neurobiology of specific peptides. The symposium appeared to be well received and constructive.

A third area of active research concerns our interest in neural coding and the complex processing of stimulus information by the nervous system. The work in this work area, over the past year, has focussed on a study of the coding of spectral information in the retinal ganglion cells of the carp

retina. In this preparation it is possible to sort out a number of chromatically distinct signals. Our analysis leads us to believe each arises from a different population of cones. While earlier analysis of receptive fields of ganglion cells demonstrated simple dual color coding, our most recent data leads us to believe that color coding is actually more complicated. Our view now includes red, green and blue inputs both in the center and in the surround of the receptive field. The data indicates that these chromatically distinct inputs can be either excitatory or inhibitory. We also know that the strength of each of the inputs can vary from cell to cell. We are attempting to develop rules which clarify this degree of complexity and its relationship to the receptive field.

Another study, recently completed and partially published, examined the receptive field properties of neurones in the visual streak of the turtle retina. Using intra and extra cellular microelectrode recording, retinal ganglion cells were found which demonstrated orientation specificity. Slits of light on the retina evoked the most vigorous response when aligned with the streak and minimal response when the slit was directed normal to the streak. We also found that horizontal cells in this retina show a similar effect although to a lesser degree. Anatomical study of these neurones show non-uniform dendritic trees. Dendrite extensions in the direction of the streak were often two or more times longer than in a direction normal to the streak. Outside the streak, the morphology of these neurones does not show an elongated axis nor do the electrophysiological responses show preference with the orientation of the slit. This potentially important finding will merit further study.

Ionic mechanisms associated with phototransduction in retinal receptors were also investigated. This focus of investigative effort has been a fruitful research objective for a number of years. The principle thrust has been to analyse the dynamics of membrane voltage and currents during excitation of the receptor by light and/or current injection. We know there are ion gradients across the membrane which are maintained by pumps. Excitation mechanisms affect the permeability of the membrane or, sometimes, the pumps. A satisfactory model of the excitation of the photoreceptor has yet to be developed. Our methodology involves analyzing changes associated with specific changes in the ionic composition of the superfusate. Our most recent effort investigated the action of the cesium ion on the light response in photoreceptors of the toad retina. This ion when present in the superfusate causes an unexpected increase in the action potential to light. The interpretation we favor is that light causes first a conductance decrease which produces a hyperpolarization. This, in turn, activates a voltage dependant mechanism which leads to a conductance increase with drop in potential. Cesium is thought to block this latter mechanism. This concept has introduced new thinking on receptor transduction mechanisms and marks a distinct new step in attempts to understand photoreceptor excitation. This is a very active field internationally and this laboratory has enjoyed attention for its considerable impact on the field.

Collaborations were active between scientists of this laboratory and those of other laboratories and institutes. Deserving particular mention were collaborations with scientists from NEI, NIA and NIMH of the National Institutes of Health and members of the faculty and scientific staff of the University of California, Johns Hopkins University, University of Texas, Georgetown University, Salk Institute, Duke University, University of Toronto, and the University Compluteus, Spain.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02019-07 LNP																																								
PERIOD COVERED October 1, 1978 to September 30, 1979																																										
TITLE OF PROJECT (80 characters or less) Electrophysiology and Neuropharmacology of Simple Cellular Systems																																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>J. L. Barker</td> <td>Medical Officer</td> <td>LNP NINCDS</td> </tr> <tr> <td></td> <td>T. G. Smith</td> <td>Medical Officer</td> <td>LNP NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>K. Futamachi</td> <td>Staff Fellow</td> <td>LNP NINCDS</td> </tr> <tr> <td></td> <td>L. M. Huang</td> <td>Staff Fellow</td> <td>LNP NINCDS</td> </tr> <tr> <td></td> <td>D. L. Gruol</td> <td>Guest Worker</td> <td>LNP NINCDS</td> </tr> <tr> <td></td> <td>J. F. MacDonald</td> <td>Guest Worker</td> <td>LNP NINCDS</td> </tr> <tr> <td></td> <td>W. Vaughn</td> <td>Computer Specialist</td> <td>STD NIMH</td> </tr> <tr> <td></td> <td>W. Sheriff</td> <td>Computer Scientist</td> <td>STD NINCDS</td> </tr> <tr> <td></td> <td>A. Huang</td> <td>Technician</td> <td>LNP NINCDS</td> </tr> <tr> <td></td> <td>L. LaGrange</td> <td>Technician</td> <td>LNP NINCDS</td> </tr> </table>			PI:	J. L. Barker	Medical Officer	LNP NINCDS		T. G. Smith	Medical Officer	LNP NINCDS	OTHER:	K. Futamachi	Staff Fellow	LNP NINCDS		L. M. Huang	Staff Fellow	LNP NINCDS		D. L. Gruol	Guest Worker	LNP NINCDS		J. F. MacDonald	Guest Worker	LNP NINCDS		W. Vaughn	Computer Specialist	STD NIMH		W. Sheriff	Computer Scientist	STD NINCDS		A. Huang	Technician	LNP NINCDS		L. LaGrange	Technician	LNP NINCDS
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SUMMARY OF WORK (200 words or less - underline keywords) Electrophysiological experiments using intracellular recording techniques and extracellular pharmacologic applications have been performed on mouse spinal neurons grown in tissue culture and on molluscan central neurons. The research has focussed on two related questions: How do endogenous and exogenous ligands alter neuronal excitability? We have found that endogenous amino acids, peptides, purines and pyrimidines and exogenous benzodiazepines and barbiturates can all produce transmitter-like effects on cultured neurons. Peptides and drugs can also modulate transmitter actions. Protons <u>per se</u> can mimic some of these actions. We have resolved some of the pharmacologic events to the most elementary level possible using fluctuation or "noise" analysis. Cultured spinal neurons clearly allow detailed study of clinically relevant neuropharmacology at the membrane level. One insight we plan to pursue is the notion that the pharmacologic actions of some drugs may be mediated through receptors for endogenous ligands.																																										

Project Description

Objectives: How are changes in excitability communicated between neurons and how do clinically important drugs affect these mechanisms?

Methods Employed: Intracellular recordings are made from individual neurons using conventional and voltage clamp techniques. Endogenous substances and exogenous drugs are applied locally to the surface of individual cells using iontophoretic and pressure methods. Electrophysiological assays of membrane events induced by various substances can thus be made relatively easily with each cell serving as its own control. The accessibility of the neurons growing in monolayer culture permits long-term stable intracellular recordings which are critical for producing accurate measurements. Experiments are designed to study membrane events with one of three levels of analysis: 1) single electrode membrane potential and conductance measurements; 2) double electrode voltage-clamp analysis of membrane current events and 3) fluctuation or "noise" analysis of membrane current fluctuations. The initial observations are made at the first level of analysis. These are followed up in more detail using the other techniques. Fluctuation analysis is a statistical treatment of membrane current fluctuations which occur as a result of the simultaneous opening and closing of many agonist-activated ion channels. It is performed off-line with the aid of a computer program from magnetic tapes of the data.

Major Findings: 1. Amino acid pharmacology. All spinal cord cells grown in culture respond to each of the neutral amino acids glycine, β -alanine and γ -aminobutyric acid (GABA) and the acid amino acid glutamate. Cultured neurons show a non-uniform distribution of responses to the amino acids suggesting the development of "hot spots" or clusters of receptors on the cell surface. The most commonly studied amino acid response is functionally inhibitory to cell excitability and consists of an increase in Cl^- conductance. At the single channel level the three neutral amino acids activate elementary conductance mechanisms with different properties. Interactions among the three under voltage clamp suggest that the amino acids may share a common Cl^- conductance mechanism. Glutamate produces functionally excitatory responses which invert at about 0 mV and thus appear to be due to activation of Na^+ and K^+ conductances.

Analysis of spontaneously occurring synaptic currents shows that one population exists whose driving force is similar to that for GABA and whose time constant of decay is similar to the average lifetime of a GABA-activated channel. The sensitivity of these currents to drugs which alter GABA responses further supports the hypothesis that the synaptic currents are mediated by GABA. If this is true, then each quantal synaptic event is comprised of about 300 individual GABA-activated channels, an estimate similar to that made at invertebrate neuromuscular synapses utilizing GABA as the inhibitory transmitter.

2. Peptide pharmacology. The opioid peptides leucine- and methionine-

enkephalin have been applied locally by microiontophoresis and by pressure, and perfused in the bathing medium. Three functionally distinct types of membrane events have been observed: 1) transmitter-like responses, including a rapidly depolarizing, rapidly desensitizing excitatory response and an inhibitory response whose null potential is similar to that of GABA and is thus likely due to an activation of Cl^- conductance; 2) modulation of amino acid transmitter-like responses independent of any other effects on membrane properties; and 3) alteration in threshold for action potential generation. All of these pharmacological effects of the opioid peptides can be blocked by bath application of naloxone, suggesting that they involve stereospecific engagement of opioid peptide receptors on these cells. Entirely similar membrane responses except the threshold effect have been seen with iontophoresis and pressure application of the undecapeptide substance P. Spinal cord cells responding to either the opioid peptides or substance P numbered less than 20% of those tested which contrasts with the ubiquitous amino acid responsivity. A major goal of future research on peptide pharmacology with this preparation will be to enrich the cultures for cells expressing functional peptide receptors.

3. Purine and pyrimidine pharmacology. Although purines and pyrimidines are important constituents of nucleic acids, they are also released from neural tissue in a Ca^{++} -dependent manner and thus may mediate certain forms of intercellular communication in the nervous system. In addition, the purines hypoxanthine and inosine displace H^+ -benzodiazepines in binding assays, leading to the suggestion that they may be endogenous ligands for benzodiazepine receptor sites. We have applied purines and pyrimidines to cultured spinal neurons and find two major, transmitter-like effects: a rapidly desensitizing depolarizing excitation similar to that described with peptide applications and a non-desensitizing inhibitory response due to an increase in Cl^- conductance. Less than 20% of the cells tested appeared to be sensitive to either type of endogenous substance. Clear interactions between the purines, pyrimidines, peptides and amino acids have not yet been demonstrated. The results provide pharmacologic evidence that these substances could mediate several forms of synaptic transmission in the central nervous system.

4. H^+ ion effects. H^+ ions applied by iontophoresis from pipettes containing HCl (pH-4) mimicked several of the membrane effects observed with iontophoretic and pressure applications of peptides, purines, and pyrimidines including 1) a rapidly desensitizing, depolarizing excitatory response, 2) antagonism of neutral and acidic amino acid responses apparently in a competitive manner, and 3) elevation of threshold for spike generation. The results may relate to rapid titration of protein groups associated with specific membrane functions (e.g., rapidly desensitizing excitatory conductance mechanisms, amino acid receptor proteins, and surface charge relevant to activation of voltage-dependent conductances). They have implications for all observations made using iontophoresis in vivo. The latter research may be contaminated by contributions of H^+ ions, especially in those instances where the pH of the drug solution in the iontophoretic pipette has been lowered so as to positively charge the drug molecules.

5. Pharmacology of Clinically Important Drugs. Several clinically important drugs have been applied to cultured mouse spinal neurons including benzodiazepines and barbiturates. Benzodiazepines have transmitter-like effects on a small percentage of the cells. These include either an increase or a decrease in Cl^- conductance. Benzodiazepines also exhibit modulatory effects, consisting of enhancement of GABA-induced Cl^- conductance. The latter action has been observed with pipettes containing as little as 500 pM flurazepam, indicating a quite potent effect whose low concentration approximates the K_d of the drug. Flurazepam also cross-desensitizes with the depolarizing, excitatory response to the purine inosine, which displaces the drug in binding assays. These observations do not immediately explain the variety of pharmacologic effects seen clinically with benzodiazepines, but they do suggest that the drug may act in part by engaging receptors normally occupied by endogenous ligands. Since purines do not enhance GABA responses, it is doubtful that benzodiazepine modulation of GABA events is mediated through activation of purine receptors. Anesthetic and anticonvulsant barbiturates have been applied to single cells with the following findings: (1) the stereoisomers of pentobarbital cause stereospecific effects, including transmitter-like excitatory and inhibitory responses, while anticonvulsant barbiturates do not share this property, (2) anesthetic and anticonvulsant drugs potentiate GABA inhibitory events and depress glutamate excitatory events with anesthetics being 2-4 fold more potent than anticonvulsants and the actions of the anesthetic pentobarbital show stereospecific requirements, (3) anesthetics and anticonvulsants potentiate inhibitory responses evoked by purines and pyrimidines, showing that the modulatory sites are not restricted to GABA receptor function, and (4) one of the pentobarbital isomers stimulates transmitter release while the other inhibits release. All of the results correlate well with the therapeutic effects reported, suggesting that these actions at the cellular level contribute to the clinical pharmacology of the drugs. The stereospecificity of barbiturate action suggests the presence of stereospecific barbiturate binding sites, or receptors. The latter implies that endogenous ligands might occupy the sites physiologically. We have thus far eliminated GABA, inosine and thymine as possible candidates for endogenous ligands capable of interacting with putative barbiturate receptors.

Significance to Biomedical Research and the Program of the Institute: Dissociated cultures of the mammalian central nervous system are proving to be an extremely useful preparation to study the physiology and pharmacology of central neurons. We have begun by focussing on several aspects of receptor pharmacology, examining the membrane effects of amino acids, peptides, purines, pyrimidines, benzodiazepines and barbiturates. We have been able to resolve details of the cellular pharmacology of these endogenous and exogenous ligands. Our research has focused on receptor pharmacology since this aspect of cell-to-cell communication is relatively easily studied and yet plays a crucial role in intercellular communication. It is clear from these initial studies that a variety of clinically important drugs affect receptor-coupled changes in excitability and that these changes may well be one of the bases for their pharmacologic effects in the CNS. The results may thus help to provide a more solid scientific basis for clinical neuropharmacology.

Proposed course: The project will continue to examine the physiology of intercellular communication in the nervous system and the pharmacology of central receptors. The neuropharmacology will proceed at the three aforementioned levels of analysis. Structure-activity-relationships of benzodiazepine and barbiturate actions on central neurons, single channel level analysis of receptor function, and correlation of synaptic events with estimates of elementary events are three particular areas of future focus. Such experiments should provide more meaningful and quantitative analyses and, hopefully a better understanding of the membrane mechanisms underlying the actions of neuroactive substances. Most of the research carried out thus far has utilized unidentified spinal cord and dorsal root ganglion cells. In the future we plan to experiment with identified cells by 1) enriching cultures for specific neurons, 2) growing cells in serum-free media, and 3) applying immunohistochemical staining techniques while leaving cells physiologically viable.

Publications:

Barker, J.L. and Smith, T.G., Jr.: Electrophysiological analysis of molluscan neurons generating bursting pacemaker potential activity. In Chalazonitis, N. and Boisson, M. (eds.): Abnormal Neural Discharges. pp. 359-387. Raven Press, New York, 1978.

Barker, J.L. and Ransom, B.R.: Amino acid pharmacology of mammalian central neurones grown in tissue cultures. J. Physiol. 280: 331-354, 1978.

Barker, J.L. and Ransom, B.R.: Pentobarbital pharmacology of mammalian central neurones grown in tissue culture. J. Physiol. 280: 355-372, 1978.

McBurney, R.N. and Barker, J.L.: GABA-induced conductance fluctuations in cultured neurons. Nature 274: 596-597, 1978.

Barker, J.L., Smith, T.G. and Neale, J.H.: Multiple membrane mechanisms of enkephalin revealed using cultured neurons. Brain Research. 154:153-158, 1978.

Macdonald, R.L. and Barker, J.L.: Enhancement of GABA-mediated post-synaptic inhibition in cultured mammalian spinal cord neurons: a common mode of anticonvulsant action. Brain Research 167: 323-336, 1979.

Barker, J.L., Evidence for diverse cellular roles of peptides in neuronal function. Neurosciences Res. Prog. Bull. 16:535-553, 1978.

Barker, J.L., Gruol, D.G., Huang, L.M., Neale, J.H. and Smith, T.G.: Enkephalin: pharmacologic evidence for diverse functional roles in the

Publications (cont'd)

nervous system using primary cultures of dissociated spinal neurons. In: Characteristics and Function of Opioids, Terenius L. and Van Ree, J.M. (eds.) Elsevier, North-Holland, pp. 87-98, 1978.

Barker, J.L. and McBurney, R.N.: GABA and glycine may share the same conductance channel on cultured mammalian neurons. Nature 277: 234-236, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02152-05 LNP
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Neural connections in the retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Kolb	Research Biologist	LNP NINCDS
OTHER:	H. Wagner	Head, Sect. Neuronal Interactions	LNP NINCDS
	R. Norman	Senior Staff Fellow	LNP NINCDS

COOPERATING UNITS (if any)

R. Nelson, LVR NEI
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LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Section on Neuronal Interactions

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2	PROFESSIONAL: 1.5	OTHER: 0.5
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(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER
 (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The vertebrate retina has two plexiform layers where synaptic connections between neurons take place. At the Outer Plexiform Layer (OPL) the interactions between the photoreceptors, horizontal, and bipolar cells lead to specific information transmitted to the next layer of neural interaction, the Inner Plexiform Layer (IPL). Here the more complex spatial and temporal properties are added by means of many varieties of amacrine cell to produce the final output in the form of the ganglion cell's receptive field. We have discovered that stratification of dendrites in the IPL is ON center and OFF center channels of the visual system. We are interested in discovering whether properties such as directional selectivity and color coding might not also have specific architectural organization in both the plexiform layers.

Project Description:

Objectives: To understand the neural circuitry of the vertebrate retina.

Methods Employed: 1. Light microscopy of Golgi-impregnated material. 2. Electron microscopy of Golgi-impregnated material. 3. Ultra-thin serial sectioning for electron microscopy. 4. Correlations with intracellular recordings and Procion marking of retinal neurons.

Major Findings: In a collaborative anatomical study of Golgi-impregnated monkey retinas, we have discovered a new type of horizontal cell with a distinctly different appearance from the hitherto described monkey horizontal cell type. The new type II horizontal cell has a profusion of fine, multibranched dendrites ending either in clusters or single terminals, and a short (100-300um length) convoluted axon which occasionally sprouts small clusters of terminals. In contrast, the type I horizontal cell has thick dendritic branches bearing large clusters of terminals, and a stout axon which travels a direct course for 2 mm before ending in a multibranched axon terminal. Type II horizontal cells have larger dendritic trees than type I cells in the foveal region but smaller dendritic trees than type I horizontal cells in peripheral retina. Golgi-EM of the new type II horizontal cells shows that the dendritic terminals contact cones and possibly some rods, while the groups of terminals on the short axon contact select cones. The photoreceptor connections of the type I monkey horizontal cells are already well documented. Calculations of space constants on the type II horizontal cell axon indicates that it probably behaves as a true axon conducting signals away from the cell body. It is hypothesized that the new HII cell contacts green and blue cones while the old HI cell may contact only red and green cones.

The morphology of physiologically identified neurons of the cat retinas has been determined by comparisons of HRP injected cells with Golgi-impregnated neurons. Cone bipolar cells that respond with a hyperpolarization to light prove to be flat cone bipolars, whereas cone bipolars responding with a depolarization to a flash of light are invaginating cone bipolars. Previously, we have shown that the dendritic branching of ganglion cells either in the upper portion of the IPL where they receive flat cone bipolar input or in the lower portion of the IPL where they receive invaginating cone bipolar input determines whether the ganglion cells will be OFF center or ON center respectively. Thus, the ON/OFF center characteristics of direct cone bipolar/ganglion cell connected pathways in the retina, must originate before the bipolar ganglion cell synapses in the IPL: probably at the cone pedicle to cone bipolar synapses in the OPL.

Several amacrine and ganglion cell types have also been studied with HRP after physiological investigation of their responses to light. Future EM analysis of the synaptic input to such marked cells is planned.

Golgi studies of carp and turtle retinas have been successful and correlations of the different cell types with physiologically identified cells will be possible. The turtle, for example, contains 8-10 bipolar types, 15-18 amacrine cell types and 18-20 ganglion cell types. We know that a large ganglion cell type branching high in the IPL is bipolar dominated whereas a large diffuse ganglion cell type is amacrine dominated from comparisons of these morphological findings with some physiological results in turtle retina. We hope in particular, to find morphological equivalents for orientation selective amacrine and ganglion cells in turtle retina by these methods.

Significance to Bio-medical Research and the Program of the Institute: Studies of the structure of the retina will provide an understanding of the cells within the retina and will in all probability relate to neural circuitry elsewhere in the CNS. Many programs of this Institute are concerned with the physiology and marking of single retinal neurons and thus knowing the morphology and connectivity of these neurons is essential for our further understanding of visual events.

Proposed Course of the Project: It is proposed to continue the study of the structure of the retina to obtain further insights into the relevance of structure to function in the CNS.

Publications:

Rosenthal, A.R., Kolb, H., Bergsma, D., Huxoll, D. and Hopkins, J.L.: Chloroquine retinopathy in the rhesus monkey. Invest. Ophthal. 17: 1158-1175, 1978.

Kolb, H.: The inner plexiform layer in the retina of the cat: electron microscopic observations. J. Neurocytol. 8: 295-329, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02330-02 LNP																					
PERIOD COVERED October 1, 1978 to September 30, 1979																							
TITLE OF PROJECT (80 characters or less) Biochemical Pharmacology of Cultured Nerve and Muscle Cells.																							
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COOPERATING UNITS (if any) R. Elde, Univ. of Minnesota; J. McKelvy, Univ. Texas Southwestern Medical School; M. K. Ticku, Univ. of Texas at San Antonio. LCS, NIMH; LC, NIA.																							
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SUMMARY OF WORK (200 words or less - underline keywords) Mammalian central neurons and avian muscle cells have been grown in tissue culture to study 1) immunohistochemically identified peptidergic neurons. 2) peptide synthesis by those neurons, and 3) the characteristics of specific receptors. Sensory and spinal neurons stain specifically for nerve-specific enolase, while non-neuronal cultured cells do not. Some of these neurons stain positively for either methionine- or leucine-enkephalin, substance P, or somatostatin. Enkephalinergic neurons can synthesize and release methionine-enkephalin when incubated with labelled methionine. Binding studies have demonstrated the existence of several classes of receptor on cultured neurons including those for GABA, opioid peptides and benzodiazepines. Cultured muscle cells have functional acetylcholine receptors which, when activated, stimulate the influx of labelled Na ions. Pentobarbital blocks this flux at a site that does not discriminate between the two isomers. The results show the utility of using cultured nerve and muscle cells as model systems to study, with neurochemical techniques, the synthesis of endogenous substances and the characteristics of their receptors.																							

Objectives: The objective of this research is to gain insight into the (1) development, disposition and functionality of central neuronal membrane receptors, (2) mechanisms of peptide synthesis, and (3) physiology of neurons identified immunohistochemically.

Methods Employed: The presence of receptors stereospecific for particular agonists is investigated using conventional receptor-binding assay techniques. The functionality of such receptors is studied by examining receptor-mediated ion fluxes. The mechanisms of peptide synthesis are studied by incubating cultures with radioactive precursor amino acid, extracting radioactively labelled peptides and submitting these to a multi-step purification procedure. Immunohistochemical identification of neurons containing specific antigens is carried out using conventional immunohistochemical fluorescence techniques applied to neurons grown on coverslips in culture.

Major Findings: 1. Immunohistochemistry of cultured neurons. A small fraction of the cells which grow in culture stain positively for nerve-specific enolase, an enzyme marker specific for nerve cells. Most of the cells in culture (fibroblasts and other background elements) do not stain for the enzyme. Intracellular recordings from those cells which stain positively for the enzyme showed that they possessed membrane properties characteristic of nerve cells *in vivo*, including excitability and spontaneous synaptic activity. Some elements whose morphology resembles the enolase-positive neurons can be stained, using immunohistochemical methods, for either of four peptides (substance P, somatostatin, leucine- or methionine enkephalin). These elements are presumed to be nerve cells. They do not appear to have distinctive morphologies. The percentage of cells staining for any of these peptides is less than 10%, making it difficult to attempt intracellular recordings of identified cells.

2. Peptide synthesis. Spinal cord and brain cultures incubated in radioactive methionine synthesize and secrete methionine-enkephalin. The baseline observations should allow us to ask questions regarding regulation of synthesis and release.

3. Receptors on cultured cells. Binding assays using labelled ligands including benzodiazepines, GABA and opiates shows the presence of stereospecific, saturable binding sites for each class of substance. The functional nature of GABA sites has been studied biochemically, using GABA-stimulated influx of ^{36}Cl . Electrophysiological observations agree with the flux data and indicate that GABA receptor function is coupled mainly to Cl^- conductance mechanisms. The functional nature of the binding sites for the drugs has only been approached with electrophysiological measurements of membrane events associated with the pharmacological actions of the drugs on individual nerve cells. The data reveal a multiplicity of actions (detailed in a separate annual report). 'Cholinergic receptors on cultured avian muscle cells have been studied using

carbachol-stimulated ^{22}Na flux. The results show that anesthetic and anticonvulsant barbiturates depress Na fluxes in a dose-dependent, reversible manner. The antagonism appears to be non-competitive. The barbiturate is not stereospecific unlike those observed on cultured spinal neurons.

Significance to Biomedical Research and the Program of the Institute: Cultured mammalian neurons and avian muscle cells appear to be useful model systems to study receptor pharmacology and peptide synthesis with biochemical techniques. The prime advantages of the preparation are 1) the lack of diffusional barriers to radioactive ligands and precursors and 2) the opportunity to carefully control the extracellular environment. Demonstration of peptide synthesis *in vivo* has been all but impossible owing to the presence of physical barriers and uptake systems. Likewise binding properties of receptors from *in vivo* material utilize fractionated membrane suspensions, while the binding experiments in culture use an intact monolayer of cells. The results achieved thus far are not only promising, but certain important baseline observations upon which future questions will be predicated. What regulates the appearance of particular receptors? Are they inserted as completed units or do they mature into functional units while in the membrane? Where are they distributed on nerve cells? How are peptides synthesized--on ribosomes or through some other synthetic mechanism? What regulates their synthesis? Finally, the combination of immunohistochemistry with electrophysiology should allow us to examine, for the first time, the physiology of particular peptidergic neurons.

Even incomplete answers to the questions raised above will advance our knowledge of the developmental biology of receptors, peptide synthesis and the physiology of identified neurons, since exceedingly little hard data exists in any of these areas today.

Proposed Course of the Project: The three projects briefly outlined - developmental biology of specific receptors, peptide synthesis and immunohistochemistry - will proceed as deliberately as possible. Once baseline observations have been obtained, the first generation of appropriate and important questions will be asked.

Publications:

Neale, J.H., Barker, J.L., Uhl, G. and Snyder, S.H.: (1978) Enkephalin-containing neurons visualized on spinal cord cell cultures. Science 201: 467-469.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02293-03 LNP
PERIOD COVERED July 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Neural Integration and Processing in the Mammalian Visual System		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: H.G. Wagner Acting Chief LNP NINCDS		
COOPERATING UNITS (if any) M.L. Wolbarsht, Duke Univeristy J. Ringo, Duke University		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION Section on Neuronal Interactions		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) A study of the <u>spectral sensitivity</u> of <u>retinal ganglion cells</u> in the cat has been made using strong <u>chromatic adaptation</u> and <u>spatial localization</u> of the stimulus to the receptive center and/or periphery. The results have shown that three independent and chromatically distinct <u>cone receptor</u> systems appear to converge on many if not all ganglion cells. Most show simple additivity rather than opponency in the same region of the <u>receptive field</u> . A rare cell did show opponency however. Blue receptor input is <u>found in a high percentage</u> of ganglion cells, although not in opponency between center and periphery.		

Project Description:

Objectives: To study neural interactions and processing in a major sensory system. This study will provide insight on how information is organized and processed by a major neural plexus (retina) in preparation for transmittal to a distant neural plexus (lateral geniculate) nucleus.

Methods Employed: Anesthetized and curarized intact experimental mammals such as the cat are placed in a special holder for stereotaxic placement of a interocular microelectrode to the retina. A modified maxwellian view optical stimulator permits precise light stimuli to be placed on the retina under direct visualization. Electrical responses are correlated with various parameters of the stimulus.

Major Findings: Cat retinal ganglion cell show three independent input systems believed to be cone systems which can be differentiated on the basis of spectral sensitivities. One, previously shown by others, has its maximum sensitivity (λ max) at 450 nm. Another even better known, has its λ max at 550. The third one has a λ max of 500 nm. As this λ max is the same as presumed for the rod, considerable attention and effort was made to determine whether it was a rod or cone system.

Confidence in our interpretation that it is in reality a cone system is based on receptive field differences, cone to rod break during dark-adaptation and that the determinations were made in the presence of background light levels well into photopic levels.

Significance to Bio-medical Research and the Program of the Institute: This study indicates that the cat has trichromacy though not well expressed in behavior. The presence of three separate wavelength independent systems may be more broadly represented in the mammalian species than previously believed.

Proposed Course of the Project: Further study will be made on color coding characteristics of the receptive fields and ganglion cell classes in cats.

Publications:

Ringo, J., Wolbarsht, M.L., Wagner, H.G., Crocker, R., and Amthor, F. Trichromatic Vision in the cat. Science Vol. 198 p. 753-755, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01690-11 LNP												
PERIOD COVERED October 1, 1978 to September 30, 1979														
TITLE OF PROJECT (80 characters or less) Rapid Scanning Microspectrophotometry in Visual Cells														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" data-bbox="158 374 922 450"> <tr> <td>PI:</td> <td>T. G. Smith, Jr.</td> <td>Medical Officer</td> <td>LNP NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>J. Oldak</td> <td>Staff Fellow</td> <td>LNP NINCDS</td> </tr> <tr> <td></td> <td>T. Colburn</td> <td>Electronic Engineer</td> <td>TD NINCDS</td> </tr> </table>			PI:	T. G. Smith, Jr.	Medical Officer	LNP NINCDS	OTHER:	J. Oldak	Staff Fellow	LNP NINCDS		T. Colburn	Electronic Engineer	TD NINCDS
PI:	T. G. Smith, Jr.	Medical Officer	LNP NINCDS											
OTHER:	J. Oldak	Staff Fellow	LNP NINCDS											
	T. Colburn	Electronic Engineer	TD NINCDS											
COOPERATING UNITS (if any) TD, NINCDS Dept. of Biomedical Engineering University of Maryland														
LAB/BRANCH Laboratory of Neurophysiology														
SECTION Section on Sensory Physiology														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) A <u>rapid scanning microspectrophotometer</u> has been designed and constructed which allows absorption spectra to be measured between 350 and 650 nm at a speed of 0.6 msec per wavelength band (10 nm). Experiments on frog (<u>Rana pipiens</u>) have been performed or are in progress to measure the effects of several variables on visual pigment (rhodopsin) <u>kinetic changes</u> . These variables include pH, availability of glucose and oxygen, temperature, divalent cations, presence and absence of pigment epithelium and degree of attachment of rod outer segments. The results have confirmed the importance of <u>pH</u> in determining the rhodopsin photoproduct pathways and kinetics. For the first time, however, the <u>metabolic</u> and <u>respiratory status</u> of the <u>retina</u> can be paramount in determining photo-product characteristics.														

Project Description:

Objectives: The objective of this research is the development of rapid scanning microspectrophotometric techniques for investigation of the chemical kinetics of excitable cells. Although these techniques are being developed to study the molecular steps coupling photoexcitation of visual pigments to the excitation of the electrical response of the cells, their more general application to cell physiology have also been considered.

Methods Employed: The microspectrophotometer sequentially samples transmittance at spectral wave bands between 350 and 650 nm, at rates of 600 microseconds per sample point. The system uses a rapid scan monochromator in which the entrance slit has been replaced by the image of a cathode ray tube face.

Major Findings: The first complete set of experiments has been completed. These experiments varied, in a systematic way, the effects of pH, metabolism and respiration on the pathways and kinetics on the rhodopsin photoproducts, metarhodopsins II and III and retinal. The pH was set at 5.5 with acetate buffer, 7.4 adding glucose to the perfusate to stimulate metabolism or 2-deoxy-glucose to block metabolism. Respiration was controlled by saturating the perfusate with oxygen or nitrogen to stimulate or block respiration, respectively.

At low pH (5.5), pH was found to be the dominant factor in controlling photoproduct characteristics, whereas at pH 7.4 and especially at pH 8.2, metabolic and/or respiratory were sometimes more important.

Of the several proposed rhodopsin photoproduct pathways, the one proposed by Bauman (J. Physiol., 222: 643-663, 1972) best fits our data. In addition, a hypothesis based on the known biochemical and physiological characteristics of vertebrate photoreceptor cells can account for all, but one, of the 32 different experimental observations.

Significance to Bio-medical Research and the Program of the Institute: This project has provided the specific instrumentation and techniques for the measurement and analyses of the transduction steps which couple the stimulus to the electrical changes in the excitable membrane of photoreceptors. It can also provide a useful tool for similar study of other molecular systems functioning within living cells, for which an increasing need had developed.

Prior to the completion of these experiments there was considerable discussion, confusion and disagreement among scientists as to what were "the true" pathways and kinetics of the photoproducts of the visual pigment rhodopsin. What these experiments have shown, for the first time, is that there is not a single set of photoproduct characteristics, but that the pathways and kinetics depend on the experimental situation. In retrospect, it appears that the varied results reported by previous investigations probably reflect differences in experimental conditions rather than "correct"

or "incorrect" results. Cleaning up this confusing situation should help set photopigments and photophysiological research along a more productive course of endeavor.

Of potential importance to central nervous system research is our paradoxical finding that the functional consequences of anoxia are less severe if glucose metabolism has been reduced (with 2-deoxy-glucose) than if functioning normally (with glucose). A similar "paradoxical" result has been reported from CNS studies; however, it has been difficult to document precisely because of the absence of easily quantifiable parameters of brain function in those experiments. Thus, the retina, a CNS-appendage, where precise, quantitative measures of biologically important functional parameters can be obtained, may prove a useful preparation for assaying CNS function in various metabolic, respiratory and other states.

Our research may also be of relevance to an understanding of the pathophysiology of retinal diseased states. Heretofore, most retinal signs and symptoms were held to be mainly neural in origin. We have demonstrated, however, that abnormal retinal conditions can lead to visual pigment and hence to photoreceptor malfunction.

Proposed Course of the Project: Since the objectives of the project have been realized, the project is, with this report, terminated.

Publications:

Resnik, J.A., Malerba, F.E., Colburn, T.R., Murray, G.C. and Smith, T.G. A novel rapid scanning microspectrophotometer and its use in measuring rhodopsin photoproduct pathways and kinetics in frog retina. J. Optical Soc. Amer., June, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02221-04 LNP
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PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Ionic Mechanisms of Phototransduction in Rods of the Vertebrate Retina.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. A. Normann	Staff Fellow	LNP NINCDS
OTHER:	E. Pasino	Visiting Scientist	LNP NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Section on General Physiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.6

PROFESSIONAL:

1.5

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The membrane mechanisms by which light is transduced into electrical signals were investigated in rod type photoreceptors of the toad using ionic substitutions and pharmacological studies. The rod photoresponse results from two processes, a light modulated mechanism and a voltage and time dependent mechanism. Techniques for isolating each mechanism and the contribution of each to the photoresponse was studied. The dark potential and the photoresponse were recorded as a function of the concentration of external sodium, potassium and chloride ions as were the effects of 4 amino pyridine, cesium, and ouabain. From these measurements, it is concluded that in the dark, the rod membrane is 10 X more permeable to potassium than to sodium and that at the peak of the photoresponse, the sodium permeability is reduced by at least a factor of 10. We estimate that the cytoplasmic sodium and potassium concentrations are equal. These ionic gradients are maintained by active Na-K pumps.

Project Description:

Objectives: The principal objective of this study is to characterize the membrane mechanisms that are involved in the transduction of light stimulation into the electrical response of the photoreceptor.

Methods Employed: Intracellular recording of electrical potential and conductance was performed in the isolated retina of *Bufo Marinus* using glass capillary microelectrodes. The retina was mounted in a chamber which allowed superfusion of the photoreceptors with solutions of various ionic compositions. Dissection of the preparation and microelectrode positioning was accomplished using infrared visualization provided with commercially available infrared to visible image converters.

Extracellular sodium, potassium and chloride concentrations were measured at the site of receptor impalement using ion specific electrodes (tip diameter $\sim 50 \mu$ diameter) made from glass capillaries whose tips were filled with ion exchange resins (for potassium and chloride) or capped with sodium selective glass. These electrodes had selectivities of about 45 mV/decade when measured in the normal superfusate solutions.

Major Findings: The primary action of light stimulation of the electrical properties of rod type photoreceptors was investigated in the retina of the toad. All experiments were performed using an isolated superfused retina. Two membrane mechanisms are involved in the genesis of the rod photoresponse; a light modulated mechanism and a voltage and time dependent mechanism. When both mechanisms are stimulated by bright test flashes in either the eyecup preparation or the isolated retina, superfused with a physiological solution, a normal response is seen which consists of an initial peak which decays into a sustained plateau.

Activation of the light modulated mechanism (which can be studied in isolation from the voltage and time dependent mechanism by increasing the potassium level in the superfusate to 10 mM or by adding 2 mM CsCl to the superfusion solution) causes the rod to hyperpolarize up to a saturation potential. Further increase in light intensity only prolongs the response. The voltage dependent mechanism (which can be studied by passing extrinsic currents through the rod via the microelectrode) acts as a degenerative element in the rod membrane. Activation of the voltage dependent mechanism with either depolarizing or hyperpolarizing currents causes the membrane potential to sag back towards its prestimulus value. These two mechanisms combine to produce the rod photoresponse.

Decreasing external sodium hyperpolarized the rod but had no effect on the peak potential. We conclude, therefore, that the rod is virtually impermeable to sodium during the peak component of the photoresponse. Increasing external potassium slightly depolarized the rod but caused a large reduction in the peak component of the photoresponse. The dark and peak potentials showed an exponential dependence on the concentrations of these ions as predicted from the Goldman equation. From the Goldman

equation and the above data, we estimate that the rod is 10 X more permeable to potassium than to sodium in the dark and that the sodium permeability is reduced by at least a factor of ten by bright light. We further estimate that the cytoplasmic concentration of sodium and potassium are approximately equal.

Significance to Bio-medical Research and the Program of the Institute: This project has significance primarily at the basic research level. Much is known about the ionic mechanisms involved in propagation of electrical signals along the nerve axons and in synaptic transmission. To date, no description of a primary sensory transducer has been presented which can account for the results described above. A comparison of the membrane mechanisms involved in the generation of the rod light response with the membrane properties of axons may provide fundamental insight into the basic mechanisms underlying neuron function.

Proposed Course of the Project: This project will be completed after a publication is completed and submitted.

Publications:

Normann, R.A., and Perlman, I. The cytoplasmic ionic composition and the plasma membrane permeability of rod photoreceptors in the light and dark. Investigative Ophthalmology, April 1978, pg. 219.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02331-02 LNP
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) A Study of the Complex Receptive Field Properties of Turtle Retinal Neurons		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	Richard Normann Henry G. Wagner Helga Kolb	Senior Staff Fellow Acting Chief Research Biologist LNP NINCDS LNP NINCDS LNP NINCDS
OTHER:	Efrem Pasino	Visiting Scientist LNP NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION Section on General Physiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.1	PROFESSIONAL: 2	OTHER: .1
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS		
<input type="checkbox"/> (b) HUMAN TISSUES		
<input checked="" type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>The <u>receptive field properties</u> of the neurons in the <u>visual streak</u> of the <u>turtle retina</u> have been studied using <u>intracellular</u> and <u>extracellular microelectrode recordings</u> and <u>injection of fluorescent stains</u>. Retinal ganglion cells of the turtle retina show <u>orientation specificity</u>; slits of light when shone upon the retina at the preferred orientation elicit a vigorous response but when shone orthogonal to this orientation evoke little response. Horizontal cells show a similar effect but to a lesser degree (the response in the optimal direction is from 1.1 to 1.5 times larger than the response in the orthogonal direction).</p> <p><u>Anatomical</u> studies done in the visual streak of the turtle retina using the <u>Golgi technique</u> show that these horizontal cells and some ganglion cells have <u>non-uniform dendritic trees</u>. Cells have <u>dendritic trees</u> which are up to <u>2 times longer</u> than they are wide and are usually oriented parallel to the streak.</p>		

Project Description:

Objectives: To characterize the receptive field properties of all five cell types in the turtle retina with particular emphasis on directional selectivity and orientation specificity.

Methods Employed: Intracellular and extracellular recordings with glass microelectrodes of the electrical potentials produced by the retinal neurons in response to stationary and moving slits of light are employed. Intracellular injection of fluorescent dyes (Lucifer) is also accomplished with these electrodes to correlate recorded function with structure.

Major Findings: The receptive field properties of the neurons in the visual streak of the turtle retina have been studied using intracellular and extracellular microelectrode recordings and injection of fluorescent stains. Retinal ganglion cells of the turtle retina show orientation specificity; slits of light when shone upon the retina at the preferred orientation elicit a vigorous response but when shone orthogonal to this orientation evoke little response. Horizontal cells show a similar effect but to a lesser degree (the response in the optimal direction is from 1.1 to 1.5 times larger than the response in the orthogonal direction).

Anatomical studies done in the turtle retina using the Golgi technique show that some horizontal cells and some ganglion cells have non-uniform dendritic trees which are up to 2 times longer than they are wide. They are oriented parallel to the streak.

Significance to Bio-medical Research and the Program of the Institute: The neurons of the primate cortex have been observed to have quite complex receptive fields. Even though these cells have been studied for over 2 decades the mechanism by which these receptive fields are generated have yet to be elucidated due to the small size of these cortical neurons and the complexity of the cortex. Our finding of cells with such properties in the turtle retina may allow the determination of these mechanisms because all 5 types of retinal neurons can be impaled with intracellular electrodes. The use of intracellular staining will allow the determination of structural-functional correlations in these mechanisms.

Proposed Course of the Project: To record and stain all given classes of retinal neurons and follow the genesis of complex receptive fields throughout the retinal pathway.

Publications:

Normann, R.A., Kolb, H., Hanani, M., Pasino, E. and Holub, R.: Orientation of horizontal cell axon terminals in the streak of the turtle retina. Nature 280: 60-62, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02339-02 LNP
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Neural Coding and Processing of Information in the Visual System		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	H.G. Wagner H. Spekrijse R. Crocker H. Kolb	Acting Chief Consultant Research Investigator Research Biologist LNP NINCDS LNP NINCDS LNP NINCDS LNP NINCDS
COOPERATING UNITS (if any) M. L. Wolbarsht, Duke University		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION Section on Neuronal Interactions		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.5	PROFESSIONAL: .4	OTHER: .1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINDRS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) A study of the spectral sensitivity of retinal ganglion cells in fish. Preliminary findings indicate it will be possible to sort out chromatically distinct signals from the various cones. The signals are broad band with max sensitivity at 450, 500 and 650 nm. These signals may be either excitatory or inhibitory influences on the ganglion cell. Complex summations are apparent depending on spectral intensity and spatial distribution of the stimulus.		

Project Description:

Objectives: To study neural processing of color information in the visual system.

Methods Employed: Isolated and oxygenated retina of suitable fish such as carp are stimulated by stimuli of known wavelength, intensity, duration and spatial configuration. Electrical response in ganglion cells and other retinal neurones are detected and analyzed with respect to the stimulus.

Major Findings: Using central and peripheral receptive field stimuli plus intense chromatic adaptation backgrounds, it has been possible to separate out a number of chromatically distinct excitatory and inhibitory inputs to the ganglion cells discharge patterns. Using processes previously worked out in the horizontal cells we believe we can synthesize the ganglion cell functions on the basis of a complex intermixing excitatory and inhibitory inputs from the several classes of cones in this species.

Significance to Bio-medical Research and the Program of the Institute: This study will help understand the processing of neural information in the nervous system.

Proposed Course of the Project: The studies initiated will be continued.

Publications: None

Project Description:

Objectives: To investigate the fine structure and function of synapses between retinal neurons.

Methods Employed: Electron microscopy combined with silver impregnations by the method of Golgi and intracellular injections of horseradish peroxidase. Electrical recordings with intracellular microelectrodes.

Major Findings: The output connections of horizontal and bipolar cell dendrites have been investigated in serial sections of the retina of the tiger salamander. Horizontal cell dendrites represent the only channel through which the information contained in the responses recorded from horizontal cell bodies can be transferred to other retinal neurons. Nevertheless, although they make electrical (gap) junctions with one another, an extensive search failed to yield evidence that they make chemical synapses on any other cell processes at the outer plexiform layer. A possible explanation for this lack of evidence would be that the horizontal cell dendrites are presynaptic only to photoreceptors, as the contacts mediating horizontal cell feedback have not yet been identified by electron microscopy. Since the cell axon terminals have been previously seen to make chemical synapses on other second order neurons, it may be that while the horizontal cell dendrites mainly feed back, the horizontal cell axon terminals mainly feed forward, a hypothesis that is compatible with the available physiological data.

Bipolar cell dendrites, on the other hand, are generally believed to represent only input sites, with output connections being made only by the axon terminals at the inner plexiform layer. Still unpublished work of this laboratory, however, has previously shown that chemical synapse of bipolar cell dendrites on horizontal cell dendrites seem to account for some of the properties of the surround response recorded from horizontal cell bodies (see 1977 annual report). The continuation of such studies show now that bipolar cell dendrites also make chemical synapses on one another, as well as on horizontal cell axon terminals and certain as yet unidentified neuronal processes found at the outer plexiform layer (see below).

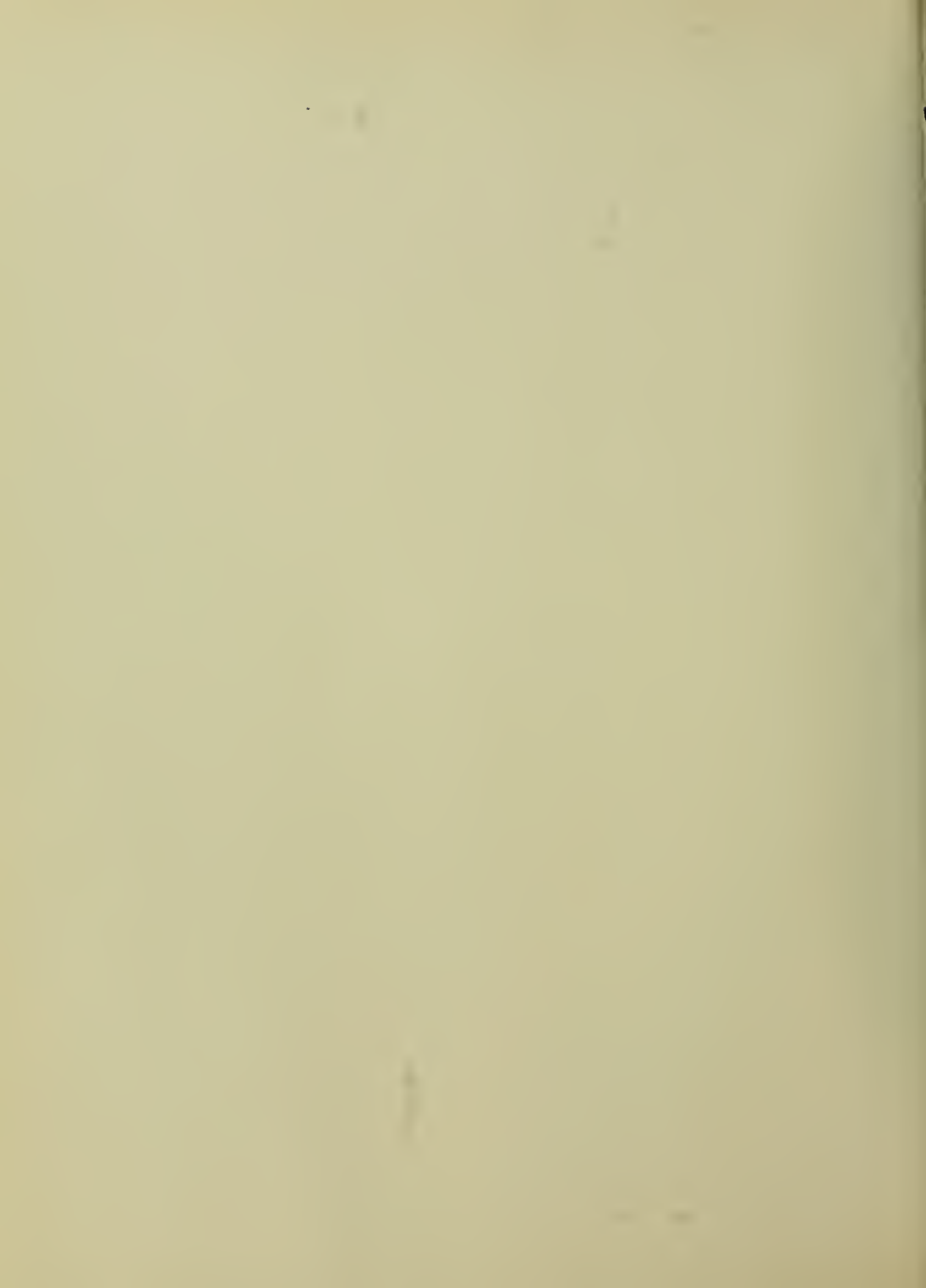
Significance to Bio-medical Research and the Program of the Institute: It is hoped that these observations will help in identifying the mechanisms of synaptic transmission between photoreceptor cells and second order neurons, and provide a better knowledge of the neuronal networks involved in the processing of visual information within the retina.

Proposed Course:

Intracellular injections of peroxidase will be continued in the hope of finding the cell of origin of certain as yet unidentified outer plexiform layer processes (mentioned in previous annual reports). Bipolar cells

had been already excluded, and during this period injections into photoreceptors failed to stain the processes under consideration. The search will now be directed towards neurons having their bodies at the inner levels of the inner nuclear layer.

Publications: None



ANNUAL REPORT

October 1, 1978 through September 30, 1979

Laboratory of Biophysics

National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report
October 1, 1978 thru September 30, 1979
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 is concerned with investigating molecular and cellular mechanisms responsible for excitation, membrane potentials, the generation of the nerve impulse, synaptic activity, 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 makes wide use of physical and chemical techniques, on-line and off-line digital computers and a variety of applied mathematical methods. 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, Mass. The Woods Hole Unit is composed of 2 sections: the Section on Neural Membranes and the Section on Neural Systems. The Bethesda unit of the laboratory is made up of the Section on Molecular Biophysics.

WOODS HOLE UNIT OF THE LABORATORY OF BIOPHYSICS:

Section on Neural Membranes.

The Section on Neural Membranes uses modern electrophysiological, electron optical, mathematical biophysical, and computer science techniques to investigate the function and structure of neural cells and tissues at limits approaching the molecular level. The general approach is to examine mechanisms that universally and fundamentally underlie all neural function. Emphasis is placed on membrane ionic channel structure and function. 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 formation are sought. The physical mechanisms involving the structures of muscle and nerve responsible for contraction and mechanotransduction are probed and these are related to both the biochemical and structural elements underlying these mechanisms. The role of glial cells in modifying axonal and neuronal function is both examined and mathematically modeled.

The project on voltage clamp and impedance measurements has been mainly concerned with a study of membrane excitation properties determined from a statistical evaluation of impulse trains. Impulse trains generated by the space clamped squid giant axon in response to, and phase locked to the current $I(t) = I_0(1 + \sin 2\pi f t)$ were modulated by a second, small amplitude sinusoidal current. The resulting impulse density was estimated for a complete period of the modulation sine wave from an impulse train with a

duration of many modulation cycles. For sufficiently small modulation amplitudes this impulse density function is itself a sinusoid with the same modulation period. Gain and phase (shift relative to stimulus modulation cycle) of the impulse density are determined as a function of the modulation frequency (Bode plots). Model studies have shown that a corner frequency and a gain resonance determine the magnitude of membrane conductance. The phase curve is a function of the conductance time course. Additional structure in the Bode plots may suggest the presence of "summing" phenomena (variables which are not reset by an impulse), and specify the relaxation times of those variables. The Bode plots of the squid axon membrane and of several models including the Hodgkin-Huxley model are qualitatively different. The necessary stimulus current and the measured conductance level are considerably higher for the axon. More importantly, the relaxation times of "recovery variables" (K-conductance and/or Na-inactivation) appear to be considerably longer for the axon when stimulated with sinusoidal currents than those of models derived from voltage clamp data and "stimulated" with the same currents.

The project on the function and structure of ionic channels has focused on potassium channel block by small inorganic and organic cations. In 1972, Bezanilla and Armstrong reported that either Li, Na or Cs, when present internally, caused a negative conductance in the positive quadrant of the instantaneous I-V curve for the K channel. Recent experiments in the Section have added to this list the organic species tris (hydroxy-methyl) aminomethane, glucosamine and tetramethylammonium, each of which produces a qualitatively similar effect. Externally, only cesium has been shown to have a similar effect, causing a steep negative conductance in the negative quadrant of the I-V relation. In earlier studies, periaxonal potassium accumulation caused the external concentration to vary depending upon the blocking species present. Since external K concentration generally affects the block, this prevented a controlled comparison of the different blocking ions. A new experimental procedure has been developed to allow a comparative study of the action of these blocking ions without this complication.

Also of interest is the observation that the three inorganic ions showed slightly steeper voltage dependence, as a group, than the three organic ions. This is consistent with the intuitive expectation that ions of smaller unhydrated radius should penetrate further into the channel, and hence also further into the membrane field.

The effect on the K channel conductance of five quaternary ammonium ions having the general formula $N(C_2H_5)_{n+1}^+$ where $n=1,2,3,4$, or 5 were investigated. Each causes a voltage dependent inhibition of axonal K conductance that becomes more pronounced as transmembrane voltage increases. The onset of block by tetramethylammonium ($n=1$) is effectively instantaneous. For compounds with $n=2$ to 5, onset is slower and causes, at appropriate concentrations and voltages, an inactivation-like decrease in the current recorded under voltage clamp over a period of milliseconds. The data show clearly, contrary to an earlier postulate by Armstrong, that it is not necessary for a quaternary ammonium ion to have the form $R-N(C_2H_5)_3^+$ to exhibit K channel blocking activity.

Analysis of the steady state block showed that the voltage dependence is similar to that for the small, instantaneously acting organic ions mentioned above, suggesting that both groups of ions act at or near the same location. The blocking potency increases as n increases from 1 to 5, with the exception that tetraethylammonium (TEA, n=2) is more potent than tetrapropylammonium (n=3). It is possible that the close correspondence between the size of TEA⁺ and the size of a K⁺ ion with a single hydration shell confers on TEA⁺ a greater blocking potency than would be expected simply from its relation to others in the tetraalkylammonium series. As suggested by Armstrong, the size of TEA probably favors its binding to the inner mouth of the channel.

These results prompt one to ask what chemical structure of the channel and its environs could allow ions of such a wide range of sizes to act in so similar a manner to block the K current.

The effect of symmetric quaternary ammonium ions upon the sodium channel conductance has to be investigated. The ions studied so far are TMA⁺ (tetramethylammonium), TEA⁺ (tetraethylammonium), TPA⁺ (tetrapropylammonium) and TPENTA⁺ (tetrapentylammonium). Of these ions, TEA has been studied most extensively. In contrast to what has been seen in the potassium channel, TEA shows a much lower blocking affinity for the sodium channel ($K_D \approx 45$ mM) and shows only weak rectification. Outward current is blocked about 10% more effectively than inward current. Observations of rectification were made with low external sodium for net outward current and with high external sodium for net inward current. TEA is not significantly permeant to the channel nor does it block sodium current when applied externally. These findings suggest that the block site may be located within the channel and that sodium ions passing inward are capable of clearing the block. A dependence of the block upon external sodium levels may be indicative of multi-ion behavior for the sodium channel.

The presence of TEA leads to a small but significant slowing of the rise of the sodium current during depolarizations. Repolarization leads to a removal of a large fraction of the block in a time fast compared to when current can be accurately measured. Subsequent decay of sodium currents under normal or block induced conditions are similar, suggesting that, unlike potassium channels, sodium channels may close normally in the presence of TEA.

These observations on TEA block of the sodium channel were made with fast sodium inactivation intact. The data differs in this respect from that of Rojas and Rudy who did not observe TEA block of sodium channels without prior removal of fast inactivation with pronase. In the course of preliminary studies it was established that incomplete inactivation (sustained sodium currents at potentials where they are expected to inactivate fully) is a normal property of the squid axon. The presence of a sustained sodium current at large, positive potentials has made it possible to look at interactions between the blocking action of the quaternary series and the "gating" transition between the early, peak sodium conductance and the

later, sustained conductance. Both peak and sustained current are blocked about equally well by TEA. In contrast, TMA, which also blocks the sodium channel but not as effectively as TEA, shows a marked change in blocking potency with the development of fast inactivation.

In the project on subcellular and extracellular neural structures, electron microscopy of the giant axon and smaller fibers of the squid Loligo pealei and of the brain system of the nudibranch Hermisenda crassicornis utilizing improved fixation methods and stereoscopic examination of relatively thick sections (0.2-0.5 μm) has allowed demonstration of a highly ordered neuroplasmic lattice in axons and other neural cellular extensions. The lattice consists primarily of longitudinally oriented neurofilaments and microfilaments, presumably actin, together with microtubules when present, linked together by a well-defined system of thin transverse filamentous bridge elements 2-3 nm in diameter with an apparent periodicity of ~ 40 nm along the axonal longitudinal axis. Internal irrigation of the squid giant axon with fixative following cannulation results in dramatically improved fixation with numerous microtubules being found in the axoplasm, particularly in the subaxolemmal cortical region. The lattice has extensive properties, with domains of order extending over several micrometers. In small axons of both Loligo and Hermisenda, the lattice domain often encompasses the whole fiber diameter. The transverse bridges appear to end at or are structurally continuous with membranous elements such as the axolemma, vesicles, endoplasmic reticulum and mitochondria. It is thought that some or all of the lattice components are involved in axonal transport processes.

The project concerned with excitation-contraction processes in voluntary muscle has continued to examine muscle stiffness. The onset of activity following stimulation of skeletal muscle is first signaled by an increased stiffness prior to tension production. However, the development of stiffness followed in time the early arrival of the stimulus to the muscle fiber interior as reported by other workers. Stiffness did not reach a maximal (tetanic) value during a twitch as was proposed by others for contractile activity evoked by single pulse stimulation. The stiffness values in active muscle decreased in proportion to myofilament overlap decrease as the muscle was lengthened. These results support the suggestion that the internal contractile mechanics are most accurately represented externally by the muscle stiffness.

The project concerned with mechano-electrical transduction in nerve has studied the effects of longitudinal stretch on squid giant axons. The structural complexities of most mechanoreceptor organs impose considerable difficulty on their investigation. Their description nevertheless can be reduced to three elements: a mechanical transformer, the mechano-electric transducer and the amplifier-transmission component. The ultimate representation of mechanoreceptor processes, however, must be limited to the relationships between mechanical input and electrical output of excitable membranes. Axon stretch invariably produced a decrease in membrane voltage (depolarization) roughly proportional to stretch amplitude. Typically, a stretch of 4-5% L_0 produced a depolarization of 3-3.5 millivolts. The time course of depolarization induced by stretch resembled the time course of stress (tension)

in the axon. Generally, the depolarization onset, peak and decay coincided with the corresponding tension changes during stretch. However, unlike the tension which returned to prestretch levels following stretch, the repolarizing membrane voltage exceeded the resting (prestretch) level and showed a brief hyperpolarization before the return. In preparations with well maintained resting membrane voltage (-55 to -60 mV), stretch amplitudes between 6 and 10% L₀ usually resulted in regenerative discharge of the membrane voltage resembling an action potential which included zero potential overshoot. Other observations affirmed that stretch rate was also a determinant in membrane depolarization. Stretch amplitudes which produced nonreversible elastic changes were also accompanied or followed by reduced electrical responses. Finally, the membrane potential, immediately following relatively large stretches or an action potential like discharge would enter a phase of prolonged depolarization which could last up to several minutes.

Section on Neural Systems.

The objective of the Section on Neural Systems is to study cellular and subcellular mechanisms by which neural tissue controls organism behavior and behavioral change in response to environmental stimuli. In pursuing this primary research objective, several principle areas of investigation have been involved. These include: sensory transduction, neural interaction, neural systems (electrophysiological and anatomic features), behavior (stereotypic and learned), neural mechanisms for associative learning, biochemistry of neural function and modification, and development of neural systems. For the last few years the major focus of the section has been an integrated multidisciplinary effort to determine a neural (and possibly a biochemical) basis for an associative learning model with the nudibranch mollusc Hermissenda crassicornis. A number of invertebrate species were considered as potential model systems to analyze cellular mechanisms of behavior and learning. These included Tritonia, Aplysia, Pleurobranchia, Helix, Elysia, and Hamonoia. The last two have been cultivated within the laboratory and subjected to preliminary electrophysiologic and histologic investigation. The nudibranch mollusc Hermissenda crassicornis, however, has proven to be a most opportune preparation in satisfying the host of constraints which arose from the questions which were asked. With Hermissenda it has been possible to define a model of associative learning with the same defining features used for vertebrate associative learning. Movement of Hermissenda toward a light source is markedly reduced after repeated pairing of a light stimulus with rotation. This behavioral change is truly associative (i.e., random light and rotation do not produce the effect), persists for at least several days after training and increases with practice. Stimulus specificity for this behavioral change was indicated by the fact that trained animals did not show changes in responsiveness to food. Because of the relative simplicity of the nervous system it has been possible to ascertain many of the invariant aspects of the three sensory pathways essential to the associative learning model: the visual, statocyst, and chemosensory pathways.

Changes have been found (within these neural systems of Hermissenda) which occurred only in animals subjected to associative learning paradigms and not to control paradigms. For example, with the first associative

training procedure used it was found that hair cells received less excitatory input from ipsilateral Type A photoreceptors after repeated stimulus pairing but not after control training paradigms. Comparable neural modification could be produced while recording intracellularly. Thus it was possible to monitor the neural changes as they were progressively produced by the associative training procedure.

Recent experimental results indicated that a primary neural change occurred (with the learning model) within the Type B cells of the Hermisenda eye. Current and voltage clamp recordings from this cell revealed the presence of a prolonged voltage-dependent Ca^{++} current during and after light steps. The voltage-dependence of this Ca^{++} -current and a Ca^{++} -dependent K^{+} current provide a neural basis for the contingency necessary to the associative learning model. The finding that this Ca^{++} current can be regulated by intracellular injection of cyclic-AMP (and not 5'-AMP) suggested biochemical studies (e.g. of protein phosphorylation and protein synthesis) which have been initiated.

In other related studies, power spectra of the voltage and current noise in hair cells have been analyzed for a variety of stimulus and treatment conditions. These analyses indicate that the resting conductances of the hair cell membrane are modulated by the rhythmic beating of the cilia. More recently, power spectra of the current noise in hair cells (obtained in voltage-clamped hair cells) have provided more accurate values of hair cell conductances at rest and during stimulation. Under voltage-clamp the filtering properties of the hair cell membrane no longer distorted the conductance measurements.

It has also been established that excitation of hair cells by Type A photoreceptor impulses persists during synaptic blockade. No resistance change was observed during this excitation when synaptic blockade is effected by perfusing with Co^{++} or Co^{+++} with reduced extracellular calcium. Additional data were also obtained which support the interpretation that the excitation of hair cells by Type A impulses arises from potassium accumulation around the Type A photoreceptor and hair cell axonal membranes. As such, this represents the first well-documented case of neurons exchanging significant information without either electrical or chemical synapses, although non-synaptic interaction has been demonstrated between "simple system" neurons and their investing tissues.

Recent biochemical studies have focused on single cell protein synthesis and phosphorylation. Those cells believed to play a primary role in effecting the associative learning have been found to undergo specific changes of protein phosphorylation related to the previous training experience of the animal.

It has also been possible to determine by microspectrophotometric techniques the spectra of visual pigments in individual Type B (see above) photoreceptors isolated from the nervous system.

Behavioral analyses of the main experimental animal Hermissenda cras-
sicornis have ranged from field observations to comparative studies of
laboratory-reared and collected species. Findings of the past year, for
instance, showed that light response involves a preference for certain
levels of intensity, and a biphasic approach/withdrawal process which depends
on an individual animal's light history. This behavior is consistent with
the predictions from a recent model of phototaxis which assumes that species
have preferences for optimum levels of ambient illumination. Field observa-
tions, on the other hand, indicate that natural Hermissenda populations
undergo diurnal vertical migrations which are determined not only by ambient
light and temperature conditions but also by food availability.

Currently, the third laboratory generation of Hermissenda is in the
juvenile stage. A model for the study of environmental genotypic interaction
may be provided by the statocyst in laboratory-reared Hermissenda. In all F_1
and F_2 generation individuals the statocysts contained a single statoconium.
In wild animals, on the other hand, the statocysts typically contain 150-200
statoconia (concretions suspended within the intracyst fluid). Only occasion-
ally were single statoconia encountered in wild Hermissenda. Conventional
and electronmicroscopic sections have been prepared of pre-metamorphosis
forms. Identification of the neural elements within the sensory pathways
investigated in the adults has been achieved. The ontogeny of the Hermissenda
nervous system, therefore, can now be followed with particular attention
being given to the temporal and spatial relationships during development of
the ganglia, eyes, statocysts and tentacles.

Another EM study, concerning adult statocyst hair cells, stained with
uranyl acetate and lead citrate or vanadomolybdate, revealed the presence of
microfilaments as lateral projections from the basal bodies. These micro-
filaments extend tangentially below the cell surface in the form of an
astral array. Their length and directionality suggest some degree of morpho-
logical polarity and the possibility of an infraciliary network. The EM
results also indicate that the smaller, more numerous statocyst support cells
are responsible for forming the statoconia concretions within the statocyst,
while the hair cells may play a passive role in their formation by acting as
a reservoir for stored carbonates.

BETHESDA UNIT OF LB:

Section on Molecular Biophysics.

The Section on Molecular Biophysics is involved in four general areas of
research: molecular mechanisms of channel gating, properties of open channels,
molecular mechanisms of drug action, and biological applications of membrane
biophysics.

In the project studying channel gating, several advances have been made.
The approach now being taken is to study gating using the patch-clamp tech-
nique pioneered by Neher and Sakmann in conjunction with the basic channel
concepts that were developed in the Section's lipid bilayer program. The

heart of the patch-clamp technique is a pipette of appropriate shape to make a tight seal against the membrane to be studied. Pipettes have been fabricated and single acetylcholine-activated channels have been observed.

Another approach, which was pioneered by Armstrong and Bezanilla is to measure "gating current" - currents caused by the movement of charge in the gates, themselves. The effect of temperature on gating currents in the squid giant axon has been determined.

A new project is the study of the development of excitability in axons by transverse mechanical stimulation. This is a follow-up of the work by Julian and Goldman. Equipment has been fabricated to apply and measure appropriate mechanical stimuli, and to record the voltage-clamped axonal response.

Other experiments have been directed at explaining the surprising linearity of the instantaneous I-V relation for sodium channels. It was found that, in general, the shape of this curve depends on the calcium concentration, since calcium tends to block the sodium channel. At the physiological calcium concentration, the nonlinearity (expected because of asymmetric sodium concentrations) and the nonlinearity caused by calcium blockage tend to cancel, giving rise to an approximately linear instantaneous I-V curve.

Another approach that is underway (in collaboration with Dr. George Weiss of DCRT) is the determination of the noise spectrum for an open channel. This "transport noise", as opposed to gating noise, may provide useful information about the nature of ionic transport through channels.

The project studying the molecular mechanisms of drug action has made several advances. The alkaloid drug yohimbine was studied in two different types of experiments. First, in collaboration with Dr. Lipicky of the Food and Drug Administration, yohimbine was applied to a squid giant axon, and a variety of voltage-clamped experiments were performed. In collaboration with Dr. Catterall, LBG, NHLBI, tissue-cultured neuroblastoma cells were used to test for competitive inhibition between yohimbine and batrachotoxin (BTX), a drug that opens sodium channels. In these experiments, varying concentrations of yohimbine and BTX were applied to different culture dishes, and the radioactive uptake of sodium ions was measured. It was found that there was competitive inhibition between the two drugs, suggesting that they both bind to the same site. Since BTX is a channel opener and so cannot occlude the channel, it is suggested that yohimbine, a drug that reduces sodium current, does so by its action on the gating mechanism, rather than by occlusion.

The study on the effects of some of the channel-opening drugs has found that scorpion venom tends to broaden the action potential in Myxicola. In the investigation on the effect of veratridine, itself, on Myxicola axons, it has been found that application of veratridine results in a steady state component of sodium current.

Using the method of radioactive uptake of sodium in the presence of BTX to compare sodium channels with different TTX dissociation constants, it was found that a number of properties related to the selectivity filter are the same in the two cell lines studies, suggesting that the difference in channel structure that accounts for the difference in binding occurs not at the selectivity filter, but at a hydrophobic site.

In the project on biological applications of membrane biophysics, a number of qualitative observations on nerve responses by means of the Hodgkin-Huxley model have been explained by showing both qualitatively and quantitatively how anodal excitation occurs.

A theoretical analysis of the second order nonlinear components of membrane admittance of the Hodgkin-Huxley model has been made, and computer programs have been developed to permit comparison of experimental results with theoretical predictions.

The sophisticated mathematics of optimal control theory has been used to describe the manner in which man executes voluntary muscular activity.

In collaboration with Dr. Rapoport of NIA, a project on the frog perineurium has been undertaken. The reasons for interest in this project are: determination of the function of the perineurium, use as a prototypical blood-brain barrier, and possibility of using perineurium impedance to diagnose neuropathies. Sucrose permeability and the impedance of the perineurium have already been measured. The effect of stretch has also been determined: 10% stretch causes a reversible increase in sucrose permeability, but 20% stretch causes irreversible damage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01950-08 LB																				
PERIOD COVERED October 1, 1978 to September 30, 1979																						
TITLE OF PROJECT (80 characters or less) Excitable Membrane Characteristics: Voltage Clamp and Impedance Measurements.																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 35%;">W. J. Adelman, Jr.</td> <td style="width: 20%;">Chief</td> <td style="width: 15%;">LB NINCDS</td> </tr> <tr> <td>Other:</td> <td>K. S. Cole</td> <td>Research Biophysicist</td> <td>LB NINCDS</td> </tr> <tr> <td></td> <td>C. Tyndale</td> <td>Electronic Engineer</td> <td>MBL</td> </tr> <tr> <td></td> <td>R. Waltz</td> <td>Mathematician Programmer</td> <td>MBL</td> </tr> <tr> <td></td> <td>J. Fohlmeister</td> <td>Guest Worker</td> <td>LB NINCDS</td> </tr> </table>			PI:	W. J. Adelman, Jr.	Chief	LB NINCDS	Other:	K. S. Cole	Research Biophysicist	LB NINCDS		C. Tyndale	Electronic Engineer	MBL		R. Waltz	Mathematician Programmer	MBL		J. Fohlmeister	Guest Worker	LB NINCDS
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COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA 02543																						
LAB/BRANCH Laboratory of Biophysics, IRP, NINCDS																						
SECTION Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)																						
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205																						
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.8	OTHER: 0.2																				
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) <p>The general aim of this project has been to improve <u>electrical measurements of excitable membrane characteristics</u> consistent with physical and chemical methods for the study of nerve membrane ionic channels. Two major approaches are used. The first involves the development of methods for <u>rapid analysis of ionic channel conductances</u> by means of <u>computer controlled voltage clamp techniques</u>. Programs for carrying out this analysis are developed. The second approach involves analysis of <u>excitable membrane characteristics</u> by means of <u>frequency analysis</u> of sinusoidally modulated action potential trains locked to a larger amplitude, higher frequency sinusoidal stimulus. Improvement of <u>bridge impedance techniques</u> to achieve greater accuracy are made. Admittance measurements on <u>giant axons</u> and an investigation of effects of polarizations for comparison with step and ramp techniques and ion conduction models are carried out. This project is supportive of a number of other projects in terms of the development of relevant <u>hardware</u> and <u>software</u>.</p>																						

Project Description:Objectives.

- 1) To develop and test a simple method for continuously monitoring membrane slope conductance during a voltage clamp.
- 2) Excitation properties of axon membrane from a statistical evaluation of impulse trains. Transfer functions resulting from small amplitude sinusoidal perturbations of stimulus currents responsible for steady state impulse trains are compared with similar functions of excitation models and evaluated in terms of membrane excitation parameters.

Methods employed.

- 1) G_K kinetics were also obtained by superposing a small jump voltage, ΔE , on the larger voltage pulse. In this case, $g_K = \Delta I / \Delta E$, ΔI being the corresponding small step in current (a method suggested by Richard FitzHugh in 1958 and applied by Cole and Moore with equivocal success as their slower voltage clamp obscured ΔI with large capacity currents). It is now possible to continuously monitor the slope conductance during a clamp pulse by superposing a small amplitude continuous square wave of constant frequency on the voltage pulse.
- 2) Squid giant axons were space clamped to sinusoidal currents. Steady state impulse trains, modulated by small amplitude sinusoidal signals, are converted to impulse density functions over the period of the modulating sine wave. Amplitude and phase of impulse density (relative to the modulation current) are sampled over a range of modulation frequencies to construct gain and phase functions (Bode plots). Axon data is compared with similar data from a number of different membrane excitation theories.

Major Findings.

- 1) In TTX-treated squid axons, we compared results obtained with this method with results from both conventional currents and two-pulse instantaneous currents. As the square wave duration (100-250 μ sec) was significantly less than the time constants of g_K (1-10 msec) and significantly greater than the voltage clamp settling time (10 μ sec), and as the amplitude of the continuous wave was small (5 mV), one could superpose a curve of the mean current between the discrete jumps (correcting for I capacity) on the equivalent unperturbed I_K curve. Using the continuous square wave monitor, $g(5)$ for a series of E_K were obtained uncontaminated by K^+ accumulation. Tail current g_K kinetics were separated from K^+ wash-out kinetics. In addition, the times and voltages at which instantaneous I/E relations had zero slopes ($\Delta I=0$) and negative slopes (phase reversal in ΔI jumps) were readily determined with an economy of measurements as compared to the two-pulse method. A paper describing these results was presented at the 1979 Biophysical Society meeting in Atlanta, Georgia.

2) Analysis leading to gain and phase points shows how harmonic (and other) distortion (typically $< 15\%$) with modulation amplitudes 10-25% of steady state values confirms the feasibility of the method. Gain and phase characteristics are a strong function of temperature (consistent with a $Q_{10} \approx 3.0$), which confirms that kinetics parameters of excitable channels are measured. Dynamic comparison with the Hodgkin-Huxley model confirmed once again that the model conductance levels are too low by perhaps a factor of 2.5 to 3.0. More importantly, the dynamic (Bode plot) characteristics of the axon is qualitatively at odds with the model: the model shows a "corner frequency" (at $f_{mod} = 15.5$ Hz) which is typical of the absence of conductance perturbations in the interspike interval in response to the modulation perturbations of the stimulus. This corner frequency is completely obscured for the axon by dynamic phenomena related to a combination of conductance perturbations and "summing" conductance phenomena, which may in turn be related to the strong adaptation properties of the axon membrane. A modification of the Hodgkin-Huxley model which incorporates the effects of K^+ -accumulation in a periaxonal space shows a hybrid response which is similar to the unmodified model for small stimulus currents and similar to, but quantitatively different from, the axon for larger stimulus currents. The quantitative difference is in the relaxation times of recovery variables (K^+ -channel activation and Na^+ -inactivation) which appear to be approximately double the model values for the axon. A paper describing this work has been submitted to the Biophysical Journal and this work will be presented at the 1979 Society for Neurosciences meeting.

Publications:

Cole, K.S.: Mostly Membranes. Ann. Rev. Physiol. 41: 1-24, 1979.

Tyndale, C. and Crow, T.J.: An IC Control Unit for Generating Random and Nonrandom Events. IEEE Transactions on Biomedical Engineering. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02087-06 LB
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PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Function and Structure of Ionic Channels: Ion Interactions and Gating Mechanisms.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Adelman, Jr.	Chief	LB NINCDS
Other:	R. J. French	Visiting Associate	LB NINCDS
	J. J. Shoukimas	IPA Fellow	LB NINCDS

COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543

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 MANYEARS:

3.3

PROFESSIONAL:

3.3

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER
- (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Voltage clamp experiments are carried out to determine the function and structure of ionic channels in single giant nerve fibers. Information is obtained about the position and properties of membrane ionic channel sites that limit conductances and determine ionic selectivity by an analysis of the interaction between current-carrying and blocking ions. The ability of various rate theory models to describe the flow of ions through open potassium channels is tested. Kinetics of ionic blocking of channels are studied to gain information about energy barriers crossed by ions entering channels, and about the nature of ion binding sites within the channels. Analogies between the kinetics of ionic currents across the nerve membrane and those seen in excitable lipid bilayer membrane preparations treated with channel forming agents are investigated. The effect of calcium ions upon sodium channel inactivation is studied.

Project Description:Objectives.

- 1) To characterize ionic binding sites within the channels from studies of the interactions of small ions with the predominant charge carriers.
- 2) To probe the ionic channels and their immediate surroundings in the membrane by use of pharmacological agents of a large range of sizes and structures.
- 3) To understand the influence of free ions and fixed membrane charges upon the gating properties of the excitable conductances.
- 4) To understand the structural basis of conductance gating by studying properties of channels that have been selectively modified by enzymatic and other chemical means.

Methods Employed.

The excitable properties of squid giant axon are studied with the voltage clamp technique. In most experiments, the axons are also perfused internally, allowing manipulation of both internal and external axon ionic environments. Experiments are controlled and data collected by an on-line computer system.

Major Findings.

1) Either Li, Na or Cs, when present internally, causes a negative conductance in the positive quadrant of the instantaneous I-V curve for the K channel. The organic species tris (hydroxy-methyl) aminomethane, glucosamine and tetramethylammonium can now be added to this series. Each of these produces a qualitatively similar effect. Externally, only cesium has been shown to have a similar effect, causing a steep negative conductance in the negative quadrant of the I-V relation. In earlier studies, periaxonal potassium accumulation caused the external concentration to vary depending upon the blocking species present. Since external K concentration generally affects the block, this prevented a controlled comparison of the different blocking ions. A new experimental procedure has been developed to allow a comparative study of the action of these blocking ions without this complication.

To quantitatively compare the effects of the blocking ions, I-V or g(conductance)-V data, obtained with each blocker present, were fitted using a simple exponential function to describe the voltage dependence of the block. Several points of interest emerge from this analysis. For external Cs at concentrations > 50 mM, the voltage dependence of the block is too steep to be explained simply by the amount of electrical work done on a single Cs ion per channel moving to a site within the membrane field. In contrast, it is not possible to exclude a single binding site model for the block by the internally active ions on a similar basis. The voltage dependence of block by internal ions is generally less steep. For both internal and external blocking ions,

changes in the concentration of permeant (K) ions, in the solution on the opposite (trans) side of the membrane from the blocking ions, modify the block. Both percentage block and steepness of the voltage dependence decrease with an increase of K concentration on the trans side of the membrane. These observations add to the growing body of evidence for a multiple site model of the K channel.

Also of interest is the observation that the three inorganic ions showed slightly steeper voltage dependence, as a group, than the three organic ions. This is consistent with the intuitive expectation that ions of smaller unhydrated radius should penetrate further into the channel, and hence also further into the membrane field.

2) We have investigated the effect on the K channel conductance of five quaternary ammonium ions having the general formula $N(C_nH_{2n+1})_4^+$ where $n=1, 2, 3, 4$ or 5 . Each causes a voltage dependent inhibition of axonal K conductance that becomes more pronounced as transmembrane voltage increases. As noted in the previous section, the onset of block by tetramethylammonium ($n=1$) is effectively instantaneous. For compounds with $n=2$ to 5 , onset is slower and causes, at appropriate concentrations and voltages, an inactivation-like decrease in the current recorded under voltage clamp over a period of milliseconds. The data show clearly, contrary to an earlier postulate by Armstrong that it is not necessary for a quaternary ammonium ion to have the form $R-N(C_2H_5)_3^+$ to exhibit K channel blocking activity.

Analysis of the steady state block showed that the voltage dependence is similar to that for the small, instantaneously acting organic ions mentioned above, suggesting that both groups of ions act at or near the same location. The blocking potency increases as n increases from 1 to 5 , with the exception that tetraethylammonium (TEA, $n=2$) is more potent than tetrapropylammonium ($n=3$). It is possible that the close correspondence between the size of TEA⁺ and the size of a K⁺ ion with a single hydration shell confers on TEA⁺ a greater blocking potency than would be expected simply from its relation to others in the tetraalkylammonium series. As suggested by Armstrong, the size of TEA probably favors its binding to the inner mouth of the channel.

These results prompt one to ask what chemical structure of the channel and its environs could allow ions of such a wide range of sizes to act in so similar a manner to block the K current.

This data was presented at the 1979 meeting of the Biophysical Society. This material is at present being prepared for publication.

3) We have studied the effect of symmetric quaternary ammonium ions upon the sodium channel conductance. The ions studied so far are TMA⁺ (tetramethylammonium), TEA⁺ (tetraethylammonium), TPA⁺ (tetrapropylammonium) and TPENTA⁺ (tetrapentylammonium). Of these ions, TEA has been studied most extensively. In contrast to what has been seen in the potassium channel, TEA shows a much lower blocking affinity for the sodium channel ($K_D = 45$ mM) and shows only weak rectification. Outward current is blocked about 10% more effectively

than inward current. Observations of rectification were made with low external sodium for net outward current and with high external sodium for net inward current. TEA is not significantly permeant to the channel nor does it block sodium current when applied externally. These findings suggest that the block site may be located within the channel and that sodium ions passing inward are capable of clearing the block. A dependence of the block upon external sodium levels may be indicative of multi-ion behavior for the sodium channel.

The presence of TEA leads to a small but significant slowing of the rise of the sodium current during depolarizations. Repolarization leads to a removal of a large fraction of the block in a time fast compared to when current can be accurately measured. Subsequent decay of sodium currents under normal or block induced conditions are similar, suggesting that, unlike potassium channels, sodium channels may close normally in the presence of TEA.

These observations on TEA block of the sodium channel were made with fast sodium inactivation intact. This data differs in this respect from that of Rojas and Rudy who did not observe TEA block of sodium channels without prior removal of fast inactivation with pronase. In the course of preliminary studies we established that incomplete inactivation (sustained sodium currents at potentials where they are expected to inactivate fully) is a normal property of the squid axon. The presence of a sustained sodium current at large, positive potentials has enabled us to look at interactions between the blocking action of the quaternary series and the "gating" transition between the early, peak sodium conductance and the later, sustained conductance. Both peak and sustained current are blocked about equally well by TEA. In contrast, TMA, which also blocks the sodium channel but not as effectively as TEA, shows a marked change in blocking potency with the development of fast inactivation.

4) The red tide organism G. breve produces potent neurotoxic fractions which give rise to hyperexcitability in a number of excitable membranes. We have studied the effect of a fraction isolated from cell culture upon Aplysia soma conductances. Application of the toxin leads to spontaneous action potential trains and oscillatory membrane potential responses. From voltage clamp studies the toxic effect results from shifts of conductance versus voltage relations in the hyperpolarized direction. The sodium conductance versus voltage relations shows a greater shift than the potassium conductance relation. Modeling of these effects with the Hodgkin-Huxley relations shows that the voltage clamp derived parameters can account for the increased excitability in the presence of the toxin.

Publications:

Shoukimas, J.J., Siger, A., and Abbott, B.C.: The Action of G. Breve Neurotoxin on Membrane Conductance. In Taylor, D.L. and Seliger, H.H. (Eds.): Toxic Dinoflagellate Blooms. New York, Elsevier/North-Holland, 1979, pp. 425-430.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02092-06 LB																
PERIOD COVERED October 1, 1978 to September 30, 1979																		
TITLE OF PROJECT (80 characters or less) Subcellular and Extracellular Structure Associated with Nerve and Muscle.																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">W. J. Adelman, Jr.</td> <td style="width: 30%;">Chief</td> <td style="width: 20%;">LB NINCDS</td> </tr> <tr> <td>Other:</td> <td>A. Hodge</td> <td>Senior Scientist</td> <td>MBL</td> </tr> <tr> <td></td> <td>L. Shiman</td> <td>IPA Fellow</td> <td>LB NINCDS</td> </tr> <tr> <td></td> <td>P. Morrison</td> <td>Research Assistant</td> <td>MBL</td> </tr> </table>			PI:	W. J. Adelman, Jr.	Chief	LB NINCDS	Other:	A. Hodge	Senior Scientist	MBL		L. Shiman	IPA Fellow	LB NINCDS		P. Morrison	Research Assistant	MBL
PI:	W. J. Adelman, Jr.	Chief	LB NINCDS															
Other:	A. Hodge	Senior Scientist	MBL															
	L. Shiman	IPA Fellow	LB NINCDS															
	P. Morrison	Research Assistant	MBL															
COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA 02543																		
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 MANYEARS: 3.7	PROFESSIONAL: 3.7	OTHER: 0.2																
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to examine the subcellular and extracellular structure of nerve and muscle and relate such structure to function. <u>Electron microscopy</u> and structural modeling. The following structures are probed: 1) <u>Neuroplasmic lattice</u> , 2) <u>neurofilaments</u> , 3) <u>microtubules</u> .																		

Project Description:Objectives.

- 1) To examine the structure and possible function of the major filamentous components found in the cytoplasm of neuronal cells: microtubules, neurofilaments, actin filaments and transverse bridges, and to study their ordered association into a transverse bridge lattice system; to investigate the role of this lattice and its possible functional involvement in axoplasmic transport phenomena.
- 2) To study the protein composition of axonal and neuronal cytoplasm, and to identify specific proteins with each of the filamentous structures referred to above.
- 3) To study the fast component of axoplasmic transport in the squid giant axon.

Methods employed.

1) This study utilized thicker sections (0.1-0.3 μm) for stereoscopic study of neural networks in conventional TEM (Philips 300) where the upper limit of section thickness is determined primarily by loss of resolution resulting from inadequate imaging of inelastically scattered electrons and by problems associated with heavy metal staining. Improved fixation was observed for squid neural tissue (including giant axons) and for the CNS of *Hermisenda crassicornis* when the initial glutaraldehyde (2-4%) phosphate-buffered fixative (pH 7.2) contained EGTA (2-40 mM) and Mg^{++} (1-5 mM) with the total osmolarity adjusted with sucrose to not less than 1100 mosm. Initial fixation (30 min.-2 hours) was followed by cold OsO_4 treatment (30 min.-2 hrs.). Specimens were dehydrated and embedded using Epon 812. Specimens were stained en bloc with either aqueous or alcoholic solutions of uranyl acetate, sometimes followed by alcoholic PTA. Often these thicker sections required further staining, usually alcoholic uranyl acetate and aqueous lead citrate for adequate contrast.

Further improvement in fixation of the squid giant axon has resulted from applying the cannulation method of Adelman and Gilbert (1964) for internal irrigation of the axon with fixative. Acquisition of a Philips EM400 with STEM mode accessories has further broadened the scope of the structural studies and allowed direct comparison of STEM and TEM images of the same specimen area. The use of STEM, particularly with LaB_6 electron sources shows promise of moving towards our goal of better resolution in 3-D with less heavy metal staining.

2) Some progress has been made toward the goal of localizing protein components of the axoplasm by an extension of the giant axon cannulation technique in which the axoplasm is first irrigated with a preparation of the S_1 fraction of myosin prior to intracellular application of fixative.

Major Findings.

1) Electron microscopy of the giant axon and smaller fibers of the squid Loligo pealei and of the brain system of the nudibranch Hermisenda crassicornis utilizing improved fixation methods and stereoscopic examination of relatively thick sections (0.2 - 0.5 μm) has allowed demonstration of a highly ordered neuroplasmic lattice in axons and other neural cellular extensions. The lattice consists primarily of longitudinally oriented neurofilaments and microfilaments, presumably actin, together with microtubules when present, linked together by a well defined system of thin transverse filamentous bridge elements 2-3 nm in diameter with an apparent periodicity of ~ 40 nm along the axonal longitudinal axis. Internal irrigation of the squid giant axon with fixative following cannulation results in dramatically improved fixation with numerous microtubules being found in the axoplasm, particularly in the subaxolemmal cortical region. The lattice has extensive properties, with domains of order extending over several micrometers. In small axons of both Loligo and Hermisenda, the lattice domain often encompasses the whole fiber diameter. The transverse bridges appear to end at or are structurally continuous with membranous elements such as the axolemma, vesicles, endoplasmic reticulum and mitochondria. It is thought that some or all of the lattice components are involved in axonal transport processes. These findings are embodied in a paper which has been accepted for publication by the Journal of Ultrastructure Research.

The application of a recently acquired Philips EM400 electron microscope with STEM capability has confirmed the characteristics of the neuroplasmic lattice and allowed more precise evaluation of lattice parameters. In particular, the capability of this instrument for aberration of thick specimens (1 - 5 μm) in both TEM and STEM mode has allowed the beginning of an evaluation of the long range characteristics of the lattice in relation to spatial distribution within the neuron network and possible correlation with neuronal functions such as transport.

Use of the STEM mode has made possible the observation of structure in sections too lightly stained with heavy metals for the attainment of adequate contrast and resolution in the TEM mode. It is hoped that application of more intense electron sources (such as LaB_6) may further extend the usefulness of this method as part of an overall goal to reduce our dependence on heavy metal staining for adequate contrast and hence resolution of fine structure.

2) A start has been made towards the goal of identifying and localizing specific neuronal protein components. The internal irrigation fixation technique using cannulations has been modified to allow the introduction of specifically reacting substances such as antibodies into the axoplasm prior to irrigation with fixative. To date, this method has been used to introduce the S_1 fraction derived from rabbit myosin into the squid giant axon in an attempt to "decorate" the presumed actin filaments. Considerable uptake of protein and stabilization of the overall lattice were observed together with some indications of decoration, as yet insufficient to allow precise deter-

mination of the f-actin distribution in the axoplasm.

Publications:

Hodge, A.J. and Adelman, W.J., Jr.: The Neuroplasmic Lattice: Demonstration and Characterization in Loligo and Hemissenda Neurons. J. Ultrastructure Research. (accepted for publication; in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02252-03 LB																								
PERIOD COVERED October 1, 1978 to September 30, 1979																										
TITLE OF PROJECT (60 characters or less) Visual pigments: a comparative study of chemical properties.																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>F. I. Harosi</td> <td>Research Associate</td> <td>LB NINCDS</td> </tr> <tr> <td>Other:</td> <td>E. F. MacNichol</td> <td>Director</td> <td>LSP MBL</td> </tr> <tr> <td></td> <td>C. Sandorfy</td> <td>Professor, Chemistry</td> <td>U. Montreal</td> </tr> <tr> <td></td> <td>Y. W. Kunz</td> <td>Professor, Zoology</td> <td>U. College, Dublin</td> </tr> <tr> <td></td> <td>D. L. Alkon</td> <td>Medical Officer</td> <td>LB NINCDS</td> </tr> <tr> <td></td> <td>P. S. Chen, Jr.</td> <td>Asst. Dir. for Intramural Affairs</td> <td>OD, NIH</td> </tr> </table>			PI:	F. I. Harosi	Research Associate	LB NINCDS	Other:	E. F. MacNichol	Director	LSP MBL		C. Sandorfy	Professor, Chemistry	U. Montreal		Y. W. Kunz	Professor, Zoology	U. College, Dublin		D. L. Alkon	Medical Officer	LB NINCDS		P. S. Chen, Jr.	Asst. Dir. for Intramural Affairs	OD, NIH
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	D. L. Alkon	Medical Officer	LB NINCDS																							
	P. S. Chen, Jr.	Asst. Dir. for Intramural Affairs	OD, NIH																							
COOPERATING UNITS (if any) OD, NIH Marine Biological Laboratory, Woods Hole, MA 02543 University of Montreal; University College, Dublin																										
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 MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0																								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords) <p>The objective of this project has been a detailed understanding of the initial processes involved in vision. Topics of study were:</p> <ol style="list-style-type: none"> 1) <u>Spectroscopic characterization of vertebrate visual pigments</u> in isolated photoreceptors. 2) Theoretical studies aimed at the elucidation of the <u>spectroscopic changes</u> following light absorption, and of the <u>molecular changes</u> leading to cellular excitation. 3) <u>Spectroscopic study of invertebrate visual pigments</u>. 4) <u>Absorption spectroscopy</u> of biological pigments in isolated cells: <u>hemoglobins</u>, <u>hemocyanins</u>, and <u>cytochromes</u>. <p>This project is herewith terminated.</p>																										

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02273-03 LB								
PERIOD COVERED October 1, 1978 to September 30, 1979										
TITLE OF PROJECT (80 characters or less) An Investigation of Electro-mechanical Coupled Interaction in Excitable Tissues.										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" data-bbox="180 332 982 393"> <tr> <td>PI:</td> <td>J. B. Wells</td> <td>Research Physiologist</td> <td>LB NINCDS</td> </tr> <tr> <td>Other:</td> <td>D. E. Goldman</td> <td>Guest Worker</td> <td>LB NINCDS</td> </tr> </table>			PI:	J. B. Wells	Research Physiologist	LB NINCDS	Other:	D. E. Goldman	Guest Worker	LB NINCDS
PI:	J. B. Wells	Research Physiologist	LB NINCDS							
Other:	D. E. Goldman	Guest Worker	LB NINCDS							
COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA 02543										
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 MANYEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0.0								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) The major portion of the research effort was concerned with <u>mechanoelectric transduction mechanisms</u> in squid giant axons. An input-output relationship was observed and present studies will further define and quantitate this relationship.										

Project Description:Objectives.

The structural complexities of most mechanoreceptor organs impose considerable difficulty on their investigation. The ultimate representation of such transduction processes, however, can be limited to the relationships between mechanical input and electrical output of excitable membranes. The general objective, therefore, was to measure the interactions between the ionic conductance phenomena and certain mechanical perturbations of nerve membrane preparations. Specifically, we attempted to establish whether simple stretch applied to the excitable membrane would evoke electrical activity and what relationships, if any, exist between the input-output signals with particular reference to the time course of events.

Methods employed.

The isolated squid giant axon preparation was used because it represents the most accessible and studied nerve membrane known. A voice coil displacement transducer was used to apply constant velocity stretches to one end of the axon cylinder. The other end was tied to an in-dwelling glass capillary electrode which was itself carried by a piezoelectric force transducer. This initial configuration which permitted simultaneous recording of electrical and mechanical events in the membrane was adapted in order to optimize the area of membrane subjected to strain.

Major findings.

1) Mechanical studies. Longitudinal stress-strain data displayed non-linear compliance to rapid stretches with marked stress relaxation observed during the course of stretch. An irreversible increase in compliance was observed when stretch amplitude exceeded 6-10% resting axon length.

2) Electrical studies. Axon stretch invariably produced membrane depolarization. Typically, 3-4 millivolts change was evoked by a stretch of 4-5% L_0 . The time course of the depolarization induced by stretch resembled the time course of stress (tension) in the axon. Stretch amplitudes between 6-10% L_0 usually resulted in regenerative discharge of the membrane voltage which resembled closely an electrically evoked action potential. The membrane potential, following large stretches or an action potential discharge, entered a phase of prolonged depolarization which could last up to several minutes.

Proposed Course of Project.

These findings indicate that isolated squid giant axon is an excellent model in which to study mechanoelectric transduction processes. Currently, an impedance bridge is being adapted to investigate qualitatively the capacitative and resistive changes produced in the membrane during stretch. Also, a voltage clamp apparatus, now available, will provide quantitative informa-

tion on specific ionic conductances during mechanical perturbations. Work in the immediate future concerning the relationship of mechanical input to electrical output of the nerve membrane will investigate the effects of strain amplitude and strain rate on electrical responses.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02329-02 LB								
PERIOD COVERED October 1, 1978 to September 30, 1979										
TITLE OF PROJECT (80 characters or less) Mechanical properties of resting and stimulated skeletal and/or locomotor muscles.										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">J. B. Wells</td> <td style="width: 35%;">Research Physiologist</td> <td style="width: 15%;">LB NINCDS</td> </tr> <tr> <td>Other:</td> <td>M. Schoenberg</td> <td>Research Physiologist</td> <td>LPB NIAMDD</td> </tr> </table>			PI:	J. B. Wells	Research Physiologist	LB NINCDS	Other:	M. Schoenberg	Research Physiologist	LPB NIAMDD
PI:	J. B. Wells	Research Physiologist	LB NINCDS							
Other:	M. Schoenberg	Research Physiologist	LPB NIAMDD							
COOPERATING UNITS (if any) Laboratory of Physical Biology, NIAMDD, Bethesda, MD 20205 Marine Biological Laboratory, Woods Hole, MA 02543										
LAB/BRANCH Laboratory of Biophysics, IRP										
SECTION Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)										
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20205										
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) The effort concerned with excitation-contraction processes in voluntary muscle investigated a possible viscous effect on the instantaneous elastic measurements used to assess crossbridge activity.										

Project Description:Objectives.

The general objective of this project is to elucidate the chemomechanical transduction processes whereby external mechanical work or tension is produced by activated muscle. The sliding filament hypothesis, currently the most widely accepted scheme of contraction is based on the observation that contractile tension output varies proportionally to the inverse of resting muscle length. It is supposed that the number of active force generators (attached crossbridges) depends on the length of interdigitation between the two populations of myofilaments. This scheme implies that the instantaneous stiffness of active muscle is proportional to the number of attached crossbridges and indeed evidence from other workers supports this suggestion. Therefore, one specific objective was to related instantaneous stiffness during both activation and contractile processes to force generation by the muscle to test the above hypotheses. Also, a problem which is relative to all elastic measurements in biological tissues is the influence of viscosity in the estimate of elastic stiffness. Thus, some experiments were conducted to specifically address this problem.

Methods employed.

Stiffness was estimated by the "transmission time" method, developed in our laboratories, which relates the longitudinal transmission velocity of mechanical impulses to the instantaneous elastic state of the muscle. Sarcomere shortening related to the presence of external (non-crossbridge) series compliance was measured using light diffraction techniques.

Major findings.

x The following major findings lead to the conclusion that the instantaneous stiffness value is a measure of attached crossbridges in the active skeletal muscle. The onset of activity following stimulation is first signaled by an increased stiffness prior to tension production. The development of instantaneous stiffness during isometric contraction precedes that of force. Instantaneous stiffness in active muscle decreases proportionally to the decrease in myofilament overlap as the muscle is lengthened.

A recent report which described a viscoelastic response in rapidly stretched resting muscle questioned the influence of viscosity on instantaneous stiffness measurements made in the active muscle. Our preliminary observations made during the transition of the muscle from the resting to the active state showed this response to be related to crossbridge activity rather than a passive viscous response. At present, no large viscous effects have been implicated in the dynamic elastic measurements of active muscle.

Proposed Course of Project:

Marine invertebrate muscle offers several advantages over vertebrate skeletal muscle in the study of excitation-contraction coupled processes. In some marine species, certain muscle fibers contain sarcomeres four to five times longer than vertebrate muscle sarcomeres. Contractile mechanisms from such muscle are slower and can be studied with more precision and less complex apparatus. It is proposed to continue these studies in marine muscle.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02151- 05 LB	
PERIOD COVERED October 1, 1978 to September 30, 1979			
TITLE OF PROJECT (80 characters or less) Information Processing in Simple Nervous Systems			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
P.I.:	D. L. Alkon	Medical Officer	LB NINCDS
Other:	T. Crow	Staff Fellow	LB NINCDS
	I. Lederhendler	Visiting Fellow	LB NINCDS
	T. Jerussi	IPA Fellow	LB NINCDS
	M. Tabata	Visiting Fellow	LB NINCDS
	J. Harrigan	Mariculturist	MBL
	J. Neary	Biochemist	MBL
	S. Leighton	Guest Worker	LB NINCDS
	D. Meyer	Guest Worker	LB NINCDS
	A. Kuzirian	Extramural Fellow	LB NINCDS
	S. Senft	Graduate Student	U. of Oregon
	M. Manning	Undergraduate	U. of Miss.
	R. Stevens	Guest Worker	LB NINCDS
COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA 02543 University of Oregon; University of Mississippi			
LAB/BRANCH Laboratory of Biophysics, IRP			
SECTION Section on Neural Systems (located at MBL, Woods Hole, MA 02543)			
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20205			
TOTAL MANYEARS: 9.0	PROFESSIONAL: 8.5	OTHER: 0.5	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) The principal objective is to study the mechanisms by which simple <u>neural networks</u> process information with particular emphasis on mechanisms of <u>learning</u> . The nervous system of <u>Hermissenda crassicornis</u> has proven to be an excellent model for <u>information processing</u> at several levels: <u>sensory transduction</u> by photoreceptors and hair cells, analysis of <u>synaptic circuitry</u> , changes in <u>synaptic circuitry</u> produced by conditioning paradigms administered to intact animals, as well as to isolated nervous systems, membrane properties modified by <u>conditioning</u> , identification of critical developmental stages for the neural networks studies, as well as stages critical for learning. Techniques employed thus far to pursue these questions include: <u>simultaneous intracellular recording</u> from multiple neural elements, paired stimulation of the visual and vestibular pathways using a rotating table, iontophoresis of fluorescent dyes and electron dense materials, automated behavioral monitoring of intact <u>Hermissenda</u> . Other methods include <u>mariculture</u> , <u>subcellular fractionation</u> <u>protein phosphorylation analysis</u> , and <u>uptake of neurotransmitter precursors</u> .			

Other Professional Personnel Engaged (continued)

R. Allen	Guest Worker	LB	NINCDS
I. Levitan	Guest Worker	LB	NINCDS
W. Rall	Guest Worker	LB	NINCDS
J. Rinzel	Guest Worker	LB	NINCDS

Project Description:Objectives.

To study the mechanisms by which simple neural networks process information with particular emphasis on mechanisms of learning. Information processing at several levels is of interest:

- a. Sensory transduction by photoreceptors and hair cells.
- b. Synaptic interactions between primary sensory receptors.
- c. Synaptic interactions between primary and higher order neural elements.
- d. Intersensory communication: e.g. synaptic interaction between the visual and gravitational sensory pathways.
- e. Changes of synaptic interaction produced by conditioning paradigms administered to the intact animals as well as to the isolated nervous systems.
- f. Membrane and synaptic properties modified by conditioning.
- g. Identification of critical developmental stages of the neural networks studied as well as stages critical for learning.

Methods employed.

1) The nudibranch mollusc Hermissenda crassicornis is the principle experimental preparation. Other marine species are also being screened to provide favorable preparations for specific experimental questions. Intracellular recording from several neural cells simultaneously has been the main technique used thus far. Means for simultaneously stimulating the chemosensory, visual and vestibular pathways while recording intracellular potentials have been developed in our laboratory. Iontophoresis of fluorescent dyes (e.g. Procion Yellow) and electron dense materials (e.g. cobalt) are also being used extensively.

2) Other methods allow biochemical electronmicroscopic and developmental approaches to the problems of interest. These include mariculture, subcellular fractionation, protein phosphorylation analysis, uptake of neurotransmitter precursors, etc. Automated behavioral monitoring now permits long-term studies of intact Hermissenda.

Major Findings.

Past work has focused on six major areas:

- a. Behavioral conditioning with neural correlates.

- b. Cellular conditioning in isolated nervous systems.
- c. Neural network analysis.
- d. Receptor physiology.
- e. Voltage clamp (and biochemistry) of Hermissenda neurons.
- f. Neural development.

Behavioral and cellular conditioning. For the last few years the major focus of the section has been an integrated multidisciplinary effort to determine a neural (and possibly a biochemical) basis for an associative learning model with the nudibranch mollusc Hermissenda crassicornis. A number of invertebrate species were considered as potential model systems to analyze cellular mechanisms of behavior and learning. These included Tritonia, Aplysia, Pleurobranchia, Helix, Elysia, and Hammonia. The last two have been cultivated within the laboratory and subjected to preliminary electrophysiological and histologic investigation. The nudibranch mollusc Hermissenda crassicornis, however, has proven to be a most opportune preparation in satisfying the host of constraints which arose from the questions which were asked. With Hermissenda, it has been possible to define a model of associative learning with the same defining features used for vertebrate associative learning. Movement of Hermissenda toward a light source is markedly reduced after repeated pairing of a light stimulus with rotation. This behavioral change is truly associative (i.e. random light and rotation do not produce the effect), persists for at least several days after training and increases with practice. Stimulus specificity for this behavioral change was indicated by the fact that trained animals did not show changes in responsiveness to food. Because of the relative simplicity of the nervous system it has been possible to ascertain many of the invariant aspects of the three sensory pathways essential to the associative learning model: the visual, statocyst, and chemosensory pathways.

Changes have been found (within these neural systems of Hermissenda) which occurred only in animals subjected to associative learning paradigms and not to control paradigms. For example, with the first associative training procedure used, it was found that hair cells received less excitatory input from ipsilateral Type A photoreceptors after repeated stimulus pairing but not after control training paradigms. Comparable neural modification could be produced while recording intracellularly. Thus, it was possible to monitor the neural changes as they were progressively produced by the associative training procedure.

Behavioral analyses of the main experimental animal Hermissenda crassicornis have ranged from field observations to comparative studies of laboratory-reared and collected species. Findings of the past year, for instance, showed that light response involves a preference for certain levels of intensity, and a biphasic approach/withdrawal process which depends on an individual animal's light history. This behavior is consistent with the predictions from a recent model of phototaxis which assumes that species have preferences for optimum levels of ambient illumination. Field observations, on the other hand, indicate that natural Hermissenda populations undergo diurnal vertical migrations which are determined not only by ambient light and temperature

conditions, but also by food availability.

c. Neural network analysis. An understanding of the basis for the cellular and behavioral conditioning previously identified for intact animals, as well as their isolated nervous systems, has been extended within the past year by an extensive study of neural organization for Hermissenda. This study has focused on two main areas: motor systems and efferent modulation of sensory pathways. Using intracellular recording and marking techniques, large central neurons have been identified as probable motor neurons within the visual pathway. We have obtained evidence that specific optic ganglion cells also serve motor neuron functions. Synaptic feedback from these central neurons onto the sensory pathways has now been defined. The importance of this feedback for the animals' behavior, particularly associative learning, has also been investigated.

Renewed attention has been given to photoreceptor optic ganglion cell interactions. Of particular interest is a positive synaptic feedback onto the Type B photoreceptors. This positive feedback increased following rotation (via a known synaptic pathway) and thus depolarized the Type B cell. This depolarization, when rotation was paired with light, caused the LLD to increase by the voltage-dependent mechanism mentioned above.

Pre-synaptic regulation of these EPSP's was therefore thoroughly investigated. Excitatory post-synaptic potentials (EPSP's) have previously been recorded from Type B but not Type A photoreceptors in the eye of Hermissenda. Removal of most of the circumesophageal nervous system by a razor cut left the eye (5 photoreceptors), ipsilateral optic ganglion (14 neurons) and EPSP's essentially unchanged. EPSP frequency decreased with injection of negative current into an ipsilateral optic ganglion cell ("S" cell) which was silent in darkness and hyperpolarized in response to light. Such injection did not affect impulse activity of the "S" cell while it significantly reduced EPSP frequency. Type B impulses eliminated EPSP's in other ipsilateral Type B cells, as well as hyperpolarized the "S" cell. These results indicate that the pre-synaptic source of the EPSP's is an ipsilateral optic ganglion cell which is electrically coupled to the "S" cell.

Techniques for iontophoretic injection of the electron dense substances, cobalt and horseradish peroxidase, have been successfully adapted for identifying synaptic junctions in Hermissenda. Histologic processing, involving both histochemical and cryostatic methods, has also been developed. Neurons within the Hermissenda nervous system have been shown to have adjoining terminal axonal membranes in close apposition when these neurons have, by electrophysiological criteria, been shown to make monosynaptic connections. This demonstration of synaptic interaction with the electronmicroscope utilized iontophoretic injection of horseradish peroxidase into pre- and post-synaptic neurons. This use of electronmicroscopic combined with electrophysiologic data provides for the first time criteria for the identification of synapses in molluscan nervous systems.

Within the past year, fixation and staining of Hermissenda nervous tissue

for the EM has been markedly improved in our laboratory. This has already made possible superb resolution of some of the neural structures which we have studied with electrophysiologic and biochemical techniques. Precise orientation of these tissues for fixation, and embedding the most likely, has begun to make it possible to visualize their complete structure.

In a recent EM study concerning adult statocyst hair cells stained with uranyl acetate and lead citrate or vanadomolybdate, the presence of microfilaments as lateral projections from the basal bodies was revealed. These microfilaments extend tangentially below the cell surface in the form of an astral array. Their length and directionality suggest some degree of morphological polarity and the possibility of an infraciliary network. The EM results also indicate that the smaller, more numerous statocyst support cells are responsible for forming the statoconia concretions within the statocyst, while the hair cells may play a passive role in their formation by acting as a reservoir for stored carbonates.

d. Receptor physiology. Power spectra of the voltage and current noise in hair cells have been analyzed for a variety of stimulus and treatment conditions. These analyses indicate that the resting conductances of the hair cell membrane are modulated by the rhythmic beating of the cilia. More recently, power spectra of the current noise in hair cells (obtained in voltage-clamped hair cells) have provided more accurate values of hair cell conductances at rest and during stimulation. Under voltage-clamp, the filtering properties of the hair cell membrane no longer distorted the conductance measurements.

e. Voltage clamp (and biochemistry) of *Hermisenda* neurons. Recent experimental results indicated that a primary neural change occurred (with the learning model) within the Type B cells of the *Hermisenda* eye. Current and voltage clamp recordings from this cell revealed the presence of a prolonged voltage-dependent Ca^{++} current during and after light steps. The voltage-dependence of this Ca^{++} current and a Ca^{++} -dependent K^{+} current provide a neural basis for the contingency necessary to the associative learning model. The finding that this Ca^{++} current can be regulated by intracellular injection of cyclic-AMP (and not 5'-AMP) suggested biochemical studies (e.g., of protein phosphorylation and protein synthesis) which have been initiated.

Recent biochemical studies have focused on single cell protein synthesis and phosphorylation. Those cells believed to play a primary role in effecting the associative learning have been found to undergo specific changes of protein phosphorylation related to the previous training experience of the animal. It has also been possible to determine by microspectrophotometric techniques the spectra of visual pigments in individual Type B (see above) photoreceptors isolated from the nervous system.

f. Neural development. Currently, the third laboratory generation of *Hermisenda* is in the juvenile stage. A model for the study of environmental genotypic interaction may be provided by the statocyst in laboratory-reared *Hermisenda*. In all F_1 and F_2 generation individuals the statocysts contained

a single statoconium. In wild animals, on the other hand, the statocysts typically contain 150-200 statoconia (concretions suspended within the intracyst fluid). Only occasionally were single statoconia encountered in wild Hermisenda. Conventional and electronmicroscopic sections have been prepared of pre-metamorphosis forms. Identification of the neural elements within the sensory pathways investigated in the adults has been achieved. The ontogeny of the Hermisenda nervous system, therefore, can now be followed with particular attention being given to the temporal and spatial relationships during development of the ganglia, eyes, statocysts and tentacles.

Proposed Course of Project:

1) Precise analysis of synaptic interactions between cells within the aforementioned neural networks will be continued with the techniques of intracellular recording and iontophoresis. Particular emphasis will be placed on electronmicroscopic visualization and reconstruction of cell contacts aided by distribution of hydrogen peroxidase within axons and terminal branches. These studies will not be limited, however, to the networks already discussed. The motor units within the sensory pathways (visual, statocyst, and chemosensory) will be identified. In addition, other more evolved animal forms with potentially analyzable neural networks and behavior will be explored.

2) Anatomic, as well as electrophysiologic, correlates of behavioral and developmental changes will be sought. Using voltage-clamp techniques, cellular mechanisms responsible for the learning model will be further analyzed. Regulation of potential-dependent currents believed to, at least in part, underly the observed behavioral changes will be given particular attention.

3) Biochemical and pharmacologic analyses of relevant neural systems will continue. Studies are also planned to identify subcellular and/or biochemical loci at which primary behaviorally-meaningful changes occur. The Type B photoreceptor will be the initial focus of this work.

In order to explore the phosphorylation events mentioned above in Hermisenda, we plan to study the level of phosphorylation of membrane proteins before and after training (paired stimulation) by using 32 -P-inorganic phosphate (32 Pi) as a marker, followed by SDS-slab gel electrophoresis and autoradiography to separate and visualize the radio-labeled phosphoproteins. We plan to identify the mechanisms leading to changes in protein phosphorylation, i.e. cyclase, phosphodiesterase, protein kinase or phosphatase activities. It may also be possible to detect changes in levels of cyclic nucleotides by immunocytochemistry. By using 32 Pi as a label, we will be able to detect protein phosphorylation by either cyclic nucleotide-dependent or independent mechanisms. Subsequent studies could then discriminate between the two processes. Initial experiments have utilized isolated nervous systems in order to establish the biochemical detection procedures in our laboratory, but more recent studies involve analysis of phosphorylation in individual Hermisenda neurons.

Other mechanisms of post-translational modification will also be

explored. Use of the high resolution, two-dimensional method of O'Farrell is planned to determine if specific proteins are methylated or demethylated during associative training.

We also plan to study the synthesis and modification of gene products in the Hermissenda nervous system by means of high resolution, two-dimensional electrophoresis. O'Farrell has shown that the 50 or so protein bands visible on conventional one-dimensional gels can be resolved into 1100 proteins by combining isoelectric focusing in the first dimension with SDS-slab gel electrophoresis in the second dimension. A modification in the basic O'Farrell technique provides for the separation of nuclear proteins.

4) Behavioral experiments will be continued to further determine the comparability of the Hermissenda associative learning model to associative learning defined for more evolved species.

We will continue ongoing time-lapse studies of behavior so that motor activity patterns and response to food, cover and conspecifics of wild, laboratory-reared, and experimentally manipulated animals can be described under a variety of illumination conditions. To aid in the analysis of the data generated by this approach, a digitizer interfaced with a computer will be used to record the data directly on tape and then analyze it. The relationship of laboratory to natural habitat behaviors will also be pursued by making additional observations of Hermissenda in the wild.

5) Hermissenda will be raised through successive generations. It is planned to combine all of the above approaches with mariculture. The existence of clearly identified neural networks in Hermissenda, together with mariculture, offer the possibility of using genetic mapping to study principles of neural organization, development, and learning.

6) The generalizability of cellular principles of learning and development determined for relatively "simple" neural systems and the behavior they control will be examined. Ultimately, mechanisms common to organisms with a wide range of evolutionary diversity and complexity may contribute to understanding the human nervous system and may motivate clinical approaches.

Publications:

Alkon, D.L. and Grossman, Y.: Long-lasting depolarization and hyperpolarization in the eye of Hermissenda. J. Neurophysiol. 41: 1328-1342, 1978.

Crow, T.J. and Alkon, D.L.: Retention of an associative behavioral change in Hemissenda. Science. 201: 1239-41, 1978.

Heldman, E., Grossman, Y., Jerussi, T., and Alkon, D.L.: Cholinergic features of photoreceptor synapses in Hermissenda. J. Neurophysiol. 42: 153-165, 1979.

Grossman, Y., Alkon, D.L., and Heldman, E.: A common origin of voltage noise and hair cell generator potentials. J. Gen. Physiol. 73: 23-48, 1979.

Crow, T.J., Heldman, E., Hacopian, V., Enos, R., and Alkon, D.L.: Ultrastructure of photoreceptors in the eye of Hermissenda labeled with intracellular injections of horseradish peroxidase. J. Neurocytol. 8: 181-195, 1979.

Alkon, D.L.: A neural basis for associative learning. Sci. Am. (In press).

Alkon, D.L.: Voltage-dependent Ca^{++} and K^+ conductances: a neural basis for contingency in an associative learning model. Science. (In press).

Crow, T.J. and Harrigan, J.: Comparison of laboratory-reared and wild molluscan responses to associative training. Brain Research. (In press).

Tyndale, C. and Crow, T.J.: An IC control unit for generating random and nonrandom events. IEEE Transactions on Biomedical Engineering. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02088-06 LB																				
PERIOD COVERED October 1, 1978 to September 30, 1979																						
TITLE OF PROJECT (80 characters or less) Function and Structure of Membrane Ionic Channels: Pharmacology and Ionic Selectivity																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">L. Binstock</td> <td style="width: 40%;">Electronic Engineer</td> <td style="width: 10%;">LB NINCDS</td> </tr> <tr> <td rowspan="5">Other:</td> <td>R.E. Taylor</td> <td>Research Physiologist</td> <td>LB NINCDS</td> </tr> <tr> <td>F. Bezanilla</td> <td>Professor</td> <td>UCLA</td> </tr> <tr> <td>L.M. Huang</td> <td>Staff Fellow</td> <td>LN NINCDS</td> </tr> <tr> <td>B.S. Wong</td> <td>Visiting Fellow</td> <td>LB NINCDS</td> </tr> <tr> <td>H. Lecar</td> <td>Research Physicist</td> <td>LB NINCDS</td> </tr> </table>			PI:	L. Binstock	Electronic Engineer	LB NINCDS	Other:	R.E. Taylor	Research Physiologist	LB NINCDS	F. Bezanilla	Professor	UCLA	L.M. Huang	Staff Fellow	LN NINCDS	B.S. Wong	Visiting Fellow	LB NINCDS	H. Lecar	Research Physicist	LB NINCDS
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COOPERATING UNITS (if any) Laboratory of Neurophysiology, IRP Marine Biological Laboratory, Woods Hole, Massachusetts UCLA School of Medicine, California																						
LAB/BRANCH Laboratory of Biophysics, IRP																						
SECTION Section on Molecular Biophysics																						
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205																						
TOTAL MANYEARS: 3.2	PROFESSIONAL: 2.5	OTHER: 0.7																				
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) <p style="text-align: center;">The long-range purpose of this project is to study the function and structure of <u>membrane ionic channels</u>. The areas of present research are:</p> <ol style="list-style-type: none"> 1) The effects of neurotoxins ("channel openers") on the <u>axonal membrane in Myxicola</u>; 2) Conductance dynamics of potassium channels (<u>Cole-Moore delay</u>) in <u>Myxicola axons</u>; 3) The measurement of gating currents of the <u>sodium channel</u> in the <u>squid giant axon</u>. 																						

Project Description

Objectives: To study the ionic current flow across the nerve membrane without the complication of excitation and propagation in terms of individual ion currents and to observe the effect of changes in normal external and internal environments. These environments are altered by addition of various chemical and pharmacological agents as well as by changing the ionic environment. The long range objectives are the interpretations of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: Standard voltage clamp techniques are employed on preparations a and b: (a) Myxicola giant axon. For many experiments on the giant axon of Myxicola, the voltage clamp is under computer control. Internal perfusion is also used. Noise measurements are made for some experiments. (b) Squid giant axon. The squid giant axon is internally perfused and signal averaging techniques are used.

Major Findings:

(a) Myxicola giant axon: Veratridine (VTD) opens sodium channels, modifies them to stay open producing slow steady state sodium current. When a channel is open VTD may possibly keep the channel open. With the application of VTD, two populations of Na⁺ channels are present, normal and so-called modified. These results were obtained by means of computer analysis as well as observation of tail currents. These experiments were made with external application of VTD. Internal application of VTD by means of internal perfusion are underway. This, with the removal of K by means of Tetraethylammonium (TEA), should determine the time course of Na both normal and steady state. Noise measurements will determine the size and number of modified channels. This method is more direct and should determine more conclusively the presence of the modified Na channel.

Various scorpion venoms, both "New World and "Old World", have been experimented with. Initial experiments showed that Leiurus (Old World) heat treated venom produced a long delay in the action potential of the frog sciatic nerve. In Myxicola axon the action potential widens and under clamp the changes in current are too small to determine any real changes. Centruroides (New World) produced no changes in Myxicola. Consequently, it was decided to purify the toxins from the crude venom and to identify the toxins electrophoretically. Some venoms have many active toxins. Old World toxins act on the slower closing or turnoff of the channel - inactivation. New World toxins are involved in the rapid opening of the channel - activation.

Some studies have been made varying the calcium concentrations in the Myxicola axon to determine the shifts in inactivation and how Ca might interfere with the scorpion toxin. This information may also be of value in the further study of VTD and other toxins.

It would be desirable to study the following:

- 1) The remaining neurotoxins, i.e. scorpion toxins (both "New World" and "Old World"), batrachotoxin and aconitine in greater detail.
- 2) To study pairs of neurotoxins to see if there are any increases in the effect of the combined toxins versus the effect of a single toxin. To see if there is competition or inhibition.
- 3) To study gating currents with the applied toxins versus control.
- 4) To study the effect of these neurotoxins on the selectivity of organic cations and other ions.

(b) Squid giant axon: A large fraction of the asymmetrical displacement current has been identified with the molecular rearrangements leading to the opening and closing of the sodium channels and are called "gating currents". A paper on the effects of temperature on these gating currents has appeared. The effects of slow inactivation were presented to the Biophysical Society in February, 1979. The principle long range goal of this work is the correlation between the gating currents and the sodium conductance changes with voltage. A system for collection and storage of live squid has been developed at UCLA and preliminary studies of the effects of zinc ions applied internally to the giant axon have been done.

Publications:

Bezanilla, F. and Taylor, R.E.: Temperature effects on gating currents in the squid giant axon. Biophys. J. 23: 478-484, 1978.

Taylor, R.E. and Bezanilla, F.: Comments on the measurement of gating currents in the frequency domain. Biophys. J. 26: 338-340, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02091-06 LB
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Mathematical Modeling		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R. FitzHugh Research Physicist LB NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Biophysics		
SECTION Section on Molecular Biophysics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS 1.2	PROFESSIONAL: 1.0	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Mathematical models for the following phenomena were studied: 1) The energy profile for an ion passing through a cylindrical <u>channel</u> in a <u>dielectric membrane</u> . 2) The second-order (non-linear) components of <u>membrane admittance</u> as measured by adding a sinusoidal signal to a step <u>voltage clamp</u> .		

Project Description

D. G. Levitt's model for an ion in a cylindrical pore in a dielectric membrane assumes that the water both inside and outside the pore is a non-conducting dielectric. A modified model which assumes that the water outside the pore is a perfect conductor is much simpler mathematically, giving an explicit solution which can be computed simply and accurately. The results from the newer model agree approximately with those from Levitt's. The actual physical situation probably lies between the two models. Further studies on both models, extended to charged pores, are expected to further the understanding of experimental results on acetylcholine channels.

Experiments by Dr. Adelman and others, in which a sinusoidal component is added to a step voltage clamp, in some cases produce a current component with double the frequency of the original sinusoid. Formulas for the second-order (nonlinear) membrane admittance have been derived for the Hodgkin-Huxley equations, and computations of the amplitude and phase of the currents await comparison with experiments.

Publications

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02218-04 LB																	
PERIOD COVERED October 1, 1978 to September 30, 1979																			
TITLE OF PROJECT (80 characters or less) Voltage-Dependent Ionic Conductance in Membranes																			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">D.L. Gilbert</td> <td style="width: 40%;">Research Physiologist</td> <td style="width: 10%;">LB NINCDS</td> </tr> <tr> <td rowspan="4">Other:</td> <td>G. Ehrenstein</td> <td>Research Physicist</td> <td>LB NINCDS</td> </tr> <tr> <td>L.M. Huang</td> <td>Staff Fellow</td> <td>LN NINCDS</td> </tr> <tr> <td>H. Lecar</td> <td>Research Physicist</td> <td>LB NINCDS</td> </tr> <tr> <td>C. Morris</td> <td>Visiting Fellow</td> <td>LB NINCDS</td> </tr> </table>			PI:	D.L. Gilbert	Research Physiologist	LB NINCDS	Other:	G. Ehrenstein	Research Physicist	LB NINCDS	L.M. Huang	Staff Fellow	LN NINCDS	H. Lecar	Research Physicist	LB NINCDS	C. Morris	Visiting Fellow	LB NINCDS
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SUMMARY OF WORK (200 words or less - underline keywords) <p>One goal of this project is to better understand the mechanisms of the <u>ionic conductances in membranes</u> which are <u>voltage dependent</u> and excitable. Another goal is to determine how <u>drugs</u> influence these channels.</p> <p>These studies involve the use of the <u>squid giant axon</u> and the <u>giant barnacle muscle fiber</u>. Previously, we presented a drug modified-channel-kinetics model. The essential idea of this model is that drugs can modify the rates of opening and closing ionic channels. Using the local anesthetic, QX-314, we have been studying the characterization of this particular drug-bound channel. We have found that this drug-bound channel exhibits slower kinetics and operates a greater depolarizing potential than does the unbound channel.</p>																			

Project Description

Objectives: The primary means of determining mechanisms by which ionic channels respond to membrane potential are to change the response of the channels by altering pressure or by applying appropriate drugs. Particular interest has been given to drugs which exhibit use-dependence. In the presence of use-dependent drugs, currents obtained by applying voltage pulses exhibit a dependence on the previous history of a repetitive series of voltage pulsing.

Methods Employed: The measurement of ionic conductances through the channels is effected by means of voltage and/or current clamping squid axons or barnacle muscle fibers.

Major Findings: Our drug model can produce use-dependent effects. Such effects are not unusual and drugs of a diverse chemical and pharmacological nature possess this property. We found that yohimbine is a competitive inhibitor of batrachotoxin (BTX) in neuroblastoma cells. Since BTX is known to be a sodium-channel-opener, and thus cannot occlude the sodium channel, it is likely that yohimbine also does not occlude the sodium channel. This leads to a model wherein yohimbine reduces sodium current by modifying the rates for opening and closing of the sodium channel. This model was found to be consistent with voltage clamp experiments on squid giant axons treated with yohimbine.

We have also shown that phenytoin, an anti-arrhythmic drug, and SKF 4547-G, an anti-myotonic drug, exhibit use-dependence. We found that a very large use-dependent effect was obtained with the anti-arrhythmic and anti-anginal drug, perhexiline.

As a concomitant of our voltage-clamp study of the barnacle giant muscle fiber, we did an experimental and theoretical analysis of the voltage oscillations observed under current-clamp stimulation. The barnacle fiber shows complex and varied oscillatory behavior which has at times been attributed to various causes such as spatial nonuniformities and Ca-induced K-conductance. We were able to show that these oscillations are a natural mode of behavior for a system having two non-inactivating voltage-dependent conductances. In the experimental studies the oscillatory behavior was characterized with varying applied current and external Ca concentration. It was then shown by phase plane analysis that a system of nonlinear differential equations based on voltage clamp data could account for most of the observed features of the oscillations.

The major voltage clamp findings to date are the instantaneous current voltage relations of the Ca-channels in barnacle fiber studied in the presence of high external Ba and Ca solutions. The data shows the rectifying behavior expected for a ligand-binding narrow pore rather than the linearity estimated by earlier workers. Detailed quantitative study of the barnacle preparation has always been hindered by the heavy tubulation of the fiber.

Experiments with glycerol and various enzymes and with an isolated membrane patch have not yet improved the situation.

Publications:

Lipicky, R.J., Gilbert, D.L. and Ehrenstein, G.: Effects of yohimbine on squid axons. Biophys. J. 24: 405-422, 1978.

Huang, L.M., Ehrenstein, G. and Catterall, W.A.: Interaction between batrachotoxin and yohimbine. Biophys. J. 23: 219-231, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02219-04 LB																
PERIOD COVERED <p style="text-align: center;">October 1, 1978 to September 30, 1979</p>																		
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Structure and function of the perineurium</p>																		
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	A. Weerasuriya	Visiting Fellow	LB NINCDS															
	N. Shinowara	Staff Fellow	GRC, NIA															
COOPERATING UNITS (if any) <p style="text-align: center;">Gerontology Research Center, NIA, Baltimore, Md. 21224</p>																		
LAB/BRANCH Laboratory of Biophysics																		
SECTION Section on Molecular Biophysics																		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205																		
TOTAL MANYEARS: <p style="text-align: center;">1.9</p>	PROFESSIONAL: <p style="text-align: center;">1.6</p>	OTHER: <p style="text-align: center;">0.3</p>																
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) <p>The purpose of this project is to determine how and to what extent the <u>perineurium</u> is involved in the maintenance and regulation of the ionic and metabolic environment of the axons of peripheral nerves. The topics of interest include: (1) <u>passive permeability</u> to electrolytes and nonelectrolytes; (2) <u>active transport of ions</u>; (3) <u>facilitated diffusion or transport of amino acids and glucose</u>; (4) <u>electrical impedance and short circuit currents</u>; (5) the determination of the normally existing composition of the <u>extracellular fluid</u> in the <u>endoneurium</u>.</p>																		

Project Description:

The extracellular space in the endoneurium of peripheral nerve is isolated by the endothelial lining of cell capillaries and by the single layer of cells in the perineurium which are connected together by tight junctions. This project is concerned with the study of the role of the epithelial cell layer in the perineurium.

The methods employed are principally the standard techniques used to study unidirectional fluxes of various substances across the isolated and perfused perineurial sheath of the frog or toad, including the use of radioactive tracers. In addition, histological techniques are employed using electron microscopy, and electrical measurements are made using internal and external voltage and current supplying electrodes.

A paper is in press concerning the results of the studies of the effects of stretch and hypertonic solutions on the permeability to sucrose.

AC impedance measurements showed the DC resistance of the perineurium to be about 400 ohm cm² and reduced by exposure to hypertonic solutions. The capacitance indicates the presence of six or more layers of cells and was unchanged by the experimental manipulations. These results were presented at the Biophysical Society meeting in February, 1979.

The sodium permeability was measured to be 1.5×10^{-6} cm/sec and appears to be entirely paracellular since inhibitors of active transport had no effect. The permeability ratios of Na/K/Cl indicate that there is no discrimination between K and Cl and that the K/Na ratio is greater than the limiting conductance ratio in free solution.

The blood-nerve barrier bears the same relation to peripheral nerve axons as the blood-brain barrier does to the brain. Very little work has been done in this area. It is not known, for example, how the composition of the fluid in the extracellular space compares with cerebrospinal fluid. It would appear that the barriers in the perineurium would be intimately involved in a number of functions of the axons and in a variety of peripheral neuropathies.

We have successfully completed preliminary experiments on the changes in permeability induced by Wallerian degeneration and recovery and on the development of the rat perineurium preparation, and we propose to study the composition of the endoneurial fluid using ion-selective electrodes. Electron microscopic studies are in progress to relate the experimental results to structural features.

Publications:

Weerasuriya, Rapoport, S.I. and Taylor, R.E.: Modification of permeability of frog perineurium to ¹⁴C-sucrose by stretch and hypertonicity. Brain Research (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02316-02 LB
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Comparison of Different Modes of Axonal Stimulation		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	G. Ehrenstein	Research Physicist LB NINCDS
Other:	G. Ganot	Visiting Fellow LB NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Biophysics		
SECTION Section on Molecular Biophysics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.9	OTHER: .4
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS	<input type="checkbox"/> (b) HUMAN TISSUES	<input checked="" type="checkbox"/> (c) NEITHER
<input type="checkbox"/> (a1) MINORS	<input type="checkbox"/> (a2) INTERVIEWS	
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>There are differences in the response of <u>excitable membranes</u> to different modes of stimulation. For example, <u>sodium channels</u> inactivate following electrical stimulation, but do not inactivate following chemical stimulation (by batrachotoxin, for example). The goal of this project is to compare and contrast the responses (especially inactivation) of ionic channels to electrical, chemical, and <u>mechanical stimulation</u>.</p>		

Project Description:

We have found that Myxicola axons respond to mechanical stimulation in a manner qualitatively similar to lobster axons. Because of experimental simplicity, we have therefore shifted our study to Myxicola. Mechanical stimulation is applied transversely to the axon using a specially designed transducer. Voltage clamp experiments have shown that this mechanical stimulation increases the electrical conductance of the axon membrane, and that this conductance increase has a very long refractory period - several minutes. Present work is centered on determining the ionic selectivity of the observed conductance.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02317-02 LB
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Excitable Membranes of Tissue-cultured Nerve and Muscle Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	L.M. Huang	Staff Fellow	LN NINCDS
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COOPERATING UNITS (if any)
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Laboratory of Developmental Neurobiology, NICHD
Immunology Branch, NCI

LAB/BRANCH
Laboratory of Biophysics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	2.4	PROFESSIONAL:	1.8	OTHER:	0.6
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ionic channels are studied in excitable cells grown in tissue culture. The unit conductances of postsynaptic ionic channels are determined from electrical noise measurements. Ionic selectivity properties are determined by voltage clamp and tracer experiments. An experiment to measure single channel fluctuations using an extra-cellular electrode is in progress. Theoretical studies of the gating process, of transport noise and of barrier models for ionic transport through pores are in progress.

Project Description

Objectives: To determine the unit conductance of post-synaptic ionic channels by measurement of agonist-induced electric current fluctuations. To determine the selectivity and barrier structures of the channels and to compare these with theoretical predictions based on simple molecular structures.

Methods Employed: An electrically isolated patch recording system is used to measure extracellular currents at the picoampere level. The isolated patch system is capable of measuring current fluctuations caused by the activation of individual ion-conducting channels within the membrane. Electrical noise analysis, microelectrode voltage clamp recording, and radioactive tracers are used for the study of channel selectivity. Theoretical analysis of gating kinetics, channel transport and membrane noise continue.

Major Findings: Single channel currents have been observed for three ion conducting systems: (a) Acetylcholine activated post synaptic channels in tissue-cultured rat and chick muscle; (b) Glutamate-activated post synaptic channels in tissue-cultured mouse CNS neurons; (c) Complement-induced ionic channels in chick muscle.

The most thorough studies so far were done on the acetylcholine channels, using suberyldicholine as an agonist. The channel conductance was found to be 49 pS at a temperature of 25°C and the open-channel lifetime was 3.5 msec. In experiments done in collaboration with C. Christian (LDN-NICHD) we studied the effects of aggregation factor on the postsynaptic receptors. Regions of high channel-fluctuation activity were shown to coincide with regions of high receptor density as measured by fluorescence of rhodamine labelled α -bungarotoxin. The aggregation factor was also shown to change the open-channel lifetime of a fraction of the channel population.

Preliminary experiments gave a value of ~40 pS for glutamate-sensitive channels in CNS neurons. The channels induced by complement are larger pores with a conductance of ~200 pS. The pores appear transiently in bursts when a complement-loaded pipette is pressed to the membrane. We are presently studying the statistics of the bursts, in an attempt to understand the complement-channel formation kinetics during the immune response.

Significance to Biomedical Research: Ionic channels are the basic units of nerve excitation. The variety of excitable-cell behavior can be understood largely in terms of the properties of a few prototypical channels and their distribution in the cell membrane. Experimental study of ionic channels in tissue-cultured excitable cells allows the characterization of the unit channels by experimental means which do not have to be designed anew for each new type of membrane.

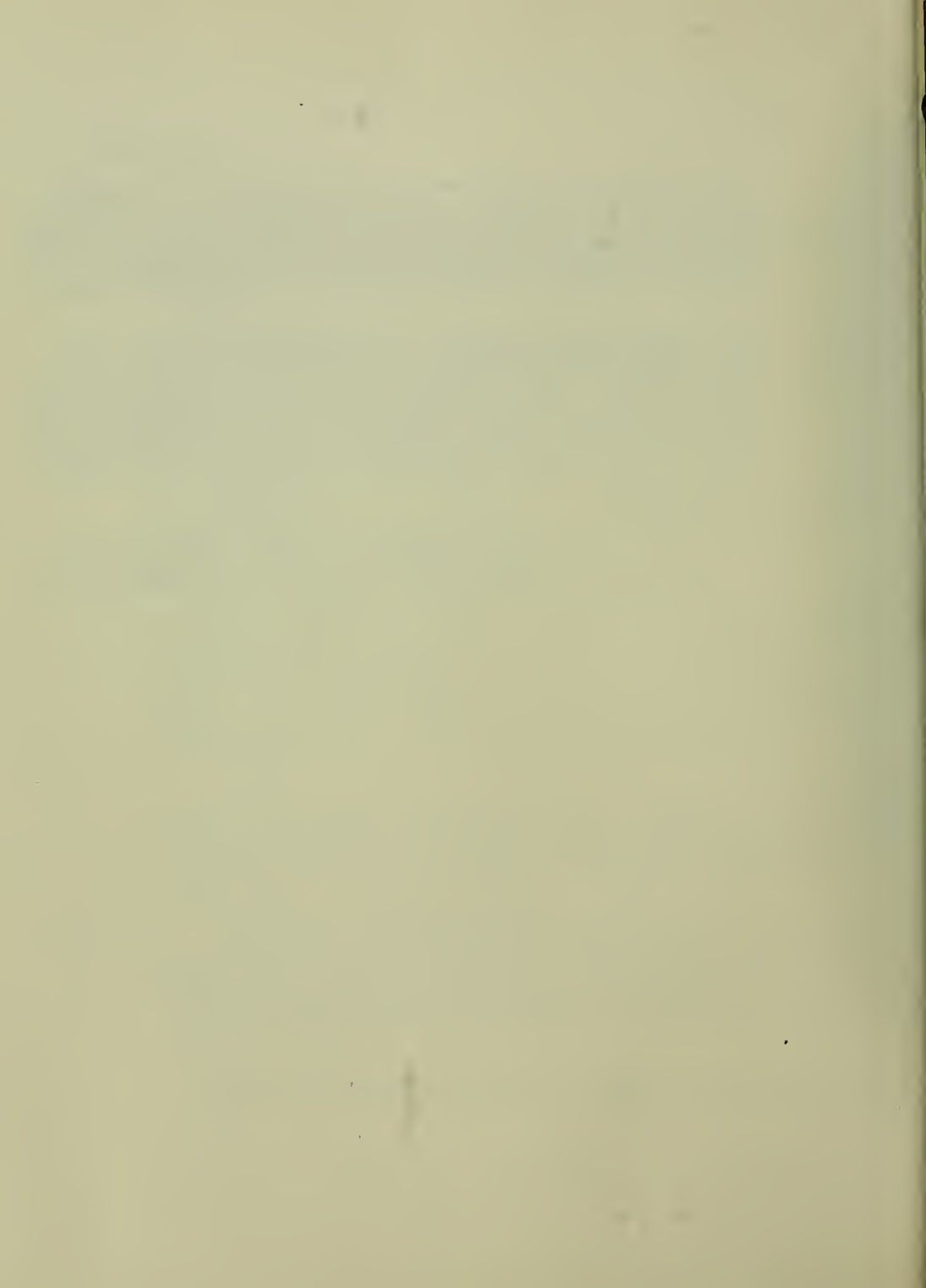
The study of the effects of aggregation factor on the distribution and lifetimes of acetylcholine channels is an attempt to understand some of the

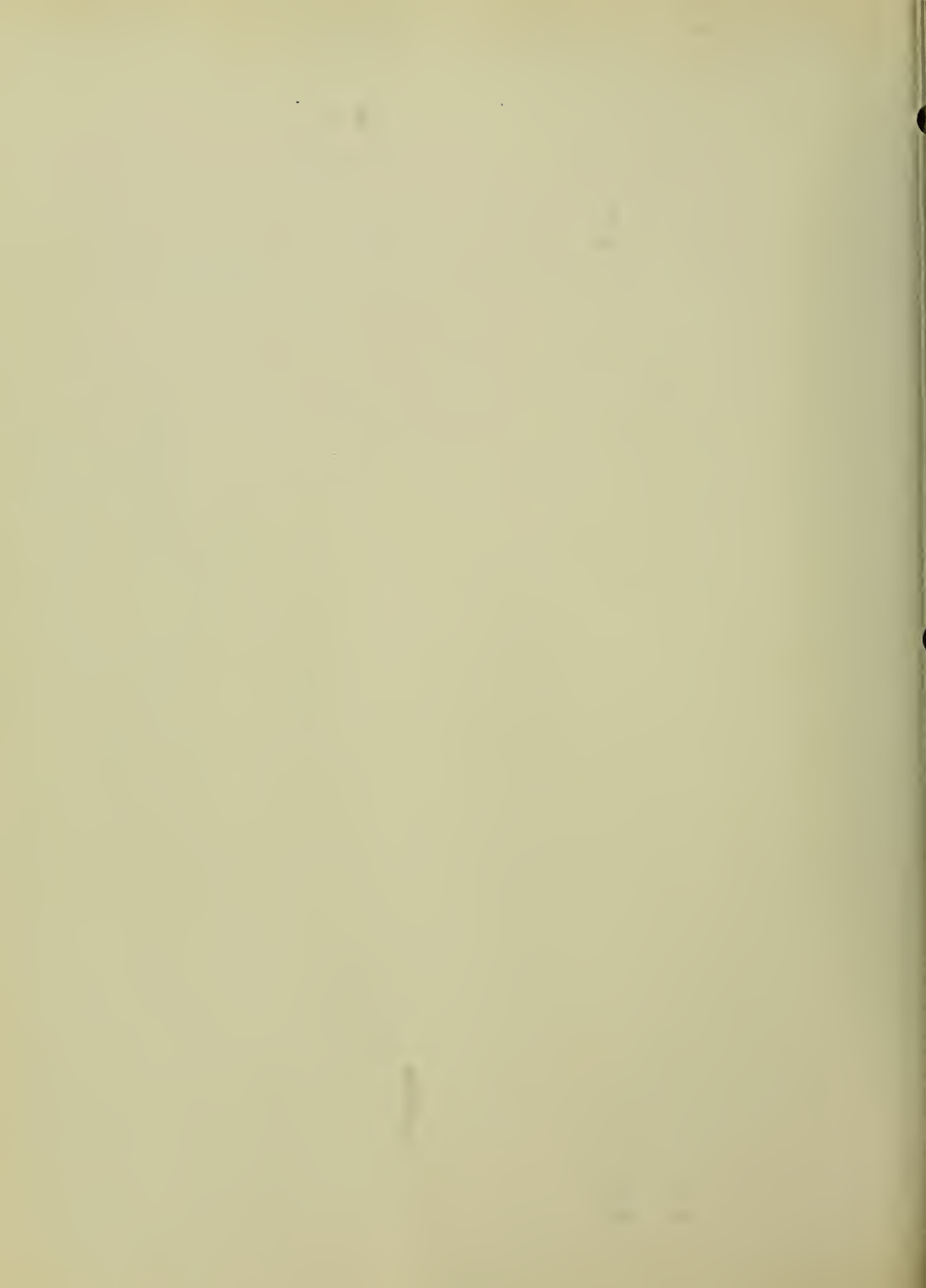
factors involved in synapse formation during development. The glutamate channel experiment allows us to use the patch technique for neurotransmitter identification, since among various putative transmitters of the CNS neurons, the fluctuations experiment allows one to choose the transmitter whose open lifetime is closest to the decay time of the postsynaptic potential. The complement experiment allows one to see details of the dynamics of channel formation from isolated subunits during the immune response.

Proposed Course of Project: The single-channel experiments on the three systems studied initially should be completed within the year. The patch recording technique developed for this project will also be used for the study of the synaptic potentials of single synaptic boutons and a variant of this method will be applied to the study of dendritic propagation. Two other applications of the patch electrode method are anticipated including a study of chemically induced Ca current noise in macrophages and a study of inter-cell channels which form during myoblast fusion.

Publications:

Lecar, H. and Sachs, F.: Membrane noise analysis. In Nelson, P.G. and Lieberman, M. (Eds.): Excitable Cells in Tissue Culture. New York, Plenum Press, 1979 (in press).





ANNUAL REPORT

October 1, 1978 through September 30, 1979

Laboratory of Experimental Neurology
National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report

October 1, 1978 through September 30, 1979

Laboratory of Experimental Neurology, Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

William F. Caveness, M. D

General Statement

The Laboratory was established within the Intramural Program, NINCDS, NIH, April 1969. It was afforded five permanent positions. These were reduced to four in 1977 and to one in 1979. Its scientific objectives have been: to provide fresh insight into the propagation of focal motor seizures in the monkey; to provide precise observations of the delayed effects of ionizing radiation, including therapeutic exposures, on the monkey brain; and to uncover the anatomical as well as functional sequelae of craniocerebral trauma in Man.

Current Scientific Effort:

1) Experimental Focal Seizures in the Monkey

The conceptual framework is that the propagation of a focal motor seizure is a highly organized process involving, perhaps in an excessive manner, elements in the sensorimotor system. Attention is directed to relatively large neuronal aggregates, connected by macro-circuitry.

To gain a global appraisal of neuronal activity in cortical and sub-cortical structures we have employed the [^{14}C] Deoxyglucose method for the determination of regional cerebral glucose utilization. The method was devised by the Chief of the Laboratory of Cerebral Metabolism, NIMH, and is based on the accumulation of 2-deoxy-D[^{14}C] glucose-6-phosphate in the tissues after an intravenous pulse of this compound. Deoxyglucose is transported across the blood brain barrier by the same carrier that transports glucose, and it competes with glucose for hexokinase, the enzyme that phosphorylates both. The rate of deoxyglucose phosphorylation is therefore, quantitatively related to the rate of glucose phosphorylation and thus to the rate of glucose utilization.

The experimental observations took place two hours after completion of the surgical preparation, and end of the general anesthesia. While the animals were fully alert every procedure was in accordance with the humane principles enunciated by the American Physiological Society.

The seizures were induced by stereotactically controlled injection

of 25,000 units of crystalline potassium penicillin G, in 0.025 ml of distilled water, into area 4 of the face-hand area of the right motor cortex at a depth of 2.5 mm.

Bipolar electroencephalographic (EEG) recording was obtained with six Beckman scalp electrodes arrayed bilaterally in three symmetrical pairs over the frontal, temporal, and occipital regions, respectively. Eight pairs of needle electrodes for electromyographic (EMG) recording were placed in the right and left masseter and orbicularis oris muscles and the flexors of the four extremities.

Following the intracortical penicillin injection and after the development of ipsilateral electrographic spikes and the beginning of the contralateral clinical expression, [^{14}C] labeled deoxyglucose, 100 $\mu\text{Ci}/\text{kg}$, was injected by vein. After thirty minutes, during which timed arterial blood samples were obtained, the animal was decapitated, and the head immediately immersed in Freon chilled by liquid nitrogen to -100°C . Subsequently the brain in the skull, was serially sectioned at 30 μm with a PMV cryo-microtome at a temperature of -20°C .

Approximately 100 sections from each brain were dried at 60°C and placed on blue sensitive x-ray film for macroautoradiographs. From the determination of the concentration of [^{14}C]deoxyglucose and glucose in arterial plasma and the concentration of [^{14}C] in the autoradiographs, the actual glucose utilization was calculated for 48 bilateral structures within and outside the sensorimotor system.

Yield to date:

Three manuscripts have been submitted to the Annals of Neurology that fairly represent the culmination of the studies on focal motor seizures in the Macaca mulatta. These may be summarized as follows.

LOCAL CEREBRAL GLUCOSE UTILIZATION IN NEWBORN AND PUBESCENT
MONKEYS DURING FOCAL MOTOR SEIZURES

ABSTRACT

The [^{14}C]deoxyglucose method was used to determine the rate of local cerebral glucose utilization (LCGU) in newborn and pubescent monkeys during focal motor seizures, induced by injecting penicillin into the face-hand area of the right motor cortex. For the controls, 3 newborn and 4 pubescent, and for the seizures, 3 newborn and 6 pubescent monkeys were used. In the controls, the pattern of glucose utilization within the structures of the sensorimotor system was quite different at the two age levels, with the newborn showing far less activity, the most evident difference being in the neocortex and striatum. In the seizure monkeys, the unilateral increase in LCGU relative to the controls was greater in the newborn than in the pubescent monkeys, except in the cerebral and cerebellar cortices. The increased utilization in the cortical and subcortical structures was ipsilateral to the discharging lesion and without the well defined pattern

seen in the pubescent monkeys. In general, the newborn was capable of supporting a focal motor seizure but lacked the precise clinical and electrographic expressions or efficient energy metabolism that accompany the maturation of the brain at puberty. See Fig. 1-4.

PROPAGATION OF FOCAL MOTOR SEIZURES IN THE PUBESCENT MONKEY

ABSTRACT

The rate of local cerebral glucose utilization was employed for the quantification of energy metabolism in macrostructures of the sensorimotor system during the propagation of focal motor seizures in the 24 month old monkey. This rate was determined in four control monkeys, in four with the seizures limited to the contralateral face, in four with the seizures extending to the contralateral face and upper extremity, and in four in which there was a bilateral expression. There was a sequential increase in glucose utilization, primarily unilateral, with propagation. This was greatest, in order, in the sensory and motor cerebral cortices, putamen and globus pallidus, with somewhat less increase in sensory and motor thalamic relay nuclei, and least in the cerebellar cortex. It was concluded that the increased rate of glucose utilization in the indicated distribution was for the transmission and restraint of the paroxysmal activity, and the essential maintenance of energy equilibrium. See Fig. 5-8.

EFFECTS OF MANIPULATION OF THE SENSORIMOTOR SYSTEM ON FOCAL MOTOR SEIZURES IN THE MONKEY

ABSTRACT

During focal motor seizures, induced by injecting penicillin into the face-hand area of the right motor cortex of 24 month old monkeys, the manipulation of the sensorimotor system was brought about by: a) the elimination, through a paralytic agent, of the proprioceptive input from contracting muscles and joints. This caused no significant alteration in the electrographic expression of the seizure and no alteration in the pattern of local glucose utilization in cortical or subcortical components of the sensorimotor system. There was however, an overall increase in the rate of energy metabolism in the paralyzed monkeys with electrographic seizures. These observations respectively underscore the strength of the integrated seizure activity, unaltered in pattern by the removal of a component of the sensorimotor system, and suggest an imbalance in excitation-inhibition mechanisms in the absence of proprioceptive input. b) The cryogenic destruction of up to 90% of the ipsilateral ventral caudal globus pallidus, was without effect on the electrographic or clinical expression of the seizure. This is another indication of the overall integrity, including that in alternate pathways, of the seizure phenomena subserved by the sensorimotor system. c) Electrical stimulation 10/sec, 30V, 0.3 mA, of the ipsilateral ventral caudal globus pallidus caused reproducible, maximum expression of the electrographic and clinical phenomena of the focal seizure for the 90 second duration of the stimulus.

This reflects the significance of this structure in the transmission of excitatory signals. See Figs. 9 through 13.

Projected Yield:

The long range future of this investigation has been entrusted to the Neurological Institute of Kyushu University, Fukuoka, Japan. It will be conducted by a cadre of scientists, specifically trained in the essential techniques while serving as members of the Laboratory of Experimental Neurology. To facilitate uninterrupted investigation, laboratory equipment that had been assembled here for this activity was made available to them through a loan from NINCDS, NIH, in January 1979. The emphasis will be shifted from focal motor seizures to seizures initiated in the temporal lobe with attention to the pattern of their spread to the hippocampus, amygdala, other parts of the limbic system and the basal ganglia. The protocols for this study of the propagation in temporal lobe seizures was carefully worked out, in conjunction with the Chief, LEN, in the Spring of 1979. The establishment of this new laboratory in Japan has the full administrative and scientific support of the Director of the Neurological Institute, Kyushu University.

Significance to Bio-Medical Research: Fresh insight into the pattern of subcortical propagation is afforded by this global appraisal of glucose utilization. Further, this established model of focal seizures will permit additional manipulation of cortical and subcortical activity by pharmacological or physical agents. This should provide new approaches to medical and/or surgical therapy for selected forms of the Epilepsies.

2) Delayed Effects of Ionizing Irradiation on the Brain of the Monkey.

Two models have been employed with focal and whole brain irradiation, respectively. Focal irradiation was used in the study of the preferential spread of vasogenic edema.

Exposure of the right visual cortex to 3500 rads of orthovoltage radiation in a single dose was followed after a period of four to five months by the rapid development of radionecrosis. An integral part of this complex lesion was a massive break in the blood brain-barrier. From the site of the lesion, the edema propagated along neuro-anatomical pathways within the geniculostriate and extra-geniculostriate visual pathways. In twelve monkeys this was determined by histological study of serial sections in six, electron microscopy in two, and solute parameters in four. The structures, ipsilateral to the lesion, that were involved in the edematous process were the lateral geniculate, the peristriate cortex, the middle and inferior temporal gyri, gray as well as white matter, but not the superior temporal gyrus, and the uncinatus fasciculus leading to the arcuate cortex. (In studies of the extra-geniculostriate visual system in the monkey, Kuypers (1965) found reciprocal anatomical connections between the visual cortex and the structures indicated above. Mishkin (1965) showed visual function in the middle and inferior temporal gyri as well as the frontal eye fields). Thus the orderly propagation of the vasogenic edema from one cortical area,

through white matter tracts, to other cortical areas or subcortical neuronal aggregates within the same neuronal system has been determined.

The whole brain was exposed to supervoltage radiation to simulate, in part, therapeutic procedures in man. Two sets of 12 monkeys each at puberty, received single and fractionated exposures, respectively. One set of 12 monkeys in adulthood, received a fractionated exposure.

Exposure to 1000 rads in a single dose, at puberty, caused no late effects. Exposure to 1500 rads caused small areas of necrosis in the forebrain white matter at 26 weeks, but a much more extensive involvement at and beyond 52 weeks that included confluent areas of necrosis in gray and white matter. Brain loss resulted in ventricular dilation. Gliomas appeared in two out of three monkeys at or beyond 52 weeks. Exposure to 2000 rads caused such a wide scatter of focal areas of necrosis, including those in the brain stem, that survival beyond 20-25 weeks was not possible. All showed enlarged ventricular systems.

Whole brain exposure, 200 rads a day, five days a week, for a course of 4000 rads, at puberty, resulted in no delayed effects. 6000 rads in a six weeks course, the simulated therapeutic exposure, caused small, less than 1 mm, widely scattered necrotic lesions with a predilection for the forebrain white matter but not excluding the central gray matter and brain stem, at 26 weeks. Although a trend toward recession in numbers of fresh lesions was apparent, complete repair never occurred. At 52 weeks, there was considerable mineralization of the lesions and widespread telangiectasia. In the developing lesions, multiple minute breaks in the blood brain barrier caused diffuse brain swelling, reflected by papilledema. The lesions from 8000 rads at 25 weeks were not strikingly different from those following 6000 rads. However, by 52 weeks, through an increase in number, size and coalescence, they had caused gross brain destruction.

Whole brain exposure to 6000 rads in a six weeks course, in the adult monkey, produced less effects than the same dose at puberty. The onset of the scattered necrotic lesions was later than expected, appearing in one out of three animals at 33 weeks, two out of three animals at 52 weeks, and two out of three at 104 weeks.

Note: A finer definition of the break in the blood brain barrier, as a part of the radiation lesion, has been provided by the use of ¹⁴C labeled L-amino-iodo-butyrlic acid. For example, it has demonstrated the lack of permanent healing: A two year old sclerotic lesion was shown to have multiple areas of extravasation.

Comment: The late biological effects of whole brain irradiation vary with the age of the host, the mode and amount of the dose and the time interval following exposure. Conceding the difficulty in interpreting these integrated factors, there are a few general conclusions that may be drawn from the described observations in the three sets of experimental animals.

1) The hallmark lesion is a minute focus of necrosis, that is widely

scattered throughout the forebrain white matter. These lesions may appear as early as four or five months or as late as one to two years, following the radiation.

At any given time, after their appearance, they are seen to be in different phases of a cycle that begins with a punched out area, passes through phagocytosis, gliosis, and ends with mineralization. Individually, one may be in the initial phase while another is in the end phase. Larger at the outset, they are diminished in size as the cycle is completed.

In the aggregate, when there are a large number of the acute lesions, part of the effect is brain swelling from multiple minute breaks in the blood brain barrier. In the aggregate, when there are a large number in the stage of mineralization, the loss in brain substance is reflected by ventricular dilation.

2) Accompanying, or perhaps preceding the discrete areas of necrosis are a variety of vascular abnormalities, the most notable of which are occasional absent or hyperplastic endothelial cells in adjacent capillaries. Quite apart from these are abnormal vascular channels making up patches of telangiectasia. These contribute to the brain damage and to the break in the blood brain barrier. The telangiectatic expression increases over time.

3) Whether the minute necrotic lesions proceed to a predominantly healed phase, or increase in number with confluence that results in gross brain destruction, depends both on the initial exposure and the length of time after radiation. For example, the lesions from 1500 rads in a single dose, 6000 rads in a fractionated dose and 8000 rads in a fractionated dose, look very much alike at 26 weeks, but at 52 weeks those from the 6000 rads are all but quiescent while those from the other two exposures have resulted in widespread brain destruction.

4) Malignant gliomas are a distinct rarity in the monkey. However, they have been found by others two or more years following whole body exposure to 600-800 rads with 55 MeV proton radiation. 1500 rads of supervoltage radiation in a single dose to the whole brain at puberty is beyond any therapeutic range, but the occurrence of neoplasms in this experimental group, alerts one to the possibility of such a complication.

5) The age of the host plays a significant part in the occurrence and the ultimate effect of the minute necrotic lesions. In the pubescent monkeys subjected to 6000 rads, fractionated dose, two out of four showed pronounced papilledema, prior to the 24th week. Subsequent optic atrophy was seen in one of these. A third showed blurred disc margins without measurable elevation in the nerve head. By contrast, in the adult monkeys subjected to 6000 rads, fractionated dose, only two out of the twelve that were killed at or beyond 24 weeks showed definite papilledema with a third showing blurred disc margins. As in the pubescent group, these funduscopic changes were prior to 24 weeks.

The scattered minute necrotic lesions were seen in all four of the

pubescent monkeys, killed at 26, 52, 68 and 78 weeks, respectively. By contrast the adult monkeys showed this phenomenon in only one out of three killed at 33 weeks, two out of three at 52 weeks and two out of three at 104 weeks. The third monkey in the last group showed eight lesions, all confined to the thalamus. Those at 33 and 52 weeks were predominantly in the intermediate phase, those at 104 weeks were predominantly in the late or mineralized phase. The increase in the telangiectatic expression over time was somewhat greater in the adult group than in the pubescent group.

6) Within groups of the same age, with similar exposures, there are individual variations in susceptibility.

Significance to Bio-Medical Research and the Program of the Institute:

The principal advantage in simulating a therapeutic regime in a monkey model is to observe the effects in a brain uncomplicated by pre-existing pathology, e.g., that resulting from a neoplasm, surgical trauma or chemotherapy. This kind of information will be useful in the planning of therapeutic efforts in man, with attention to "risk-benefit" factors, and in the interpretation of fresh neurological findings following therapy. Further, there is a direct relevance to a major program of the Cancer Institute, i.e., the Brain Tumor Study Group, and we are privileged to share in their findings as this group is made aware of our results.

3) Structural and Functional Sequelae of Penetrating Head Injury, Phase I.

A registry for Head and Spinal Cord Injuries, as they occurred in military combat in Vietnam, was developed at the request of the Surgeon General of the U. S. Navy and was implemented with the cooperation of the Surgeons General of the U. S. Army and U.S. Air Force. The purpose was to insure uniformity of data collection and identify cases for present and future studies. The yield from field surgeons, 1967 to 1979, was 2,043 entries that included 1,683 head injuries, 329 spinal cord injuries and 31 combinations of the two. A rigorous appraisal demonstrated the uniformity and completeness of 1,540 head injury forms. Ninety-two percent of these were the result of explosively propelled missiles, 8%, the result of vehicular accidents, falls, or blunt objects. In 83% there was local brain destruction, as evidenced by penetration of the dura with cortical laceration. Sixty percent were accompanied by immediate loss in consciousness, by history. Twenty-five percent were in coma responding only to pain at the time of examination, that was generally within six hours of injury. This provided an opportunity to group selected cases by regions of focal damage, with and without alteration in consciousness. These categories constitute a background against which the natural history of sequelae may be determined.

A contract proposal entitled, "Structural and Functional Sequelae of Penetrating Head Injury, Phase I", was submitted to NINCDS in May 1975. A Feasibility Study for Phase I was approved in March 1976, and implemented in July 1976, with support from the Stroke and Trauma program, NINCDS. The conduct of this plan has updated the clinical data on 1221

selected cases from the Registry, by a review of the accumulated Military and Veterans Administration hospital records since the time of injury. The assembly of the records was carried out in cooperation with the Medical Follow-up Agency of the National Research Council, NAS. Abstracting the pertinent data, coding, transfer to magnetic tape, and analyses of sequelae are being conducted in cooperation with the Physical Sciences Laboratory of Computer Research and Technology, NIH, and specially qualified professionals at the Eastern Virginia Medical School, Vanderbilt University Medical School, South Alabama Medical School and Harvard Medical School, respectively. Attached figures 14-17 indicate the organization for Phase I, injury characteristics, sequelae identified and the duration of the follow-up from the medical records. To date there have been two publications from the records review, one manuscript accepted for publication and three manuscripts in preparation.

Concomitant with the completion of Phase I, anticipated for the Autumn of 1979, there was planned a definitive protocol development for Phase II, i.e., an in-hospital examination of 1000 of these head injured casualties with techniques not previously available, including computerized tomography for the determination of brain loss. The protocol was to have been an integral part of a formal application for the support of Phase II.

In November 1978, an interim appraisal was imposed by the Director, NINCDS. This was carried out by an ad hoc panel under the supervision of the Director and Clinical Director of IRP in February 1979. The panel did not recommend the support of Phase II. On the other hand the Surgeons General of the Air Force, Army and Navy have agreed to provide the transport of the patients, beds, and computer tomography for the conduct of Phase II. Operational funds, primarily for biostatistical support and technical help, were sought from the Veterans Administration. After a careful review, the Director, Medical Research Service, in July 1979 recommended that the Veterans Administration provide funds for the Protocol Development and, depending upon the approval of this planning phase, operational funds for the conduct of the full study.

Significance to Bio-Medical Research and the Program of the Institute:

The observations during the acute phase of injury in these cases are more uniform and probably more accurate than any previous series of comparable size. This provides an extraordinary opportunity for studies of prognostic factors and the natural history of disabilities in central nervous system trauma in man. The recent development of new techniques for evaluating functional deficits, e.g., those regarding language and memory and a mode for determining alterations in brain structure, i.e., the CT Scan, will afford a unique opportunity in Phase II for fresh insight into posttraumatic sequelae and into the function of the remaining brain.

There will be provided a thought provoking supplement to the acute head injury studies being supported by the Stroke and Trauma Program of NINCDS. Further, there will be identified subsets of the head injured group that should be of interest for subsequent studies within the Clinical Center Program, e.g., an estimated 100 cases with intractable seizures.

Publications in the Reporting Period

Caveness, W.F., Kemper, T.L., Brightman, M. W., Blasberg, R. G., and Fenstermacher, J. D.: Directional character of vasogenic edema. In J. Cervos-Navarro et al. (eds) Advances in Neurology, Vol. 20, New York, Raven Press, 1978, pp 271-291.

Caveness, W.F., and Carsten, A.L.: Late effects of whole brain irradiation within the therapeutic range. In Late Biological Effects of Ionizing Radiation. Vol. II. International Atomic Energy Agency, Vienna, 1978, pp 221-239.

Caveness, W.F.: Energy metabolism in focal seizures. In B.B. Mrsulja, et al. (eds) Pathophysiology of Cerebral Energy Metabolism, New York, Plenum Press, 1979, pp 267-280.

Caveness, W. F., Meirowsky, A. M., Rish, B. L., Mohr, J.P., Kistler, J.P., Dillon, J. D., and Weiss, G.H.: The nature of posttraumatic epilepsy. J. Neurosurg. 50: 545-553, 1979.

Rish, B. L., Dillon, J. D., Meirowsky, A.M., Caveness, W.F., Mohr, J.P., Kistler, J. P., and Weiss, G.H.: Cranioplasty: A review of 1030 cases of penetrating head injuries. Neurosurg. Vol. 4, No. 5: 381-385, 1979.

Caveness, W. F.: Incidence of craniocerebral trauma in the United States in 1976 with trend from 1970-75. In R.A. Thomson, J.R. Green (Eds.) Advances in Neurology, Vol. 22, New York, Raven Press, 1979, pp 1-3.

Wakisaka, S., O'Neill, R. R., Kemper, T.L., et al: Delayed Brain Damage in adult monkeys from radiation in the therapeutic range. Radiat. Res. (in press).

Meirowsky, A. M., Caveness, W.F., Rish, B. L., Dillon, J. D., Mohr, J.P. Kistler, J. P., and Weiss, G. H.: Definitive care of cerebral missile injuries crossing the midline. J. Milit. Med. (In press).

Seizure Development

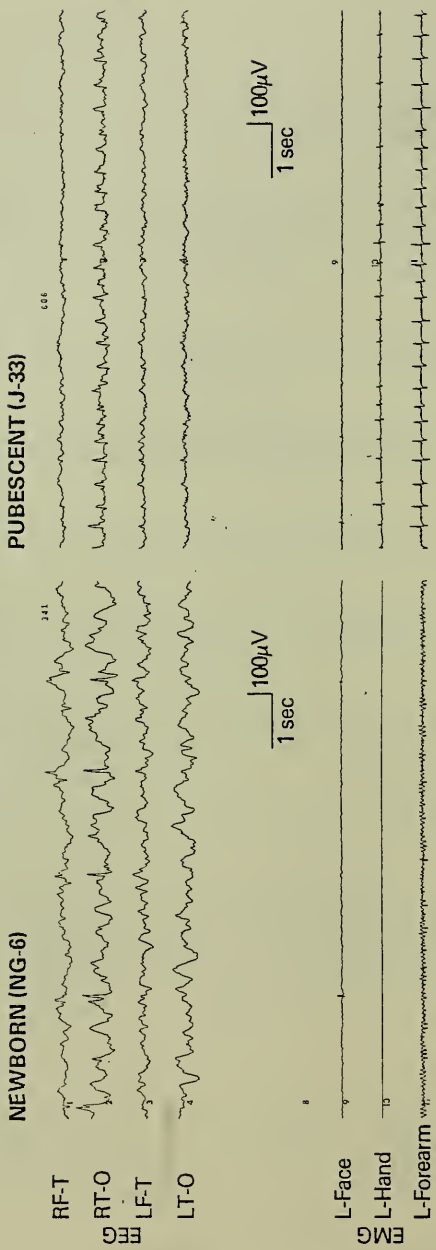
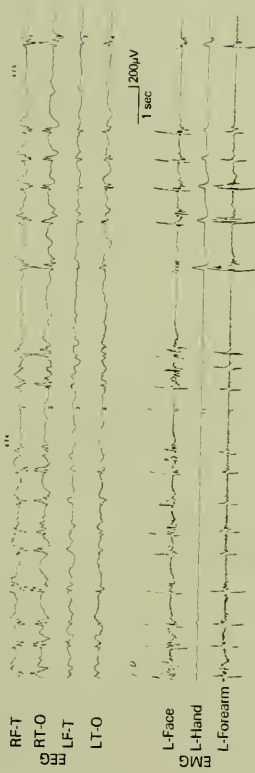


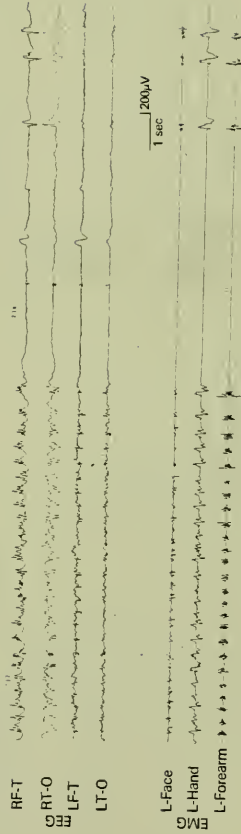
Fig. 1

Cessation of Seizure Episode

NEWBORN (NG-6)



PUBESCENT (J-33)



CEREBRAL GLUCOSE UTILIZATION CONTROL

Newborn (NGC 2, NGC 3, NGC 4) vs Pubescent (JC 6, JC 9, JC 10, JC 11)

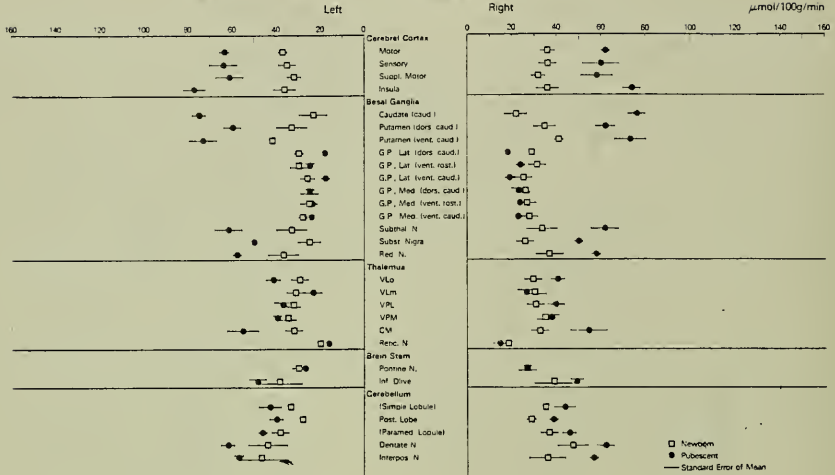


Fig 3

CEREBRAL GLUCOSE UTILIZATION

CONTRALATERAL FACE AND HAND SEIZURE

Newborn (NG 4, NG 5, NG 6) vs Pubescent (J 20, J 25, J 31, J 32, J 33, J 38)

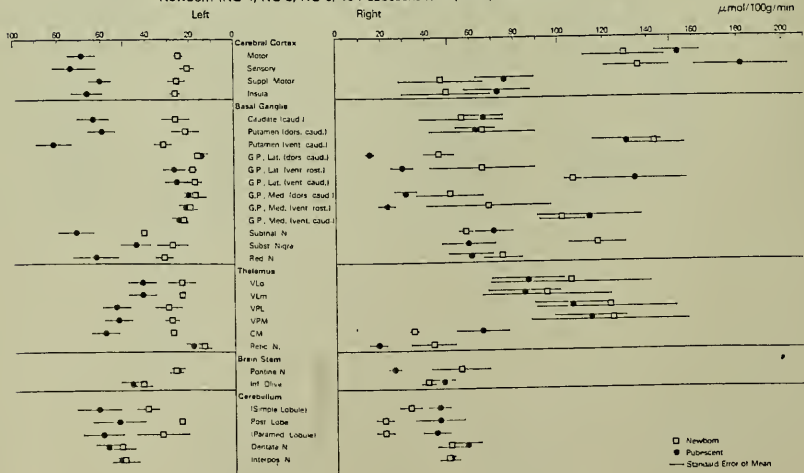


Fig. 4

CEREBRAL GLUCOSE UTILIZATION

CONTROL (JC-6, JC-9, JC-10, JC-11)

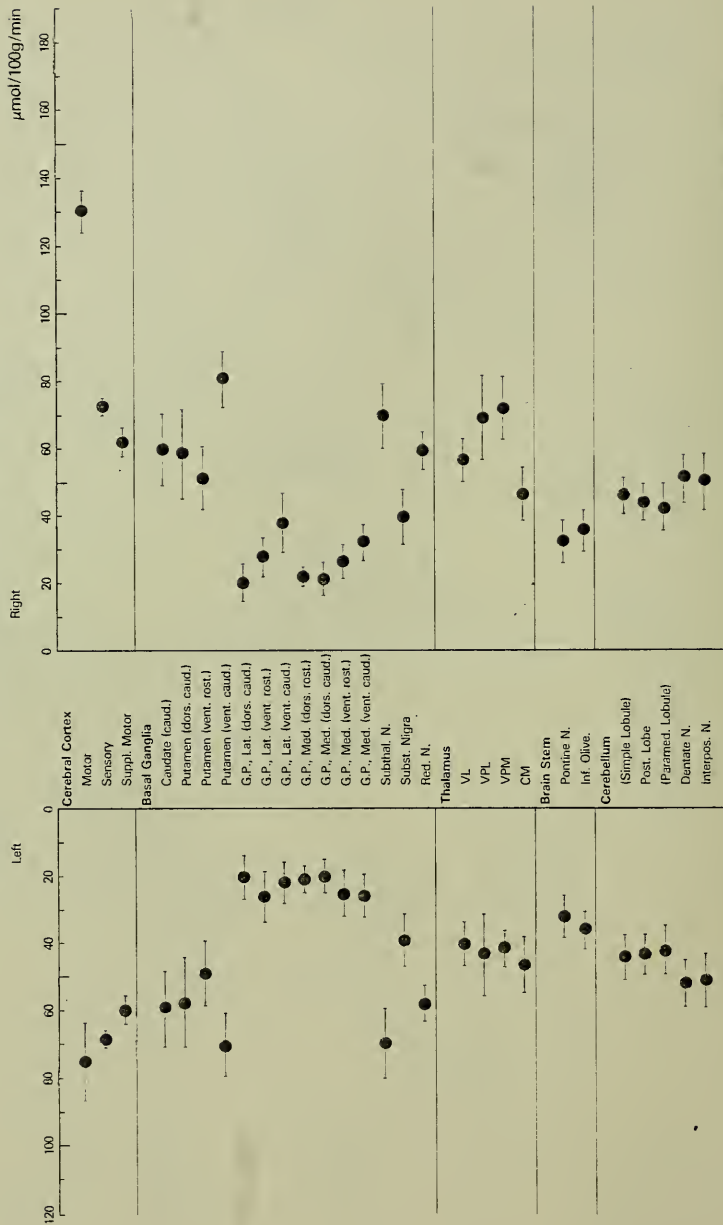


— Standard Error of Mean

Fig 5

CEREBRAL GLUCOSE UTILIZATION

CONTRALATERAL FACE SEIZURE (J-15, J-16, J-18, J-19)



— Standard Error of Mean

Fig. 6

CEREBRAL GLUCOSE UTILIZATION

CONTRALATERAL FACE AND HAND SEIZURE (J-20, J-31, J-32, J-33)

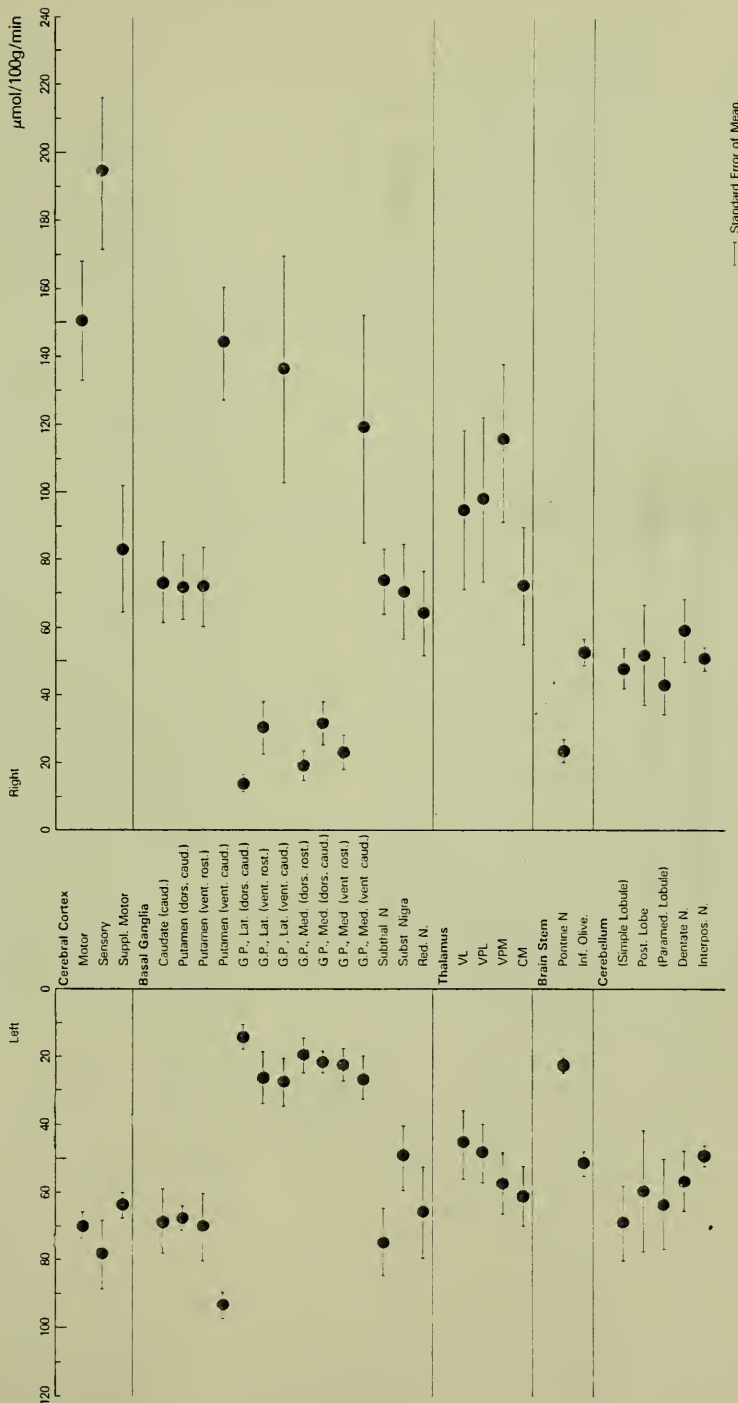


Fig 7

CEREBRAL GLUCOSE UTILIZATION BILATERAL SEIZURE (J-30, J-40, J-42, J-46)

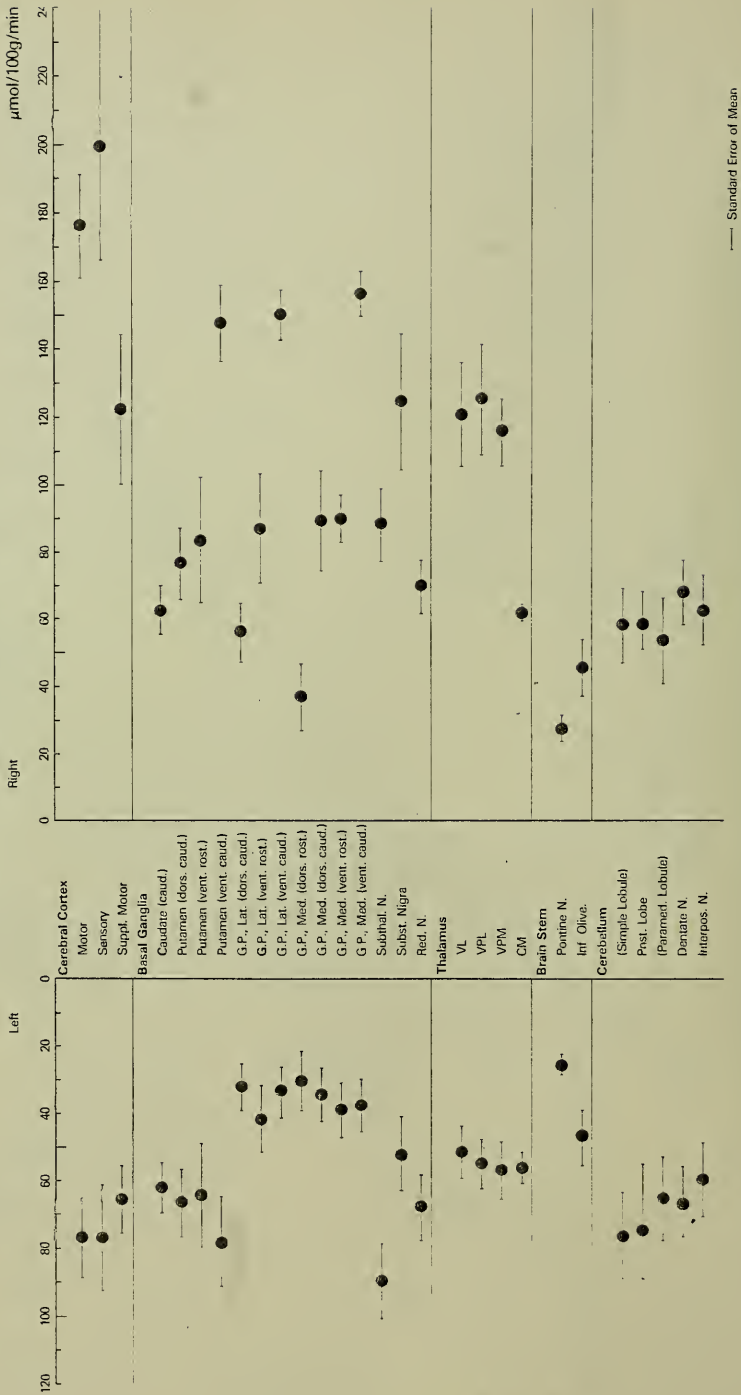
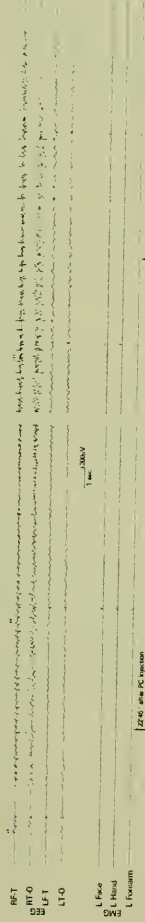


Fig. 8

Séizure Episode Paralyzed vs Non-Paralyzed

PARALYZED MONKEY J36



NON PARALYZED MONKEY J33

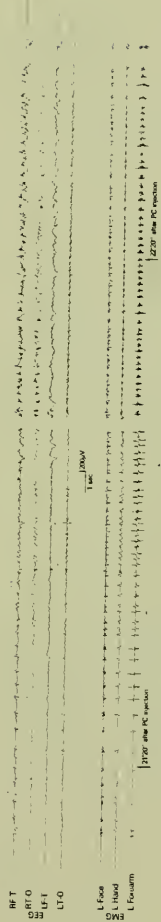


Fig 9

CEREBRAL GLUCOSE UTILIZATION

SEIZURE MONKEYS

NON-PARALYZED (J 20, 32, 33, 38, 40, 46) vs. PARALYZED (J 34, 35, 37)

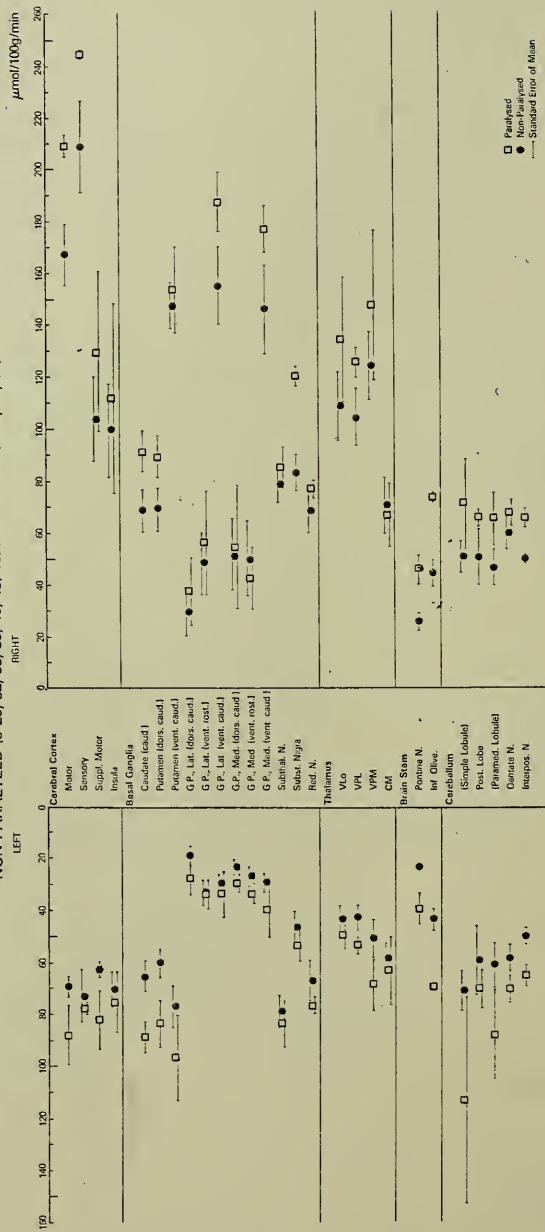


Fig. 10

CRYOPROBE IN PUTAMEN AND
GLOBUS PALLIDUS

K-107

(face-hand seizure)

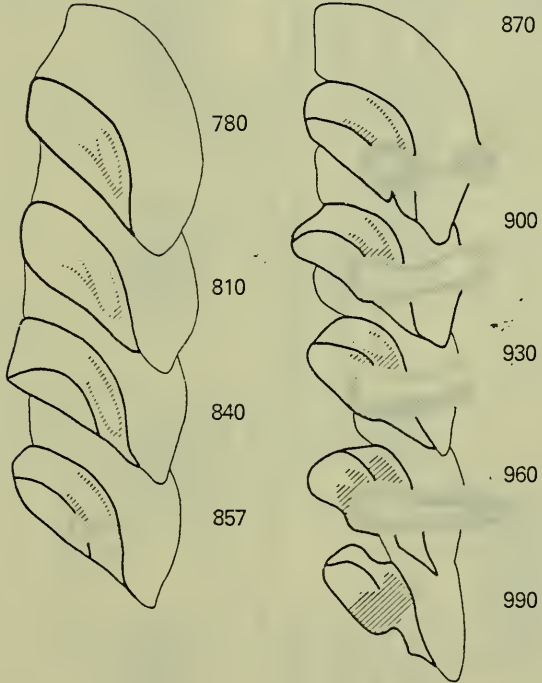
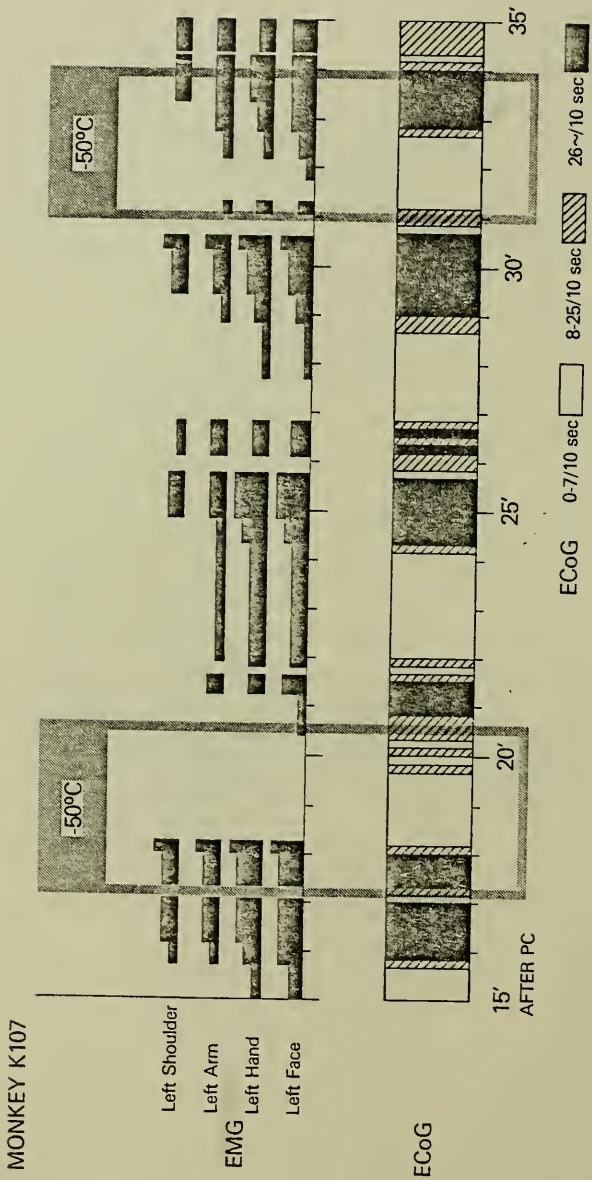


Fig 11

Fig. 12



MONKEY K114

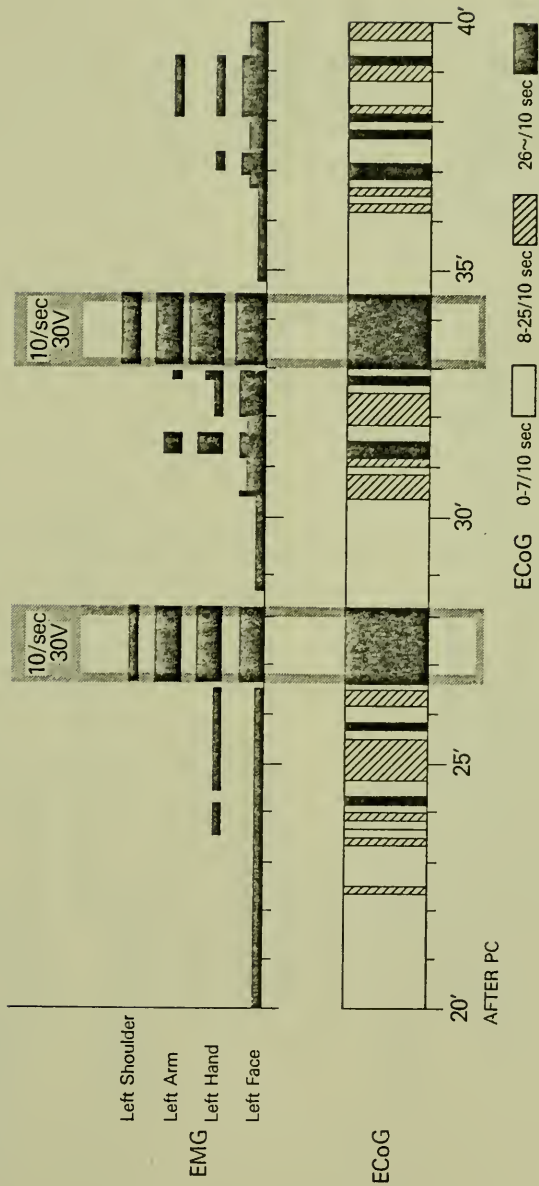


Fig 13

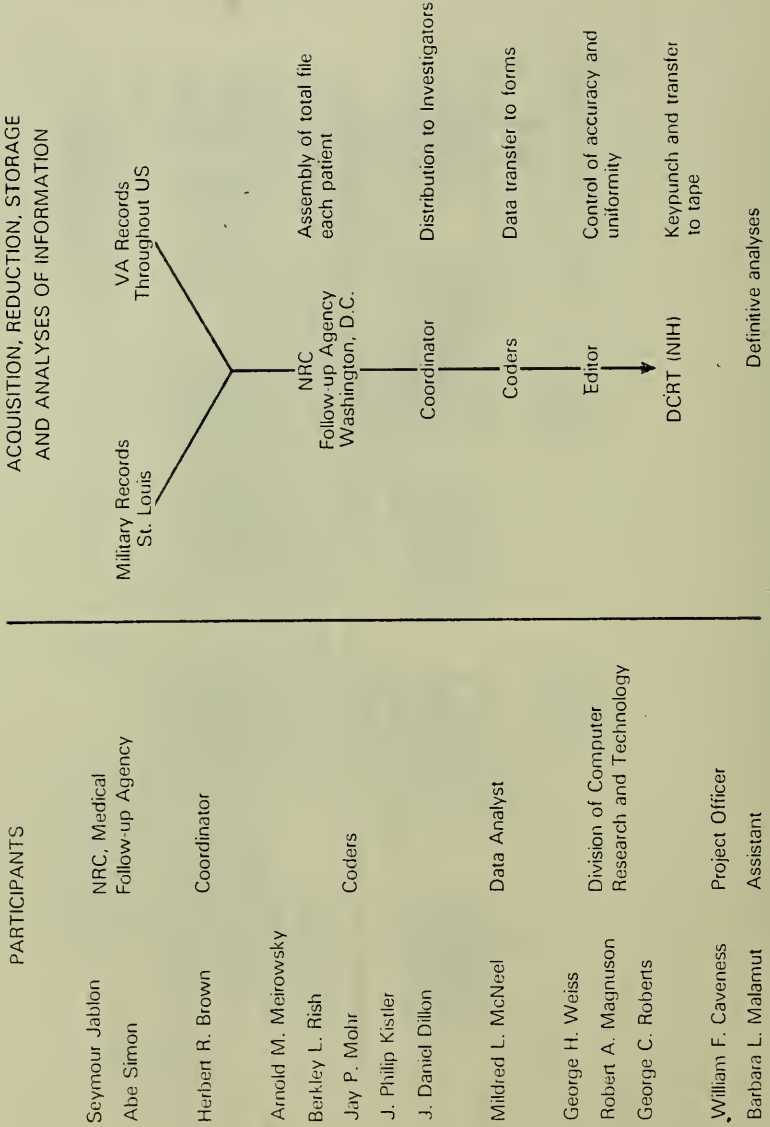


Fig. 14

VIETNAM REGISTRY DATA INTERIM APPRAISAL,

(N = 1221)

Age at Injury

Mean 21.3 years

Standard deviation 3.4 years

Agent of Injury

Missile Fragments	940	(77.0%)
Gun Shot Wounds	193	(15.8%)
Vehicular	49	(4.0%)
Other	39	(3.2%)

Severity of Injury

Alteration in Consciousness

Alert	664	(54.4%)
Responds to Command	255	(20.9%)
Responds to Pain	302	(24.7%)

Depth of Injury

Fractures	210	(17.1%)
Single Lobe	510	(41.8%)
Multiple Lobes	501	(41.0%)

Fig 15

Deficits	First Status Report		Last Status Report	
	Number of cases	Percent	Number of cases	Percent
Sensori-motor	504	<u>41.3</u>	381	<u>31.2</u>
Special sensory, visual	420	<u>34.4</u>	383	<u>31.4</u>
Dysphasia	249	<u>20.4</u>	163	<u>13.3</u>
Abnormal behavior	281	<u>23.0</u>	444	<u>36.4</u>
Abnormal memory	146	<u>12.0</u>	332	<u>27.2</u>
Organic Brain Syndrome	194	<u>15.9</u>	434	<u>35.5</u>
Posttraumatic Epilepsy			396	<u>32.4</u>

Fig. 16

FOLLOWUP (N = 1221)

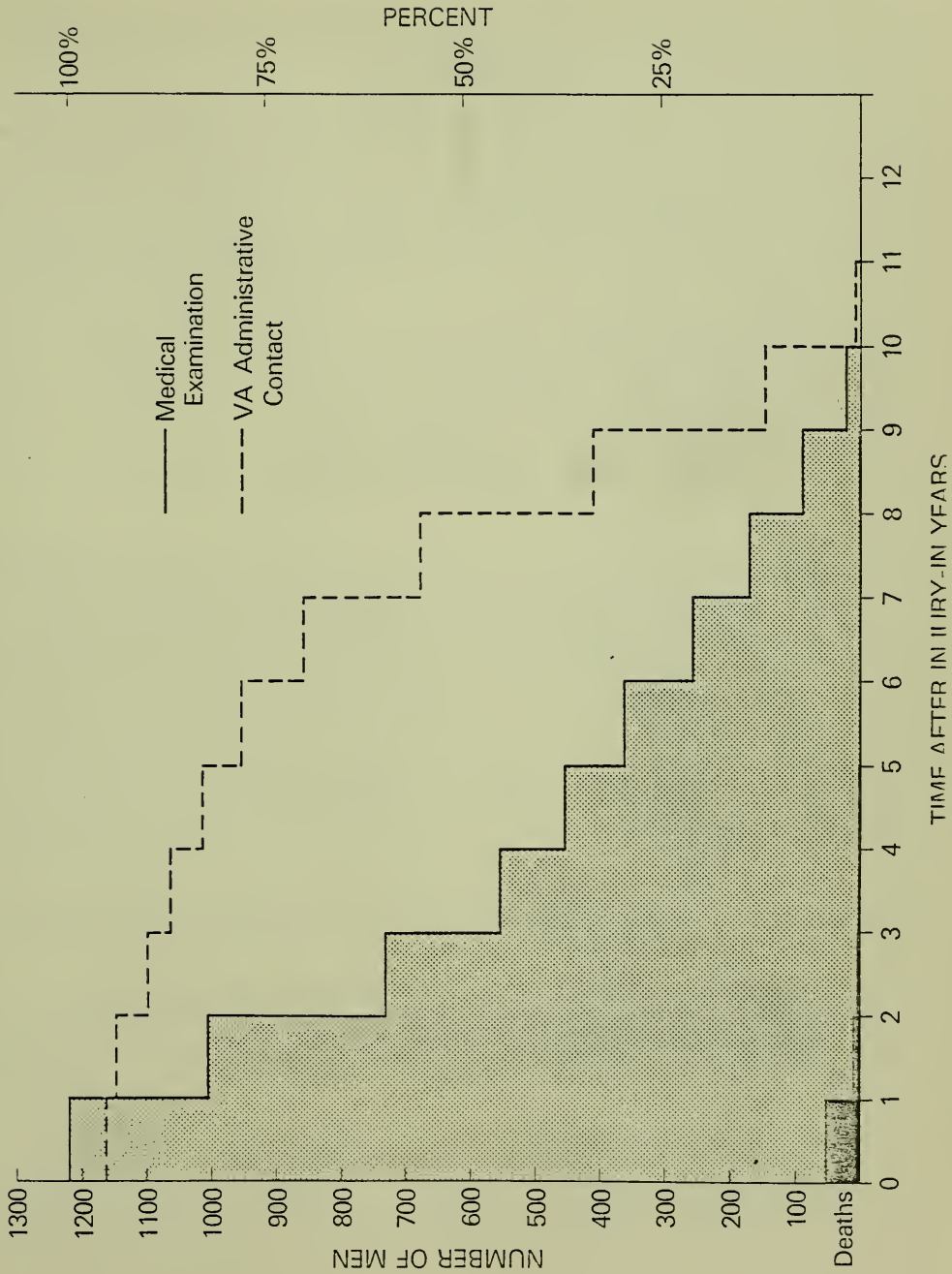


Fig 17

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER <u>Z01 NS 02189-04 LEN</u>
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Anatomical and Functional Sequelae of Penetrating Head injury, Phase I

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: W.F. Caveness	Chief	LEN	NINCDS
G. H. Weiss	Chief	PSL	DCRT
A.M. Meirowski	Assoc. Prof. Neurological Surg.,	Vanderbilt University School of Medicine	
B.L. Rish	Assoc. Prof. of Neurosurgery, Eastern Virginia Med. Sch.		
J.P. Mohr	Prof. of Neurology, University of South Alabama Medical School		
J.P. Kistler	Instructor in Neurology, Harvard Medical School		
J. D. Dillon	LCDR (MC) USN, Portsmouth, VA		

COOPERATING UNITS (if any)

Physical Sciences Laboratory, DCRT, NIH
Stroke and Trauma Program, NINCDS - US Naval Hospital, Portsmouth, VA
Eastern Virginia, Vanderbilt Univ., So. Alabama and Harvard Medical Schools

LAB/BRANCH

Laboratory of Experimental Neurology

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

3

PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER
- (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Objective: To determine the loss in brain substance and the alteration in brain function, 8-10 years after brain damage incurred during the Vietnam War. The resource for this is a Registry of 1500 Head Injuries compiled in the field by Military Surgeons from 1967 to 1970. The characteristics of the injury and the initial disability is recorded in detail, according to a prospective plan.

To achieve this objective, a plan was developed, titled Phase I, and a team assembled to a) collect, review and analyze the intervening medical records and b) plan phase II. The latter will utilize an unique approach to the loss or alteration in structure, i.e., computerized axial tomography, and other techniques, not available in previous studies of functional sequelae.

Project Description

Objectives: To determine the loss in brain substance and the alteration in brain function, 8-10 years after brain damage incurred during the Vietnam War.

Background: A registry for Head and Spinal Cord Injuries, as they occurred in military combat in Vietnam, was developed at the request of the Surgeon General of the Navy and was implemented with the cooperation of the Surgeons General of the U.S. Army and U.S. Air Force. The purpose was to insure uniformity of data collection and to identify cases for present and future studies. The yield from field surgeons, 1967 to 1970, was 2,043 entries that included 1,683 head injuries, 329 spinal cord injuries and 31 combinations of the two. The average age at time of injury was 21.6 years. A rigorous appraisal demonstrated the uniformity and completeness of 1,540 head injury forms.

Current Methods: A contract proposal entitled, "Structural and Functional Sequelae of Penetrating Head Injury, Phase I", was submitted to NINCDS in May 1975. A Feasibility Study for Phase I was approved in March 1976, and implemented in July 1976. The conduct of this plan has updated the clinical data on 1221 selected cases from the Registry, by a review of the accumulated Military and Veterans Administration hospital records since the time of injury. The assembly of the records was carried out in cooperation with the Medical Follow-up Agency of the National Research Council, NAS. The abstracting of pertinent data, coding, transfer to magnetic tape, and analyses of sequelae, so identified, is being carried out in cooperation with the Physical Sciences Laboratory of the Division of Computer Research and Technology, NIH, and specially qualified professionals at the Eastern Virginia Medical School, Vanderbilt University Medical School, South Alabama Medical School and Harvard Medical School, respectively.

Major Findings

The characteristics of the injuries in the 1221 cases were as follows:

Agent of Injury

Missile Fragments	940	(77.0%)
Gun Shot Wounds	193	(15.8%)
Vehicular	49	(4.0%)
Other	39	(3.2%)

Severity of Injury

Alteration in Consciousness

Alert	664	(54.4%)
Responds to Command	255	(20.9%)
Responds to Pain	302	(24.7%)

Depth of Injury

Fractures	210	(17.1%)
Single Lobe	510	(41.8%)
Multiple Lobes	501	(41.0%)

Comment: Local brain destruction and/or loss in consciousness are traditionally used as criteria for severity of head injury. While there are regional brain stem effects, loss in consciousness is the most reliable indicator available for diffuse brain injury. The dynamic aspects of physical disabilities, e.g., rate and degree of recovery, may well be dependent upon local or diffuse injury alone or in combination. The implication in combinations are self-evident for intellectual impairment and posttraumatic epilepsy.

The material at hand includes 83% with local brain destruction as evidenced by penetration of the dura with cortical laceration. 45% were accompanied by immediate loss in consciousness. 25% were in coma, responding only to pain at time of examination, within six hours of injury. This makes possible the selection of cases by regions of focal damage, with and without functional evidence of diffuse brain damage. Such categories provide a background against which the natural history of early and late sequelae may be determined.

The functional sequelae that have been identified from the medical records are as follows:

Deficits	First Status Report		Last Status Report	
	Number of cases	Percent	Number of cases	Percent
Sensorimotor	504	<u>41.3</u>	381	<u>31.2</u>
Special sensory				
visual	420	34.4	383	31.4
Dysphasia	249	<u>20.4</u>	163	<u>13.3</u>
Organic Brain Syndrome	194	<u>15.9</u>	434	<u>35.5</u>
Posttraumatic Epilepsy			396	<u>32.4</u>

The duration of follow-up from injury to last recorded medical examination has been as follows:

<u>Time after injury</u>	<u>Number of Cases followed</u>	<u>Percent</u>	<u>Number of cases lost during year</u>	<u>Number of deaths (N=83)</u>
1 year or less	1221	100.0	213	52
2 years	1008	82.6	277	11
3 years	731	59.9	176	4
4 years	555	45.5	102	2
5 years	453	37.1	90	3
6 years	363	29.7	105	2
7 years	258	21.1	87	3
8 years	171	14.0	181	3
9 years	90	7.3	67	3
10 years	23	1.8	23	0

Comment: It is apparent that 83% have been followed for two years, 37% for five years, and 7% followed for nine years. Therefore, comprehensive evaluation of sequelae from the medical records is limited to development or recession over a two year period. In approximately one third, this period may be extended to five or six years. Caution as to bias is necessary in evaluating those with extended medical coverage.

Reports to date: A report on posttraumatic epilepsy, published in May 1979, indicated that the incidence of seizures has changed very little since World War I; that a concerted prophylactic effort in Vietnam was without demonstrable effect; and that missile and non-missile civilian injuries may be comparable in onset of attacks. A report on Cranioplasties, also published in May 1979, indicated the relationship between morbidity and complications of the initial injury; the need for a delay of at least one year before cranioplasty for optimum results; and the acceptability of acrylic as a prosthetic material.

A report accepted for publication indicates the hazard for delayed complications and delayed neurological deficits in missile injuries crossing the midline. A report submitted for publication deals with the relation between recovery from language and from motor deficits. Reports in preparation concern the effects of retained metallic fragments; craniotomies versus craniectomies for penetrating injuries; and factors that influence survival time in those that have died, respectively.

Current and future activities: Full exploitation of the 1221 cases assembled on tape for analyses of sequelae is underway. The limitations of unequal follow-up from records compiled by diverse examiners are recognized. These can only be remedied by a uniform examination of the subjects by a single team of investigators.

Initial agreements for implementing Phase II: The Surgeons General of the Air Force, Army and Navy have agreed to provide the transport of the head injured veterans by Aeromedical Evacuation System; beds, lab and office space at Walter Reed Army Medical Center; and computed tomography at the National Naval Medical Center, respectively. Operational funds were sought from the Veterans Administration. After a careful review, the Director, Medical Research Service, in July 1979, recommended that the Veterans Administration provide funds for the Protocol Development and depending upon the approval of this planning phase, operational funds for the conduct of the full study.

Significance to Bio-Medical Research and the Program of the Institute: The observations during the acute phase of injury in these cases are more uniform and probably more accurate than any previous series of comparable size. This provides an extraordinary opportunity for studies of prognostic factors and the natural history of disability in central nervous system trauma in man. The recent development of new techniques for evaluating functional deficits, e.g., regarding language and memory and new techniques for determining alterations in brain structure, e.g., Computed Tomography, will afford a unique opportunity in Phase II for fresh insight into posttraumatic sequelae.

This project is consistent with the efforts to improve the understanding and management of the effects of trauma now being sponsored by the Stroke and Trauma Program of NINCDS and the General Trauma Program of the Institute of General Medical Sciences.

Publications:

Caveness, W.F., Meirovsky, A.M., Rish, B.L., Mohr, J.P., Kistler, J. P., Dillon, J. D., and Weiss, G.H.: The nature of posttraumatic epilepsy. J. Neurosurg. 50:545-553, 1979.

Rish, B. L., Dillon, J. D., Meirovsky, A.M., Caveness, W. F., Mohr, J.P., Kistler, J. P., and Weiss, G.H.: Cranioplasty: A review of 1030 cases of penetrating head injuries. Neurosurg. Vol. 4, No. 5: 318-385, 1979.

Meirovsky, A. M., Caveness, W.F., Rish, B.L., Dillon, J.D., Mohr, J.P., Kistler, J. P., and Weiss, G.H.: Definitive care of cerebral missile injuries crossing the midline. J. Milit. Med. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02159-05 LEN						
PERIOD COVERED October 1, 1978 through September 30, 1979								
TITLE OF PROJECT (80 characters or less) Whole Brain Irradiation Within The Therapeutic Range								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 33%;">PI: W. F. Caveness</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">LEN, NINCDS</td> </tr> <tr> <td>OTHER: S. Wakisaka</td> <td>Visiting Associate</td> <td>LEN, NINCDS</td> </tr> </table>			PI: W. F. Caveness	Chief	LEN, NINCDS	OTHER: S. Wakisaka	Visiting Associate	LEN, NINCDS
PI: W. F. Caveness	Chief	LEN, NINCDS						
OTHER: S. Wakisaka	Visiting Associate	LEN, NINCDS						
COOPERATING UNITS (if any) T. L. Kemper, Harvard Medical School D. M. Verrelli, Armed Forces Radiobiology Research Institute								
LAB/BRANCH Laboratory of Experimental Neurology								
SECTION --								
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205								
TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.1	OTHER: 1.2						
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) As a part of a continuing series of studies on the <u>delayed effects</u> of <u>ionizing irradiation</u> on the brain of the monkey, the current effort sequential observations after whole brain exposure to <u>6000 rads</u> of super-voltage radiation, in divided doses over a six-week period. The protocol simulates as closely as possible that used by twelve medical centers in therapy of malignant gliomas in humans. The current phase is primarily concerned with converting the assembled data to manuscript form for publication in scientific journals.								

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02190-04 LEN
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PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Thermal Manipulation Of Paroxysmal Neuronal Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. F. Caveness	Chief	LEN, NINCDS
OTHER:	M. Kato	Visiting Scientist	LEN, NINCDS
	S. Wakisaka	Visiting Associate	LEN, NINCDS
	T. Iguchi	Visiting Associate	LEN, NINCDS

COOPERATING UNITS (if any)
NONE

LAB/BRANCH
Laboratory of Experimental Neurology

SECTION
--

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5
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CHECK APPROPRIATE BOX(ES)

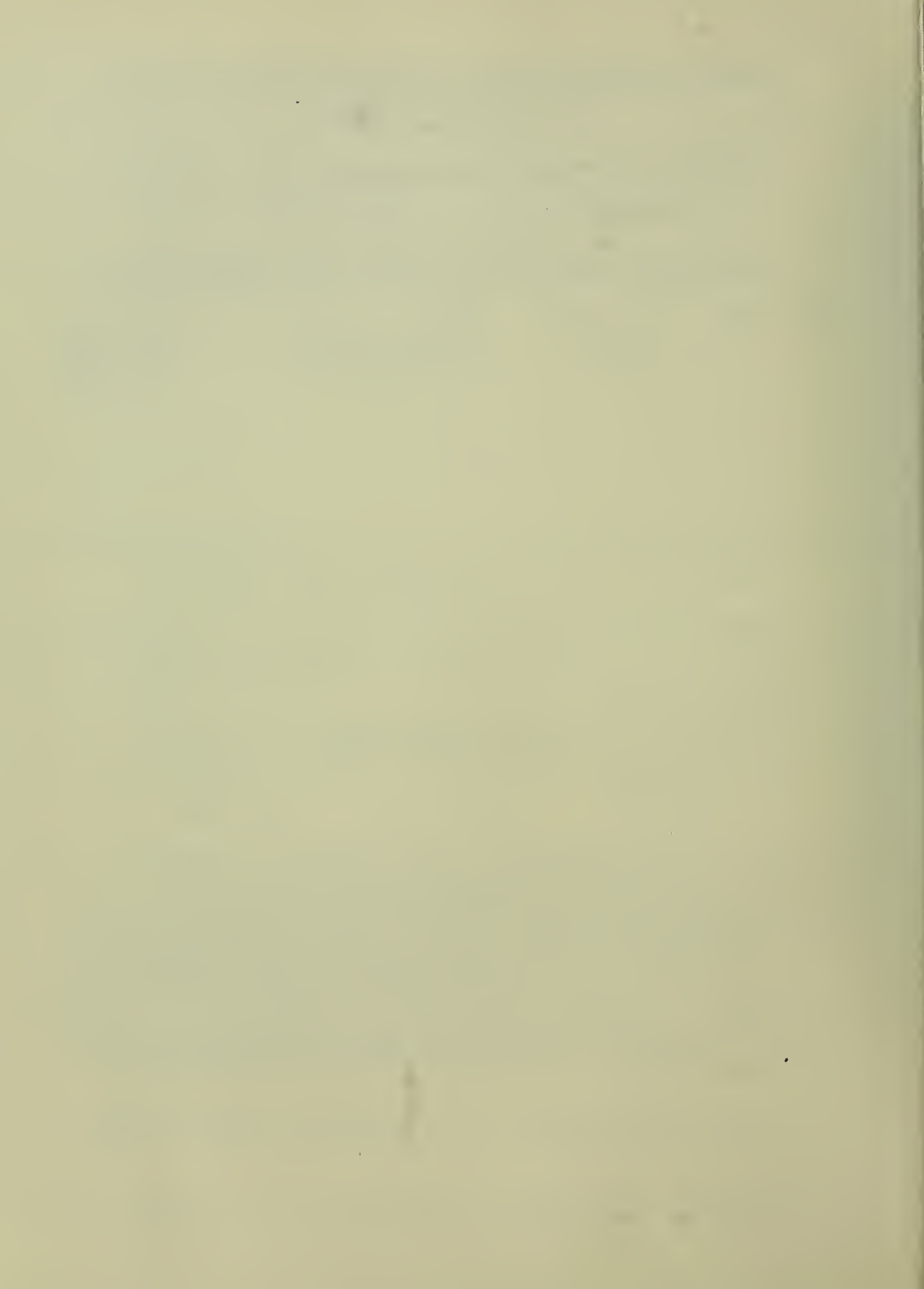
(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

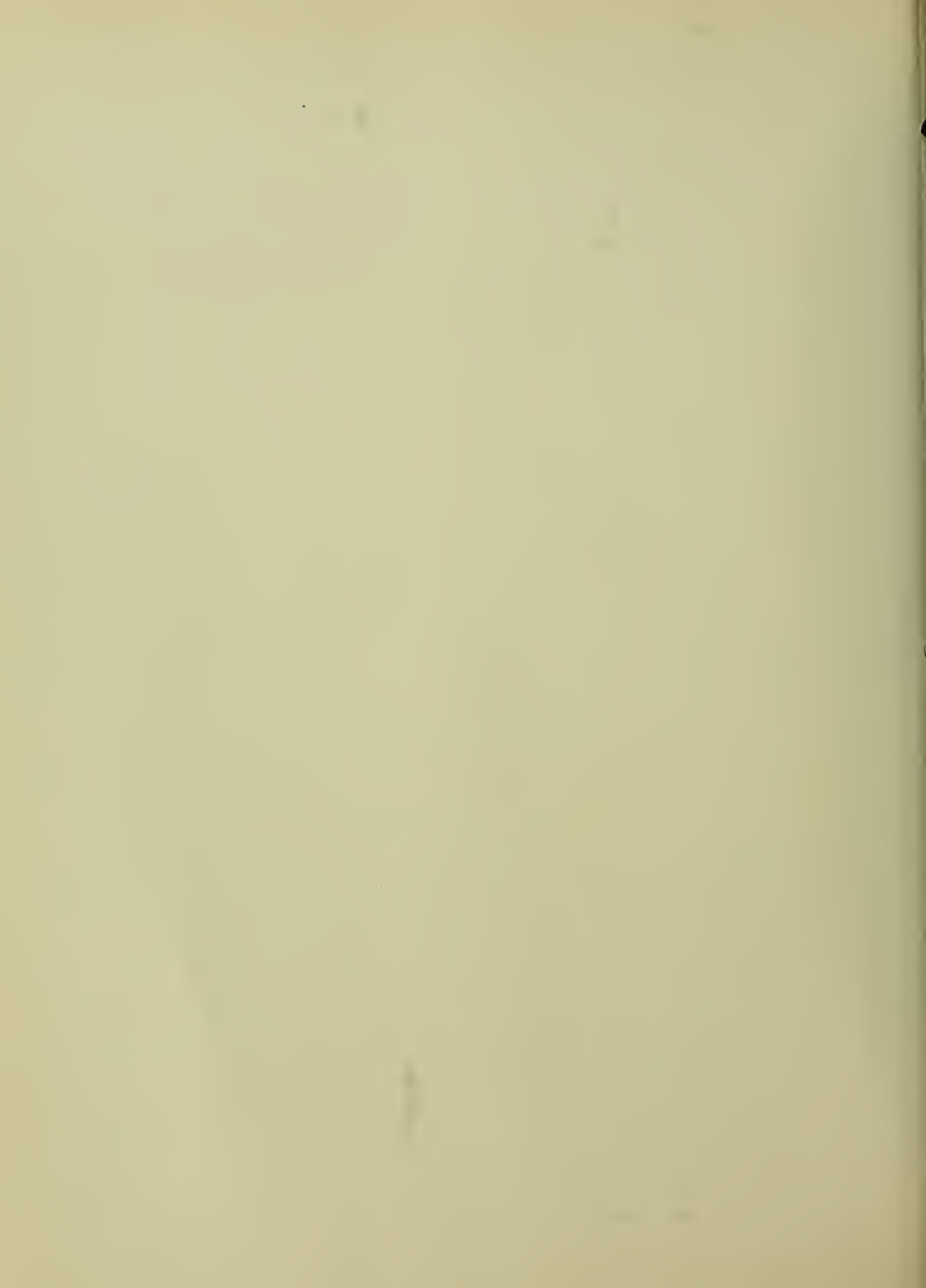
(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the study of subcortical structures that are brought into play with the propagation of paroxysmal activity from a penicillin focus in the motor cortex of the monkey, a question of importance is: In what way are neuronal aggregates essential to the full development of the experimental focal seizure? In seeking an answer, neuronal blockade of structures with previously demonstrated involvement was brought about by a stereotactically controlled cryoprobe. The instrument at hand permits graded cooling at its tip that is 0.1 cm in diameter and monitored by a microthermocouple. By using destructive degrees of cooling in selected subcortical areas, the objective was to temporarily interrupt the progression of the paroxysmal activity, thereby demonstrating the importance of these areas in fit production.

The current phase is primarily concerned with converting the assembled data to manuscript form for publication in scientific journals.





ANNUAL REPORT

October 1, 1978 through September 30, 1979

Laboratory of Neurochemistry

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1978 through September 30, 1979
Laboratory of Neurochemistry, Intramural Research
National Institute of Neurological and Communicative
Disorders and Stroke
Janet V. Passonneau, Chief

The Laboratory of Neurochemistry is composed of three sections, the Section on Cellular Neurochemistry, the Section on Enzymes, and the Section on Neuronal Development and Regeneration. These sections are engaged in a variety of projects.

Section on Cellular Neurochemistry

The Section on Cellular Neurochemistry has continued its efforts to determine the role of energy and cyclic nucleotide metabolism in normal and pathological states of the brain. Investigations have been pursued on the effects of anticonvulsants on both electrically- and chemically-induced seizures on ischemia in the cerebral cortex of the gerbil, on metabolism in the brains of hibernating hamsters and on the metabolism of tumor cells of neural origin. In the past year, a greater emphasis has been placed on a more discrete sampling of brain tissue. These studies include the determination of 1) guanyl nucleotides in retinal layers, 2) cyclic nucleotide and energy metabolites in layers of the cerebellum and cerebral cortex and 3) glucose, ATP and P-creatine in single cells of both the cerebral cortex and cerebellum. A renewed interest in cells grown in culture centers on a rat primary glial cell that can be grown in monolayer.

The effects of experimental seizures on brain metabolism have been investigated in a given region, in layers of that region and finally in single cells of that region. In the first series of experiments, mice were given electroshock (MES) with and without phenytoin pretreatment. In studies on the gross regions of the brain, it appeared that a locus of action for phenytoin was the cerebellum and quantitative histochemical studies on layers of the cerebellum tended to confirm this observation. In the layers, the normal accumulation of cyclic nucleotides which follows MES was essentially obliterated in the presence of phenytoin. In another study, the convulsant, isoniazid, was administered alone and in combination with the anticonvulsant, sodium valproate. A series of metabolites were measured in 5 layers of the cerebral cortex and 4 layers of the cerebellum. Sodium valproate not only prevented the isoniazid-induced seizure, but also blocked the elevation of cyclic GMP and depression of GABA resulting from the administration of isoniazid. In these studies, there did not appear to be a localization of a drug effect to a given brain region or even to a specific layer within a region. In another study on MES-induced

seizures, metabolites were measured in pyramidal cells from the parietal cortex, in purkinje cells from the cerebellar vermis and in adjacent neuropil to both of these cell types. An exciting discovery was that after MES the purkinje cells were less affected in terms of energy depletion than the other cell type or areas. Conceivably, this is a deleterious effect. The preservation of the energy status of the Purkinje cells after MES indicates that the cells are not stimulated to fire. The low levels of inhibitory output of these cells probably would not offset the massive hyperexcitability resulting from the seizure. Therefore, this condition may be permissive to seizure activity. These types of investigations of discrete regions and single neurons indicate that certain metabolic events may be obscured by gross sampling of the tissue.

Rapid fixation of the brain is a necessary prerequisite for the determination of brain metabolites. Routinely, the animals are plunged intact into liquid nitrogen and because respiration ceases at that time, deeper regions of the brain which are exposed to longer periods of hypoxia are not an accurate reflection of metabolic events in vivo. To overcome this problem, in situ brain freezing of conscious animals, a modification of the funnel freezing technique first described by Kerr (1935), has been tried. Spontaneous respiration is maintained for up to 60 sec and cerebral circulation continues to those regions of the brain not yet frozen. The results from this technique suggest that the maintenance of both respiration and circulation during fixation preserved the energy status at all depths of the brain. Examination of the deeper regions of the brain with one minimal fixation artefact can for the first time be examined. A comparison of fixation methods indicated that focused, high power (5.5Kw) microwave irradiation and freezing in situ are the best. However, no commercially available ovens possess the necessary power.

Using in situ fixation, deeper regions of the gerbil brain were examined after 20 minutes of unilateral ischemia and after 30 minutes of recovery following 20 minutes of ischemia. The contralateral side was only minimally affected. On the ipsilateral side, there was a pronounced decrease in ATP and P-Creatine in the cerebral cortex, hippocampus and the caudate-putamen. The loss of high-energy phosphates was not as great in the thalamus and hypothalamus. Of all the regions examined, the restoration of metabolites was only compromised in the thalamus. Use of in situ fixation will now permit us to examine the metabolism of the deeper regions of the brain which have been reported to be selectively vulnerable to ischemia.

Studies are continuing on retinal metabolism using quantitative histochemical techniques. A profile of guanine nucleotides has been obtained in the light exposed and dark adapted retinal layers of the frog. In the dark, cyclic GMP is elevated in the outer segments of the retina. Following 2 minutes of light exposure, the concentration of cyclic GMP increases dramatically in the outer plexiform layer; whereas, in the photoreceptors GTP and cyclic GMP levels decrease with a concomitant

rise in GDP, such changes suggest that guanine nucleotides may play a role in photoreception.

Another project is the study of the metabolic profiles of rat glial astrocytes, transformed astrocytes and other cell lines of neural origin. Of particular interest is one cell line which has relatively low levels of glycogen and an aberrant phosphorylase b which apparently is not stimulated by 5'AMP.

Section on Neuronal Development and Regeneration

The Section on Neuronal Development and Regeneration is re-evaluating the use of nerve allografts (i.e., a graft exchanged between genetically different members of the same species) to aid in the repair of injured peripheral nerve tissue. Previous work has recognized that a nerve allograft evokes an immune reaction by the host and that it is this reaction which prevents or retards host nerve fiber regeneration through the nerve allograft. Studies have therefore been undertaken to find ways to eliminate or reduce the antigenicity of an allograft and to prevent the host from developing an immune reaction to the allograft (i.e., immunosuppression). Since it is well known that the speed with which an allograft is rejected may depend on whether the allogenic cells contain major and minor or only minor transplantation antigens, tissue-typed nerve allografts (i.e., ones that bore major and minor or only minor antigens) were used to determine whether there was any difference in the ability of host nerve fibers to regenerate through typed-nerve-allografts. Inbred, tissue-typed rats were employed and 2 or 4 cm lengths of nerve allograft inserted between the cut ends of an excised segment of the host peroneal nerve. Host nerve fibers were able to functionally regenerate (i.e., grow through the graft and reinnervate denervated muscles) through 2 cm but not 4 cm allografts regardless of whether they contained major and minor or only minor transplantation antigens. Regeneration through a 4 cm nerve allograft could be achieved, however, if the host rat was made immunologically tolerant to donor antigen. By using the tolerant rat model it appeared possible to determine what might happen to host nerve fibers when the allogenic Schwann cells which surrounded and myelinated them were now rejected. The tolerant model might be equated in man to suppressing allograft rejection with drugs, stopping the medication, and allowing sensitized cells to develop. The rejection of allogenic cells in a tolerant rat can be effected by injecting the tolerant rat with lymphoid cells which are sensitized to alloantigen in the allograft. When this was done, allogenic Schwann cell rejection and demyelination occurred in the nerve allograft, but host nerve fibers did not degenerate, or else they degenerated and regenerated since muscle contraction and histological evidence of innervated muscle fibers were observed 3 weeks after allogenic Schwann cell rejection. Earlier time studies will be conducted to determine whether nerve degeneration occurred. In addition, longer time studies will be performed to determine whether innervation persists in axons deprived of myelin and whether host Schwann

cells will migrate into the graft region and remyelinate axons. Preliminary studies with the immunosuppressive drugs cyclophosphamide and antithymocyte serum showed that each drug could inhibit the immune reaction to nerve allografts. Studies with these immunosuppressive drugs are in progress to determine which dosages permit functional nerve regeneration through nerve allografts without producing dangerous side effects (e.g., greater risks of infection) to the host.

Another project is concerned with determining how nerve fibers exert their trophic effect on end-organs. In one study denervated tongue was reinnervated by sensory nerve fibers which do not support taste buds. These non-taste sensory fibers were able to induce bud formation indicating that neurons which normally do not perform a given trophic function can do so when permitted to reinnervate the appropriate end-organ. Studies in the rat with congenital absence of the peroneal nerve demonstrated that the muscle was not deficient since it became innervated when a normal nerve was implanted into it. Central to the issue of trophic nerve function is the concept that the nerve elaborates and transports a neurochemical to the end organ which is required for end organ development and/or maintenance. In tissue culture it was observed that a soluble protein could be extracted from 21-day degenerating nerve, which could maintain differentiated chick muscle, just as a nerve with intact axons. It remains now to be determined which cells (motor neurons or Schwann cells) produce this neurochemical in the normal nerve. Future studies will attempt to devise a way to grow taste buds in culture and find out if the same or different neurochemicals support diverse organs like muscle and taste buds.

Section on Enzyme Chemistry

a) Transient kinetic studies of the $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$. We have extended our studies of the kinetics of phosphorylation and of diphosphorylation of the ATPase. Part of the anomalous " K^+ insensitive phosphorylation previously observed has been explained in terms of the presence of a fraction (up to 20%) of the enzyme present in the form of inside-out vesicles that are only slowly permeable to K^+ .

However a small fraction (less than 5% of the total) of phosphoenzyme is resistant to K^+ and not associated with these vesicles. This may result from either a "dead-end" side reaction or an unknown contaminant protein. Computer simulation of the results of our quenched-flow studies of dephosphorylation kinetics have demonstrated that, in most cases, the amount of K^+ -sensitive phosphoenzyme is sufficient to account for the total rate of ATP hydrolysis.

However, after long pre-incubation with K^+ , the enzyme initially hydrolyses ATP at a rate that appears to be too rapid to occur solely by way of the phosphoenzyme pathway. This suggests that such preincubation may convert the enzyme to a form that hydrolyses ATP directly in the first cycle.

b) Comparative studies of the stoichiometry of ouabain, phosphate and nucleotide binding sites on the $(\text{Na}^+ + \text{K}^+)$ -ATPase. Na^+ transport ATPases from different sources have yielded different ratios of binding sites for ouabain, ATP and phosphorylation. A recent study by P. Jorgensen in Denmark yielded a 1:1:1 ratio for these sites on a purified $(\text{Na}^+ + \text{K}^+)$ -ATPase from rabbit kidney. We have carefully measured the ratio of ouabain to phosphorylation sites in the electric organ ATPase and find a ratio of 1:2. We are currently preparing purified enzyme from several sources to further investigate these differences.

c) Subunit structure of $(\text{Na}^+ + \text{K}^+)$ -ATPases. In addition to the discrepancies from different laboratories with respect to ligand-binding site stoichiometries, there is also uncertainty about the subunit composition of the enzyme. Most purified preparations produce two major bands on SDS-PAGE with about 100 K and 50 K molecular weight, termed α - and β -subunits.

Recently two laboratories have detected a 12K hydrophobic polypeptide that is specifically labelled with photo-activated cardiac glycoside derivatives. We have also detected the presence of "proteolipid" in our enzyme preparations.

In addition we have developed methods for dissociating the ATPase into subunits in the absence of ionic detergents. This permits examination of the subunits by the isoelectric focusing techniques.

These studies have revealed heterogeneity in both α - and β -subunits. This raises the possibility of a more complex structure of the ATPase or the presence of isozymes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE	PROJECT NUMBER
	NOTICE OF INTRAMURAL RESEARCH PROJECT	701 NS 00813-18-LNC

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Enzymological Aspects of Neural Functions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. W. Albers	LNC	NINCDS
	A. Hobbs	LNC	NINCDS
	N. Krishnan	LNC	NINCDS
	S. Chock	LNC	NINCDS
	J. Froehlich	NIA	Baltimore, MD.

COOPERATING UNITS (if any)
Gerontology Research Center (GRC), NIA, Baltimore, MD

LAB/BRANCH
Laboratory of Neurochemistry

SECTION
Enzyme Chemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5.3	PROFESSIONAL: 3.8	OTHER: 1.5
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER
 (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation of the mechanism and structure of the $(Na^+ + K^+)$ -ATPase is proceeding along three lines: (a) determination of the transient kinetics of phosphorylation and dephosphorylation of the enzyme; (b) ligand-binding studies; and (c) subunit structure studies. The transient kinetic studies have shown that the mechanism of hydrolysis proceeds through the phosphorylated intermediate, which in turn produces a conformational transition. The ligand binding studies have shown that the stoichiometry of ouabain-binding sites relative to phosphorylation sites in the Electrophorus electric organ preparation differs from that of the mammalian enzymes and we are investigating the basis for this difference. The subunit studies have developed higher resolution techniques for separating the peptide chains and shown that the purified α - and β -chains are heterogenous by these techniques. We now seek to determine whether this is an indication of complex structure of isozymes.

Project Description:

Objectives: These studies are currently aimed at developing detailed knowledge of the structure of the Na^+ -transport ATPase and of molecular events associated with sodium transport. At present there are three sub-projects with related goals:

- a) transient kinetic studies to elucidate molecular events associated with the active transport process;
- b) ligand-binding studies aimed at determining the number and function of binding sites on the ATPase and possible species and tissue-specific variations;
- c) sub-unit studies directed at determining the number, topography and functions of the individual polypeptide chains that constitute the transport ATPase.

Methods:

- a) The transient kinetic studies employ specialized instrumentation including the rapid-quenching device designed by Dr. J. Froehlich of N.I.A. and stopped-flow photometric and fluorometric devices.
- b) The ligand-binding studies employ, among other methods, a technique developed in this laboratory which employs the miniature ultra centrifuge to measure binding with high precision and sensitivity under equilibrium conditions.
- c) The subunit studies as well as the preceding work depends upon the production of purified $(\text{Na}^+ + \text{K}^+)$ -ATPase from a variety of tissues. New modifications in the preparation of plasma membrane fractions and of solubilized ATPase have been developed in the current year.

Major Findings:

- a) Previous work has shown that the enzyme is phosphorylated by ATP in a reaction that requires Na^+ and Mg^{++} . The phosphorylated enzyme is hydrolysed in a K^+ -dependent step. When this latter reaction was studied by the rapid quenching technique, a fraction of the phosphoenzyme was found to be only slowly hydrolysed after addition of K^+ . We can now explain this largely by the existence of a proportion of the ATPase in membrane vesicles that are only slowly permeable to K^+ . In the presence of a permeant anion and a K^+ ionophore (valinomycin), this slow component is not seen.

The overall results of our transient kinetic studies to date are consistent with the phosphorylation-driven conformational ion pumping mechanism that has been our working hypothesis.

The only exceptional observations that remain are experiments in which ATPase has been incubated for prolonged periods with K^+ as the only ligand. In such cases the initial hydrolysis of ATP is anomalously high relative to the rate of enzyme phosphorylation. These results suggest that a transient direct hydrolysis of ATP without the intervening phosphoenzyme may occur under these conditions. In addition a small (< 5%) fraction of K^+ -insensitive phosphoenzyme is observed which probably represents an impurity.

- b) We have re-investigated the ratio of phosphorylation sites to ouabain-binding sites using more precise methods than previously available. Our new results confirm our earlier findings that in the Electrophorous electric organ ATPase, the ratio is 2:1. Measurements with ATPase preparations from mammalian brain and kidney have given 1:1.

These results are derived not only from different tissues but from different methods of enzyme purification and, in most cases, from different laboratories. We are, therefore, attempting to eliminate these variables. In particular, membranes from mammalian tissues have high levels of other ATPases and a marked tendency to form closed vesicles. This has led to use of ionic detergents and chaotropic salts to eliminate these problems. We have made some progress in developing a single procedure that can be applied to tissues from different sources, to examine the reality of differences in ligand-binding stoichiometry.

In addition, through a contract made with the University of South Alabama, Dept. of Chemistry, we have developed a biologically active para-magnetic derivative of a cardiac glycoside that we expect will be useful in studying the molecular environment of the ouabain binding site.

- c) Sub-unit structure of the $(Na^+ + K^+)$ -ATPases. We have devoted some effort toward developing improved methods for the dissociation of the polypeptide subunits and their quantitative evaluation. We have been able to react the purified ATPase quantitatively with an amino-reagent and to separate the derivatized peptides on polyacrylamide gels. Quantitative scanning of the gels together with the amino acid analysis of each peptide will permit calculation of the number of peptides per enzyme molecule, which is still in some dispute. We hope to apply the same technique to the recently discovered proteolipid that is associated with the ATPase.

The higher resolution of iso-electric focusing has demonstrated that the sub-unit structure is more complex than the $\alpha_2 \beta_2$ structure that is usually assumed. Although we have resolved components of the subunits with different isoelectric points, the correlation of these data with the molecular weights from SDS-PAGE is incomplete.

Proposed Course:

- a) The transient kinetic studies have so far been considered in terms of a monomeric phosphorylation site. This may be approximately true at low ATP levels, but there are data suggesting dimeric interactions at high ATP levels which are, in fact, nearer the physiological range. It will therefore be of interest to attempt to study the mechanism of this interaction, possibly by the use of substrate analogs. These studies may be aided by the comparative and structural studies outlined below.
- b) The ligand-binding studies will be continued in line with our present work. That is, we will redetermine the stoichiometries several different ATPase preparations made under, as nearly as possible, identical conditions. Based upon the results of these studies, we will design experiments to discover the reason for the species and tissue differences. This may relate to the subunit studies.
- c) We plan to determine the basis for the apparent heterogeneity of ATPase subunits: are they caused by adventitious co-purifying proteins, artefactual changes that occur during isolation or real differences within the α - and β -peptide classes. If the latter, does this result from ATPase isozymes or from further complexity in the oligomeric structure? Beyond this we plan to investigate the relation of the associated proteolysed peptides to ATPase activity.

A new aspect will be developed by a post doctoral fellow, L. Amende, looking at the evolutionary relationships of the transport ATPases.

Significance:

The $(\text{Na}^+ + \text{K}^+)$ -ATPase is the enzymatic machinery of active Na^+ transport. This generates the cellular membrane potential and maintains the principal cellular ionic gradients. These form the basis for the nerve action potential, neurotransmitter reuptake, Ca^{++} efflux and numerous other Na^+ -dependent transport functions. The sum of these functions comprise the major metabolic work load of the brain.

A detailed knowledge of the molecular events of the Na^+ active transport system is part of the information necessary to understand brain energy requirements. Little is as yet known about the mechanisms that control the rate of Na^+ transport, which must be matched to the many different processes that are coupled to it.

The structural and binding studies should provide an explanation for some of the functional differences among $(\text{Na}^+ + \text{K}^+)$ -ATPases in different tissues and the physiological regulation of the important process of Na^+ transport.

Publications:

Goldman, S. S. and Albers, R. W.: Cold resistance of the brain during hibernation: changes in the microviscosity of the membrane and associated lipids: *J. Neurochem.* 32: 1139-1142 (1979).

Hobbs, A. S., Brumback, R. A. and Festoff, B. W.: Monovalent cation transport in myotonin dystrophy. *J. Neurol. Sciences* 41: 299-306 (1979).

Swann, A. C. and Albers, R. W.: ($\text{Na}^+ + \text{K}^+$)-Adenosine Triphosphatase of Mammalian Brain: Catalytic and Regulatory K^+ sites distinguishable by selectivity for Li^+ . *J. Biol. Chem.* 254: 4540-4544 (1979).

Albers, R. W. and Krishnan, N.: Application of the Miniature Ultracentrifuge in Receptor Binding Assays. *Analytical Biochemistry* 96 (in press) (1979).

Froehlich, J. P., Albers, R. W. and Hobbs, A. S.: Kinetics of the Ligand-induced Transitions between the E_1 and E_2 Conformations of ($\text{Na}^+ + \text{K}^+$)-ATPase Studied by the Quench-Flow Technique. Proc. of the Second International Conference on the Functions and Properties of ($\text{Na}^+ + \text{K}^+$)-ATPase (in press) (1979).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01586-12-LNC
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Trophic Neuronal Function in the Peripheral Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. A. Zalewski LNC NINCDS

COOPERATING UNITS (if any)

T. H. Oh, Department of Anatomy, University of Maryland

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Neuronal Development and Regeneration

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.6

PROFESSIONAL:

0.2

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine how nerve fibers exert their trophic effect on end-organs. In one study, denervated tongue was reinnervated by sensory nerve fibers which normally do not innervate taste buds. These sensory fibers were able to induce taste bud formation. In muscle, it was found that denervated muscle in the rat with congenital absence of the peroneal nerve could become reinnervated by a normal nerve indicating that an abnormality in the nerve and not muscle is responsible for the neurological defect. In tissue culture it was observed that a soluble protein could be extracted from 21-day degenerating nerve, which like a protein from intact nerve, could maintain muscle cells in culture (joint work with Dr. Oh). Studies with muscle allografts bearing major transplantation antigens indicated that these allografts could survive and become reinnervated in recipient rats rendered immunological tolerant with bone marrow but not lymph node cells.

Project Description

Objective: The development, maintenance and/or regeneration of tissues like taste buds and muscle requires the presence of an intact innervation. In these situations, it is believed that neuron synthesizes and transports a neurochemical(s) to the end organ which, alone, or in combination with nerve impulse activity, regulates the integrity of the end-organ. The purpose of the project is to conduct experiments which elucidate the nature, specificity and plasticity of trophic nerve function. In studies of muscle, the ability of bone marrow cells was investigated as a cell source to induce immunological tolerance to a muscle allograft which contained cells bearing major and minor transplantation antigens. Previously, we showed that donor lymph node cells could induce tolerance to a muscle allograft with minor but not major antigens. Another muscle experiment involved determining whether the nerve or muscle was responsible for the congenital absence of the peroneal nerve that was recently discovered in a mutant rat. It seemed possible that some aspect of trophic nerve function might be deficient in this rat (e.g., lack of muscle receptor for the neurotrophic factor). An initial experiment involved implanting a normal nerve into the denervated tibialis anterior (TA) muscle (denervated due to the absence of the nerve) and determining if this muscle was capable of accepting any innervation. A histological examination was also performed on the spinal cord of the mutant rat to find out if the motoneurons which normally make up the peroneal nerve were absent. A taste bud study sought to demonstrate whether a denervated tongue papilla would give rise to taste buds if it was reinnervated by sensory nerve fibers which normally do not support taste buds.

Methods Employed:

Muscle Study: The extensor digitorum longus (EDL) muscles were exchanged between inbred Lewis (LE) and Brown Norway (BN) rats. These rats were tissue-typed and known to differ from each other in major and minor transplantation antigens. Normal LE and BN rats that were neonatally made immunologically tolerant to donor antigen were used. EDL grafts were removed 21-150 days after transplantation and examined for the presence of any cellular infiltration, muscle fibers and neuromuscular junctions. A branch of the tibial nerve was implanted into the denervated TA muscle in the rat with no peroneal nerve. Three months later this TA muscle was removed and compared to a TA muscle that did not receive a nerve implant.

Taste Bud Study: The vallate papilla of a rat was denervated and the main vagal nerve trunk cut near the clavicle. The proximal end of the cut vagus nerve was joined to the cut distal glossopharyngeal nerve (the nerve that normally innervates taste buds in the vallate papilla) to provide a pathway for regenerating vagal fibers to reach the denervated papilla. Chronically denervated or vagally reinnervated

papillae were examined 90-100 days later with a variety of histochemical stains in order to detect the presence of nerve fibers and taste buds.

Major Findings:

Muscle Study: EDL muscle transplanted between normal LE and BN rats were rejected by 21 days. These muscles were massively infiltrated by mononuclear cells and no muscle or nerve fibers were present in them. On the other hand, LE or BN rats that were tolerant of each other accepted the muscle allograft and muscle fibers with neuromuscular junctions were present. Some foci of mononuclear cells were present around blood vessels in muscle allografts in tolerant rats, but these were not associated with any notable loss of muscle fibers. The denervated TA muscle in the mutant rat became reinnervated after implanting a normal nerve into it. In these muscles, muscle fiber size was near normal, muscle fiber-types were present and neuromuscular junctions could be demonstrated. Only a few, thin, undifferentiated muscle fibers were present in TA muscles not receiving a nerve implant. Histologic examination of the mutant rat's spinal cord did not reveal any gross abnormalities or obvious neuronal cell loss.

Taste Bud Study: Chronically denervated papillae lacked taste buds whereas vagally reinnervated papillae had numerous taste buds. Nerve fibers could always be demonstrated in association with regenerated buds.

Significance:

Muscle Study: The results indicate that bone marrow cells are capable of inducing neonatal tolerance to a muscle allograft when the muscle cells contain major transplantation antigens. This finding differs from that of a nerve allograft with major antigens in which tolerance to nerve can be induced by either lymph node or bone marrow cells. The observation that the TA muscle in the rat with the absent peroneal nerve could become reinnervated shows that TA muscle fibers are not defective in responding to the neurotrophic influence of motoneurons. Studies need to be performed to determine if motoneurons are present or absent in peroneal segments of the spinal cord and if they ever made contact with TA muscle.

Taste Bud Study: The induction and maintenance of taste buds by sensory nerve fibers which normally do not perform a trophic taste function means that the neurotrophic influences of sensory nerves on taste buds is a non-specific one. Moreover, the present results do not resolve the question of whether different sensory neurons normally make a trophic taste factor or whether the tongue tissue causes nerve to newly synthesize the trophic agent.

Proposed Course of Project:

Muscle Study:

- 1) Determine the capability of immunosuppressive drugs to inhibit the rejection of muscle allografts. Drugs will be tested on muscle allografts which bear major or minor transplantation antigens. If successful, treatment with immunosuppressive agents will be stopped to determine if the muscle allograft survives or is now rejected.
- 2) Continue to investigate the mutant rat with the absent peroneal nerve. Since most rats have a unilateral absence of the nerve it would be possible to inject horseradish peroxidase (HRP) into the normally innervated TA muscle and, after retrograde transport of the enzyme, identify the location of peroneal motoneurons in the spinal cord. These labeled, normal peroneal motoneurons would then serve as the standard with which to compare the presence, number and distribution of motoneurons on the contralateral side of the spinal cord in which the peroneal nerve is absent. A similar HRP study of sensory neurons would also prove useful.

Taste Bud Study:

- 1) Attempt to cause taste bud formation in tissue culture. Nerve extracts will be added to already established cultures of tongue tissue to see if a neurochemical is actually present in nerve which causes taste bud formation (experiment to be done in collaboration with Dr. Oh, Dept. of Anatomy, Univ. of Maryland).
- 2) Separate the epithelium and dermis from tongue and skin and recombine them (e.g., skin epithelium and tongue dermis) to determine what role these tissue play in taste bud formation.
- 3) Determine if the central nerve process of sensory neurons, like the peripheral nerve process, can induce taste bud formation.

Publications:

1. Zalewski, A. A.: The distribution of alkaline phosphatase activity in normal and cross-species regenerated rat and mouse taste buds. Anat. Rec. 194: 283-292, 1979.

Project Description:

Objectives: To determine if either pharmacological or physiological alterations of brain metabolism would affect the steady-state levels of cyclic AMP and/or cyclic GMP in vivo.

Methods Employed: Mice were rapidly frozen in liquid nitrogen at the appropriate times following treatment. The brains were removed at -25° , weighed and extracted in perchloric acid. The neutralized PCA extracts were used in all subsequent metabolic measurements. Cyclic nucleotides were measured by the immunoassay method of Steiner.

Maximal electroshock (MES) was applied by corneal electrodes at an intensity of 50mA for a duration of 0.2 sec. The electroshock produces a convulsive response manifested by a) a tonic extensor phase (0-15 sec), b) an intermittent clonic phase (15-30 sec) and c) a postictal depressive phase.

Hamsters were placed in a 4° cold room for approximately 3 months. One group hibernated and another did not; the latter, cold-adapted hamsters, served as controls.

Major Findings: Anoxia. In decapitated mice or in gerbils with bilateral carotid artery ligation, cyclic AMP increased and cyclic GMP decreased in the regions of the brain examined. Restoration of the gerbil blood circulation produced an additional large post-ischemic rise in cyclic AMP (up to 100-fold greater than control) and a 4-fold increase in cyclic GMP in the cerebral cortex.

Studies on the levels of cyclic nucleotides in the layers of the cerebellum during seizures clearly indicated that when the cyclic nucleotides do change, the response was the same in all the layers. However, other labs have shown using immunocytochemistry that there is a localization of the cyclic nucleotides in certain cell types of the cerebellum. Using quantitative histochemistry, the cyclic AMP was uniformly distributed in the layers of the properly fixed cerebella and the reported localization of cyclic AMP to granule and Purkinje cells was probably an effect of ischemia. Following decapitation, the accumulation of cyclic AMP was greater and more prolonged in the granular layer. In contrast, cyclic GMP in properly fixed cerebella exhibited a concentration gradient; high in the molecular layer and low in the white layer. During ischemia, there is a uniform 50% reduction in cyclic GMP in all cerebellar layers. Using immunocytochemistry, cyclic GMP was distributed throughout the cerebellum in low concentrations, but this probably reflects the depleted state of cyclic GMP during ischemia. These studies demonstrate the importance of proper fixation in determining the levels of cyclic nucleotides in vivo.

Fixation. The cyclic nucleotide concentrations have been determined at various depths of the brain using different methods of fixation: 1) plunging animals intact into either liquid nitrogen or isopentane, 2) decapitation and freezing head in liquid nitrogen, 3) in situ fixation with liquid nitrogen and 4) low (1.25kW) and high (5.5kW) power microwave irradiation. For superficial regions of the brain, all methods except decapitation were comparable. In deeper regions, only microwave irradiation and in situ fixation were acceptable. Of particular interest was the very low levels of cyclic AMP using low power microwave irradiation. Although, this normally would indicate very good fixation, every other criteria of proper fixation indicates quite the

opposite. The low levels of cyclic AMP, thus, appear to be an artefact of microwave irradiation and should not be used as a criterion on which to judge the efficacy of fixation.

Seizures. Both cyclic nucleotides increased in both the cerebellum and the cerebral cortex following MES. Cyclic AMP increased 3-fold in the cerebellum to a peak at 10 sec and thereafter decreased toward control; whereas, the cyclic AMP increased 6-fold in the cerebral cortex to a maximal concentration at 30 sec after MES and remained elevated for up to 4 min. Cyclic GMP levels also increased in both regions, but unlike cyclic AMP peaked at 60 sec. The temporal disparity in the 2 cyclic nucleotide responses suggest a separate and distinct role for the two cyclic nucleotides.

Chemically-induced convulsions elicit a somewhat different pattern than that for MES. In studies on cerebellum and cerebral cortex, cyclic AMP did not change during the seizures induced by pentylenetetrazol (PTZ), bicuculline or isoniazid (INH). However, increases in cyclic AMP were observed during seizures in discrete layers of both regions after treatment with INH. Cyclic GMP increased in both regions following INH or PTZ in the preconvulsive state as well as during the convulsion. The MES response can probably be attributed to the supramaximal stimulus used.

Anticonvulsants. All anticonvulsants with the exception of Diamox decreased cerebellar cyclic GMP, but had no effect in the cerebral cortex. Cyclic AMP was not affected in either region. Pretreatment of mice with both anticonvulsant and convulsant prevented not only the seizures but also the convulsant-induced elevation of cerebellar cyclic GMP. A dissociation of seizures and the elevated cyclic GMP was observed after a combination treatment of bicuculline and valproate; cerebellar cyclic GMP was depressed but the mice still convulsed.

Pretreatment of mice with phenytoin prior to electroshock prevented the tonic extension which was replaced by a bilateral clonic movement. Biochemically, the effect of phenytoin was predominantly in the cerebellum; the elevation of cyclic nucleotides were both substantially reduced.

Anesthesia. The effect of a 15 minute exposure to the inhalation anesthetic, Halothane, on the levels of cyclic nucleotides in three regions of the nervous system was investigated. The levels of cerebellar cyclic GMP decreased to less than 10 percent of the control values at a halothane concentration of 1.5 percent or greater. In contrast, at the same doses of halothane the levels of cyclic GMP in the cerebral cortex increased almost 5-fold. In the spinal cord, the levels of cyclic GMP decreased slightly. Over a 4 hour exposure of Halothane anesthesia, the cerebellar cyclic GMP levels remained depressed. In contrast, the elevated cyclic GMP decreased toward control value after the 30 min peak, even though the mice were still anesthetized. The changes in cyclic GMP in both regions mimics the response reported following the treatment with atropine.

Hibernation. Cyclic GMP is essentially depleted in both cerebellar and cerebral regions of the hibernating hamster. During arousal, there is a biphasic increase in cortical cyclic GMP from a body temperature of 4° to 34°. In the cerebellum, the cyclic GMP levels are proportional to the body temperature. Cyclic AMP levels are only slightly depressed in both regions of the hibernating hamster.

Significance to Biomedical Research and the Program of the Institute:
The steady state levels of the cyclic nucleotides are affected by a variety of experimental treatments. Anoxia by decapitation or ligation of the carotid arteries results in a decreased cyclic GMP and an increased cyclic AMP in both cerebellum and cerebral cortex. Since freezing the animals in liquid nitrogen is time-dependent giving rise to a period of anoxia, interpretation of the cyclic nucleotide results should be made with caution recognizing the potential hazards of the fixation artefact. Further, any treatment that potentially could produce an hypoxic component such as deep anesthesia should also be viewed cautiously. For example, the cyclic nucleotide elevation following MES has been attributed by others to be a result of the apnea-induced anoxia which occurs during tonic extension. Since anoxia decreases cyclic GMP, this argument is not valid for cyclic GMP. The cyclic AMP increase could be triggered by anoxia. However, a reduction of the anoxic period by phenytoin had only a minor effect on the cyclic AMP response in the cerebral cortex. Thus, the cyclic nucleotide changes after MES are probably related to the seizure.

The studies with pharmacological agents tend to confirm the MES data. Convulsants increase cyclic GMP in both regions and anticonvulsants decrease cyclic GMP in the cerebellum. The absence of a cyclic AMP response is the only major difference from the MES studies. Cyclic GMP is generally more responsive than is cyclic AMP. Cyclic nucleotide metabolism may be useful not only in understanding the molecular events involved in seizures, but also in the prevention thereof.

Cortical cyclic GMP increases after MES and treatment with convulsants. Why halothane at anesthetic doses produces an effect similar to that observed with convulsants is presently unclear. However, the response is also similar to that for atropine and this effect may be useful in the explanation of certain actions of halothane; effects unrelated to the anesthetic state.

The electrical activity in the brain of the hibernating hamster is essentially undetectable. It is interesting to note that the cyclic AMP levels are 80% of control but cyclic GMP is depleted in hibernating hamsters. The energetic status of the hibernating brain is maintained, so anoxia is not a factor. Therefore, these results are consistent with a relationship between the concentration of cyclic GMP and the level of brain excitability.

Proposed Course of Project: Presently, the primary emphasis on the relationship of cerebellar cyclic GMP to the different types of anticonvulsants and their different anticonvulsive activities (anti-PTZ, anti-MES, anti-bicuculline, etc.). Also, more extensive studies on the seizure-dependent rise of cyclic nucleotides, whether chemically-or electrically-induced, are being undertaken in other regions of the brain and in distinct layers of those regions. To evaluate the significance of the cyclic nucleotide responses in the cerebellum, cyclic nucleotide metabolism will be examined in a mutant mouse which exhibits a complete loss of Purkinje cells in the cerebellum.

Publications: Brooks, B.R., Lust, W.D., Andrews, J.M. and Engel, W.K.:
Decreased spinal cord cGMP in murine (wobbler) spontaneous
lower motor neuron degeneration. Arch. Neurol. 35: 590-591
(1978).

Krzanowski, J.J., Polson, J.B., Anderson, W.H., Lust, W.D. and
Szentivanyi, A.: A simple method for sampling murine pulmonary
and cardiac tissues for analysis of cyclic nucleotide levels.
Naunyn Schmiedeberg's Arch. Pharmacol. 303 #1, 55-62 (1978).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02006-07 LNC
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Regulation of Metabolism in Glioma and Neuroblastoma Cell Lines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.V. Passonneau	Head, Sect. on Cellular Neurochem.	LNC	NINCDS
OTHER:	C.J. Cummins	Staff Fellow	LNC	NINCDS
	W.D. Lust	Research Pharmacologist	LNC	NINCDS

COOPERATING UNITS (if any)
None

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Section on Cellular Neurochemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.3	OTHER: 0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are being carried out on primary cultures of astrocytes and on several transformed glial cell lines in vitro. Primary astrocytes are derived from neonatal rats, and other cell lines were derived from rodents either by viral transformation of primary cultures (TRPG, S22) or from tumors induced in situ and subsequently established in cell culture (C6, B9, B82).

The levels of key metabolites, glucose, lactate, pyruvate, ATP, P-creatine, GABA, glutamate and cyclic nucleotides were measured in cells and medium after refeeding confluent cells with MEM + glucose. Primary astrocytes, HSV-1 transformants derived from primary astrocytes (TRPG) and C6 cells all show a rapid increase in glycogen content after refeeding, while other glial cell lines, B9, B82 and S22 synthesize relatively low levels of glycogen.

The various lines have been examined for a cyclic nucleotide response to beta-adrenergic agonists, purinergic agonists and others. All cell lines examined to date exhibit a response to adrenergic stimulation, but only the primary astrocytes exhibit a response to adenosine or adenosine analogs.

Project Description:

Objectives: To investigate the anaerobic metabolism of glial cells in culture; to determine the effects of viral and chemical transformation on the anaerobic metabolism of glia; and to elucidate the mechanisms controlling glycogen metabolism in glial cells in vitro.

Methods Employed: The cells are grown in plastic dishes using modified Eagle's medium containing 10% fetal calf serum in a humidified atmosphere of 95% air-5% carbon dioxide at 37°. Extracts of the cells are analyzed for glycolytic metabolites, as well as for the cyclic nucleotides and pyridine nucleotides and for enzymes such as glycogen synthetase and phosphorylase. All of these analytical methods have been applied to the measurements in whole brain and are thus easily adaptable for use with cells in culture.

Major Findings: Energy and Anaerobic Metabolism. Primary cultures of astrocytes, S22, C6 and TRPG glial cell lines are similar in the levels of selected metabolites and in the rate of glucose consumption. ATP and P-creatine remain stable for 24 hours after feeding. Glucose levels are transiently high immediately after feeding and the half-time for glucose consumption is 3-4 hours for all cell lines examined. The preponderance of lactate and pyruvate appears in the medium even at short times after feeding, and in all lines examined lactate continues to increase until medium glucose is exhausted. The primary astrocytes show no significant pyruvate re-uptake and pyruvate concentrations in the medium continue to increase in parallel with lactate. In the transformants, significant pyruvate re-uptake occurs, and the levels of pyruvate are depleted after glucose is quantitatively consumed.

Cyclic Nucleotide Metabolism. The basal levels of cyclic AMP and cyclic GMP do not change after refeeding with MEM + glucose. The levels of both cyclic nucleotides in primary and transformed astrocytes increases 50-100 fold after stimulation by beta-adrenergic agonists (either norepinephrine or isoproterenol at 100 µM concentration). Only primary astrocytes show a cyclic nucleotide stimulation by 2-chloroadenosine or adenosine (100 µM). Dopamine, acetylcholine and histamine were without effect.

Glycogen Metabolism. The time course and maximal levels of glycogen synthesis and the time course of catabolism are comparable for primary astrocytes, TRPG and C6. The levels of synthetase and phosphorylase are also comparable. Three cell lines, S22, B9 and B82 have lower steady state levels of glycogen. To date, only the S22 cells have been characterized. The synthetase activities are near those of the permissive cell lines, but the phosphorylase a activity is high and the phosphorylase b is low, indicating a defect in phosphorylase regulation. Glycogenolysis is promoted in permissive cell lines by treatment with 100 µM isoproterenol, but the involvement of cyclic AMP is unexplored as yet.

Significance to Biomedical Research and the Program of the Institute. A study of the regulation of metabolism in the brain is complicated by the presence of several cell types and the inability to determine in which cells metabolic alterations are occurring. Such studies are facilitated by the use

of primary and transformed glial cell lines. Although cells in culture may substantially differ from their counterparts in situ, they offer a first approach to the problems of regulation of metabolism of brain which might be pursued in normal dissociated brain cells.

Since glial tumors are a major oncotype in brain tumors in man, an understanding of the metabolism of transformed glia may elucidate important mechanisms relevant to etiology and treatment. Comparisons of transformant with primary cultures, and the experimental transformation of primary cultures may yield important information on the metabolic conditions necessary for, or consequences of, the transformation process.

Glycogen synthesis occurs in both neurons and glia in the intact nervous system. The basic mechanism of the control of glycogen synthesis and degradation are well worked out, and the use of glial cells deficient in select aspects of glycogen synthesis may give further insight into the control of glycogen synthesis at the cellular level and may provide a model system for the study of glycogen storage diseases at the cellular level.

Proposed Course of Project: Present investigation is continuing into the responsiveness of various glial cell lines to putative agonists of cyclic nucleotide synthesis. Future plans include measurement of agonist binding, measurement of adenylate and guanylate cyclases and characterization of cyclic AMP dependent protein kinase.

The kinase and phosphatase systems which regulate glycogen synthetase and phosphorylase will be examined in detail in the permissive and non-permissive glial cell lines. An attempt will be made to analyze the control of glycogen metabolism by mutagenizing wild type permissive glial lines and selecting out for non-permissive variants.

Publications: none

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02142-05 LNC
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PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemical Changes During both Ischemia and the Recovery Following Ischemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. D. Lust	Research Pharmacologist	LNC NINCDS
Other:	J.V. Passonneau	Head, Sect. on Cellular Neurochem.	LNC NINCDS
	A. Wheaton	Biological Lab Technician (Micro)	LNC NINCDS
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COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Section on Cellular Neurochemistry

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.5

PROFESSIONAL:

1.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINDS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various biochemical parameters were investigated in the gerbil cerebral cortex both during and after occlusion of the common carotid artery. Ischemia produced large changes in the energy metabolites, cyclic nucleotides and certain putative neurotransmitters. There is an initial dramatic change in both energy metabolites and cyclic nucleotides after which the levels remain essentially constant. Recovery from the ischemic insult was marked by an apparent rapid restoration of energy metabolites. The results on the cyclic nucleotides and neurotransmitters seem to indicate that the recovery process is more than a mere reversal of the ischemic-induced events. The cyclic AMP fluctuations during ischemia and recovery were also duplicated in gerbil brain slices. Using in situ fixation, the effects of ischemia were also evaluated in deeper regions of the brain, including the caudate-putamen, thalamus and hypothalamus. Studies on short-term anoxia and recovery were performed and the effects were qualitatively similar to those for ischemia.

Project Description:

Objectives: To determine the alteration in energy metabolites and putative neurotransmitters during unilateral and bilateral ischemia in the gerbil cerebral cortex; and further to monitor the recovery of these metabolites following varying periods of ischemia. To relate the changes in the biochemical parameters to the impairment of function which occurs after ischemia.

Methods Employed: Mongolian gerbils were anesthetized, the common carotid arteries were exposed and looped with suture. As the gerbils emerged from the anesthesia, the artery (ies) was ligated. Those animals which exhibited positive neurological signs were frozen at various times following ligation in liquid nitrogen.

The cerebral cortex was removed at -20° and extracted in perchloric acid. In the unilateral ischemia studies the left hemisphere served as the ischemic side and the right as the control. ATP, P-creatine, glucose, glycogen, glutamate, citrate and GABA were determined enzymically. Cyclic nucleotides were measured by radioimmunoassay.

In the recovery studies, the carotid artery was occluded with an aneurysm clip for the appropriate time and then released. At various times following release, the gerbils were frozen and the brains removed as described above.

In the in vitro studies, the gerbils were decapitated (zero-time of ischemia), the cerebral cortex was removed, and the brain slices were made using a McIlwain tissue chopper. The slices either were fixed with perchloric acid (ischemia) or incubated in phosphate-buffered saline with glucose and a 95% oxygen-5% carbon dioxide atmosphere and then fixed (recovery).

In situ fixation is a modification of the funnel-freezing technique first described by Kerr (1935). The conscious gerbils continued to breath for up to 60 seconds as the brain was being frozen with liquid nitrogen. The advantage of this procedure is that oxygenation of the blood and cerebral circulation were maintained during the fixation process. By minimizing the fixation artefact, deeper regions of the brain could then be sampled.

Major Findings: The investigation of ischemia is split into two groups: 1) unilateral ligation for long-term ischemia studies and 2) bilateral ligation for short-term studies. Although the duration of unilateral ischemia usually exceeded those periods currently thought to be compatible with survival, both approaches contributed to our understanding of the biochemical events which occur during ischemia.

Unilateral Ischemia. By most of the criteria previously established in the decapitated mouse brains, the left cerebral cortex following ligation of the left common carotid artery is, in fact, ischemic. From 1/2 to 6 hours after ligation, the ATP, P-creatine, glycogen and glucose were decreased by 60% or more and remained low for the entire period. The right or control cerebral cortex was essentially the same as a sham-operated control for up to 6 hours.

Cyclic AMP in the ischemic side increased 7-fold to a maximum, at 2 hours and thereafter decreased to control values by 6 hours. GABA increased with time in the ischemic cerebral cortex to a maximum 5-fold greater than control at 6 hours. In addition, dopamine, norepinephrine and serotonin decreased with increasing periods of ligation. The decrease in dopamine and norepine-

phrine was significant within 30 minutes, while that for serotonin was only significant after 3 hours of ischemia.

In the recovery studies, the metabolites were measured at 5 minutes, 1, 5 and 20 hours after either 1 or 3 hours of ischemia. All the metabolites measured were essentially back to control levels by 1 hour of recovery. Cyclic AMP increased an additional 5-fold over the already elevated cyclic AMP levels after 5 minutes of recovery; but was restored to normal values by 1 hour of recirculation.

Bilateral ischemia. The results for bilateral ischemia of 1, 5 and 20 minutes were qualitatively similar to those observed with unilateral ischemia. However, since the first sampling in unilateral ischemia was at 30 minutes after ligation, the biochemical events which occurred in the earlier stages of ischemia were obscured. The levels of ATP, P-creatine, glycogen and glucose were already maximally depressed after 1 minute of bilateral ischemia. Cyclic AMP levels increased 19-fold in the first minute of ischemia and thereafter decreased to a concentration approximately 5-fold greater than control values at 5 minutes of ischemia. Based on both the results from uni- and bilateral ligation, the time course of the cyclic AMP changes is marked by an early large rise, followed by a drop of cyclic AMP to a plateau some 5-fold greater than control which persists for up to 2 hours and finally by a gradual decrease to control levels at 6 hours of ischemia.

Upon recirculation, restoration of P-creatine and glucose after both 1 and 5 minutes of recovery was essentially complete in all groups. In the 5 and 20 minute ischemic group, there was a marked overshoot of the metabolites during recovery. Although the levels of ATP in the 1 and 5 minute groups approached control values during recirculation, restoration was never complete. In the 20 minute ischemic group, the recovery of ATP was severely compromised, but the overshoot of glucose and P-creatine was still evident after 30 minutes of recirculation. All of the gerbils in this group died by 60 minutes of recirculation.

Anoxia. Gerbils exposed to a 100% nitrogen atmosphere exhibited generalized seizures (onset at 7 seconds) and stopped breathing at 31 seconds. After 1 and 2 minutes of anoxia, the gerbils had to be resuscitated for 72 and 142 seconds, respectively. P-creatine, ATP, glucose and cyclic GMP decreased, while cyclic AMP and lactate increased during anoxia. This response is similar to that observed after ischemia; however, it should be noted that the seizure activity may also have contributed to the response. After the 5 minutes of recovery including the time necessary for resuscitation, the metabolites with the exception of glycogen exhibited marked restoration to the control state.

Hypoxia. In preliminary studies, the cerebral metabolites in animals exposed to 5 minutes of 5% oxygen without other treatment were unaffected. Another group of gerbils were submitted to unilateral ligation followed by a period of low oxygen tension (5%). In those animals which did not exhibit neurological signs a subsequent five minute exposure to a 5% oxygen atmosphere induced neurological signs in 67% of the animals. Based on the presence or absence of neurological signs, as well as the time of onset, there appear to be distinct metabolite responses which may be a function of the anoxic or hypoxic insult.

Brain Slices: Brain slices at 1, 2 and 10 min after decapitation were incubated in an oxygenated phosphate-buffered saline for up to 30 min. The recovery of ATP and P-creatine was somewhat faster in the 1 and 2 min groups, but the magnitude of recovery, approximately 50% of the values *in vivo* was about the same in all groups. The large ischemic and post-ischemic rise in cyclic AMP observed *in vivo* could also be demonstrated in the brain slices.

Pharmacology: Based on the mortality at 3 days after 20 min of bilateral ischemia, thiamylal and phenytoin decreased the mortality from 91% to 43 and 73%, respectively. In spite of this protective effect, the metabolic response both during and after ischemia was similar to untreated gerbils. Theophylline, an anti-adenosine compound, enhanced the mortality after a 5 min bilateral ischemic insult. The cyclic AMP increases were reduced. In addition, the recovery of ATP and P-creatine was also compromised.

Regional Response to Ischemia: It has been established that *in situ* freezing of conscious gerbils uniformly fixes the entire brain. In one group, of gerbils, the right common carotid artery was clamped for 20 minutes and in another group, recirculation was permitted for a 30 minute period after 20 minutes of ischemia. Only gerbils which exhibited positive neurological signs of ischemia were used. During ischemia, the loss of ATP and P-creatine was greatest in the cerebral cortex, hippocampus and the caudate-putamen; whereas, in the hypothalamus and thalamus the reduction was not as great. In contrast, the cyclic AMP accumulation was highest in the cerebral cortex, caudate-putamen and hypothalamus, while in the thalamus and hippocampus the increases were only 50% of that in the other regions. Thus, there does appear to be a regional variation in the response to an ischemic episode. The ability of the tissue to restore the metabolite concentrations to control levels also exhibits regional variation; recovery is compromised most in the thalamus.

Significance to Biomedical Research and the Program of the Institute: The gerbil model for the investigation of ischemia is quite useful for evaluating the role of energy metabolism and cyclic nucleotides in irreversible brain damage. Because a substantial fraction of the gerbils have an anomaly of the arterial circulation in the brain, these animals can be rendered ischemic by carotid ligation. The size of the gerbil permits relatively rapid fixation of the brain in liquid nitrogen for the investigation of brain metabolites. In both unilateral and bilateral ischemia, there is a large derangement of energy metabolites, cyclic nucleotides and certain putative neurotransmitters. Only in the case of the neurotransmitters is the change in concentration proportional to the ischemic duration. After the initial rapid changes in energy metabolites and cyclic nucleotides, the concentrations remain essentially constant. Perhaps the best indicator of the extent of ischemia is the rate of recovery of the energy metabolites. In fact, the restoration process may be an important factor to understanding critical events which lead to the loss of function.

The large changes in cyclic nucleotides probably reflect the severe biochemical perturbations which occur in the brain both during and after ischemia. It is quite evident that certain events must occur in the ischemic cerebral cortex between 1 and 5 minutes of ischemia which permit the large post-ischemic accumulation of cyclic AMP observed at 5 minutes of recirculation after 5 but not 1 minute of ischemia. The current concept is that certain adenylate cyclase agonists are being liberated during the first five minutes of ischemia,

but that their expression is not manifested (in terms of cyclic AMP synthesis) because of the low levels of the substrate for adenylate cyclase, ATP. Upon recirculation, the ATP levels are rapidly regenerated and in the presence of the available agonists a burst of cyclic AMP production ensues. Thus, a proper temporal relationship between the loss of ATP and the liberation of adenylate cyclase agonists is critical to the cyclic AMP accumulation during recirculation. Cyclic GMP levels decrease during ischemia and remain low during recirculation except for 1 and 5 minutes of recirculation when there is a 4-fold increase. Based on electrophysiological results from other laboratories, it would appear that neuronal excitability is reduced by cyclic AMP and increased by cyclic GMP. Therefore, the elevated cyclic AMP levels and the reduced cyclic GMP levels during and after ischemia would collectively have an inhibitory influence on cortical excitability.

Proposed Course of Project: The major emphasis is presently on the biochemical characteristics of recovery. In addition, the molecular events involved in the enormous increase in cyclic AMP in the post-ischemic period will be investigated. Similar studies on hypoxia of various degrees are planned.

Publications: Murakami, N., Lust, W.D., Wheaton, A.B., and Passonneau, J.V.: Short-term unilateral ischemia in gerbils: a reevaluation. In Mrsulja, B.B., Rakic, L.M., Klatzo, I. (Eds): Pathophysiology of Cerebral Energy Metabolism. Plenum Press, 1979, pp. 33-46.

Lust, W.D., and Passonneau, J.V.: Cyclic nucleotide levels in brain during ischemia and recirculation. In Palmer, G. (Ed): Neuropharmacology of Cyclic Nucleotides. Urban & Schwarzenberg, 1979, pp. 229-253.

Passonneau, J.V., Lust, W.D., and McCandless, D.W.: The preparation and analysis of biological samples for the measurement of metabolites. Techniques in Life Sciences. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	J.C. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02254-03 LNC
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) The Use of Neurological Grafts to Repair the Injured Peripheral or Central Nervous System		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: A. A. Zalewski LNC NINCDS		
COOPERATING UNITS (if any) W. K. Silvers, Department of Human Genetics, University of Pennsylvania		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION Neuronal Development and Regeneration		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.4	PROFESSIONAL: 0.8	OTHER: 1.6
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Peripheral nerve allografts were inserted between the cut ends of nerve to determine whether host nerve fibers would regenerate through the <u>nerve graft</u> and reinnervate <u>denervated muscle</u> . Host nerve fibers were found to functionally regenerate through 2 cm but not 4 cm nerve allografts regardless of whether the nerve allograft bore major and minor or only minor transplantation antigens. Functional nerve regeneration did occur through a 4 cm allograft if the recipient was made <u>immunologically tolerant</u> to donor transplantation antigen. Preliminary studies have indicated that <u>cyclophosphamide</u> or <u>antithymocyte serum</u> could prevent the rejection of a nerve allograft but appropriate doses of the drugs which would not produce toxic side effects and yet permit functional reinnervation have to be determined.		

Project Description:

Objective: The purpose of this project is to determine the extent to which a peripheral nerve allograft (a graft between genetically different members of the same species) can be used to aid in the repair of injured nerve tissue. In the present study we have investigated whether host nerve fibers would regenerate through a nerve allograft and reinnervate denervated muscles. Previous work has recognized that transplantation antigens of the cells in a nerve allograft evoke an immune reaction by the host and that this immune response may prevent host nerve fiber regeneration. Earlier studies, however, did not take into consideration the fact that the speed with which an allograft is rejected may depend on whether the allograft contains major and minor or only minor transplantation antigens. The type antigens present on an allograft can be determined by tissue-typing and, knowing this fact is important, since an allograft with major antigens is rejected rapidly whereas an allograft with only minor antigens may survive longer. In the situation with nerve, longer graft survival might allow host nerve fibers to regenerate through a nerve allograft before rejection occurs. In the present study nerve allografts bearing major or minor antigens were compared in normal (nonimmunosuppressed) rats and in rats rendered immunologically tolerant to donor transplantation antigens. In addition, an attempt was made to reduce the antigenicity of a nerve allograft by removing cells (i.e., sheath cells of the epineurium and perineurium) and preliminary studies using the immunosuppressive drugs cyclophosphamide and antithymocyte serum were conducted.

Methods Employed: Inbred Brown-Norway (BN), Lewis (LE) and Fischer (FR) rats were used. The LE and FR rats differ only in minor antigens whereas BN rats differ from the LE and FR rats in major and minor transplantation antigens. FR rats served as recipients of nerve allografts; normal FR rats received a 2 or 4 cm FR, LE, or BN nerve allograft while similar lengths of nerve allografts were placed into FR rats rendered neonatally tolerant to LE antigens. Injury to nerve tissue which resulted in the denervation of the extensor digitorum longus (EDL) and tibialis anterior (TA) muscles was produced by removing a segment of the peroneal nerve from the leg of the FR rat and replacing it with a 2 or 4 cm FR, LE, or BN nerve graft. A variety of histological stains were performed on frozen sections to evaluate the nerve grafts and muscles. In other studies, the sheaths (epi- and perineurium) were stripped from 2 and 4 cm LE or BN nerve grafts that were subsequently grafted in FR recipients. Since all cells contain transplantation antigens the intent of this experiment was to reduce the volume of graft antigen to see if this delayed the consequences of any immune reaction to the graft thereby permitting host nerve fiber regeneration to occur through a greater length of nerve graft. Preliminary studies with cyclophosphamide (a drug immunosuppressive of B and T lymphocytes) and antithymocyte serum (a drug with antibodies cytotoxic to T lymphocytes) were undertaken to determine the capability

of these drugs to prevent the immune response to nerve allografts.

Major Findings: FR nerve fibers were able to regenerate through 2 cm nerve allografts and reinnervate denervated muscle regardless of whether they contained major and minor or only minor transplantation antigens. No FR nerve fiber regeneration occurred, however, through 4 cm nerve allografts and muscles associated with these grafts resembled the chronically denervated muscles in rats in which no nerve graft was inserted between the cut ends of the excised peroneal nerve. On the other hand, FR nerve fiber regeneration through a 4 cm nerve allograft could be achieved if the FR rat was made immunologically tolerant to donor antigen. Only FR rats made tolerant to the minor antigens of LE rats were studied, and these tolerant rats showed nerve fibers throughout the length of the 4 cm LE nerve graft and reinnervated muscle comparable to that seen when a 4 cm nonantigenic FR nerve graft was used. It is of interest that the EDL muscle was 80-90% the weight of the contralateral normal EDL whereas the TA muscle was consistently only 50-75% of normal. Removing the nerve sheath cells from 4 cm BN or LE nerve grafts did not permit FR nerve fiber regeneration through them. Short-term studies (3-4 weeks) with the drugs cyclophosphamide and antithymocyte serum demonstrated that both drugs prevented the immune response from developing. Stopping the drugs after short-term immunosuppression did not allow regeneration through 4 cm nerve allografts. The lack of regeneration was not due to drug inhibition of nerve fiber growth as FR rats grew fibers through crushed nerves while receiving the immunosuppressive drugs.

Significance:

The present results demonstrate that in a normal rat a 2 cm nerve allograft with major or minor transplantation antigens permits host nerve fiber regeneration through it whereas a 4 cm allograft does not. The failure of a 4 cm allograft was undoubtedly due to an immune reaction since regeneration did occur through a 4 cm LE nerve graft when it was transplanted into an FR rat tolerant of LE antigen. This finding indicates that there is no incompatibility between cells of the LE nerve and FR nerve fibers in permitting FR nerve fibers to grow through long distances of a foreign nerve. It is interesting that after injecting FR lymphocytes, which were sensitized to LE antigen, into normal or tolerant FR rats which had functional 2 cm LE nerve grafts (i.e., nerve fibers were present in the nerve graft and muscles were reinnervated) no immune reaction was seen in nerve grafts in normal FR rats while extensive mononuclear cell infiltration and demyelination was observed in the LE graft of tolerant rats. The observation means that in the normal (i.e., non-tolerant) FR rat the LE Schwann cells in the nerve graft survived long enough to permit regeneration and then they were rejected and replaced by FR Schwann cells which then myelinated the FR axons. More importantly, however, is the fact that when LE Schwann cells were rejected in tolerant FR rats no massive FR nerve degeneration or signs of muscle denervation occurred. This finding coupled with the

observation that the immunosuppressive drugs could inhibit nerve allograft rejection might indicate that immunosuppressive drugs need only be given until host nerve fibers grow through the nerve allograft. The data therefore indicates that nerve allograft immunosuppression would not require the indefinite use of drugs so that complications of chronic immunosuppression (e.g., infection) might be avoided. The discovery that removing nerve sheath cells did not delay immunological rejection sufficiently longer to permit regeneration through 4 cm nerve allografts means that other cells in the allograft (i.e., nerve, Schwann, blood cells, blood vessel cells, fibroblasts, etc.) possess potent antigens. Nevertheless, it seems worthwhile to pursue other ways of eliminating antigen from allogenic tissue (e.g., irradiation, temporary tissue culture) since this may allow the use of reduced amounts of immunosuppressive drugs to accomplish regeneration over greater lengths of a nerve allograft.

Proposed Course of Project:

- 1) Repeat the tolerant study but this time with rats tolerant to major transplantation antigens. Use longer lengths of nerve allograft and after breaking-tolerance (i.e., injecting sensitized cells) look for an immune response, demyelination, nerve degeneration, and signs of muscle denervation. Most importantly nerve allografts will be examined 3-4 months after breaking-tolerance to determine (a) if nerve regeneration ensues should nerve degeneration occur earlier, (b) if host Schwann cells will migrate into the graft after foreign Schwann-cell rejection and demyelination occurs and remyelinate axons, and (c) if muscle function is altered or restored.
- 2) Begin long-term immunosuppressive studies with cyclophosphamide, antithymocyte serum, and if available the new immunosuppressive-cyclosporin. Nerve allografts with major or minor antigens will be used since the drugs may have different success depending on the potency of the antigen being immunosuppressed.
- 3) Determine if altering allograft antigenicity alone or in combination with immunosuppressive drugs allows regeneration over longer distances.
- 4) Investigate the potency of γ -chromosome determined transplantation antigens to determine if male nerve allografts behave differently than female nerve allografts after transplantation to female recipients.
- 5) Determine whether any regeneration occurs in a recipient previously sensitized to antigens in a nerve allograft.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02255-03-LNC
PERIOD COVERED October 1, 1978 to September 30, 1979			
TITLE OF PROJECT (80 characters or less) GABA and Nucleotide Metabolism in Brain Mitochondria			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R. W. Albers LNC NINCDS			
COOPERATING UNITS (if any) None			
LAB/BRANCH Laboratory of Neurochemistry			
SECTION Enzyme Chemistry			
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER:	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) As reported last year, the co-principal investigator, Dr. Lopes-Cardozo, returned to Utrecht in June of 1978 and the project has been suspended except for the preparation of data for publication.			

Publications:

Lopes-Cardozo, M. and Albers, R. W.: Relationship between the 4-Aminobutyrate Bypath and the Oxidation of 2-Oxoglutarate in Rat Brain Mitochondria. J. Neurochem. (in press) 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02256-03 LNC															
PERIOD COVERED October 1, 1978 to September 30, 1979																	
TITLE OF PROJECT (80 characters or less) Metabolic Profiles in Normal and Diseased Retina																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">J.V. Passonneau</td> <td style="width: 30%;">Head, Sect. on Cellular Neurochem.</td> <td style="width: 10%;">LNC</td> <td style="width: 10%;">NINCDs</td> </tr> <tr> <td>OTHER:</td> <td>W.D. Lust</td> <td>Research Pharmacologist</td> <td>LNC</td> <td>NINCDs</td> </tr> <tr> <td></td> <td>F. de Azeredo</td> <td>Visiting Fellow</td> <td>LNC</td> <td>NINCDs</td> </tr> </table>			PI:	J.V. Passonneau	Head, Sect. on Cellular Neurochem.	LNC	NINCDs	OTHER:	W.D. Lust	Research Pharmacologist	LNC	NINCDs		F. de Azeredo	Visiting Fellow	LNC	NINCDs
PI:	J.V. Passonneau	Head, Sect. on Cellular Neurochem.	LNC	NINCDs													
OTHER:	W.D. Lust	Research Pharmacologist	LNC	NINCDs													
	F. de Azeredo	Visiting Fellow	LNC	NINCDs													
COOPERATING UNITS (if any) None																	
LAB/BRANCH Laboratory of Neurochemistry																	
SECTION Section of Cellular Neurochemistry																	
INSTITUTE AND LOCATION NINCDs, NIH, Bethesda, Maryland 20205																	
TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0.0															
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) <p>Studies are in progress on <u>retinal metabolism</u> in whole retinas and frozen-dried sections of retinal layers. The effects of "<u>spreading depression</u>" on <u>high-energy phosphate compounds</u> and <u>cyclic nucleotides</u> in being investigated; ATP, P-creatine, cyclic GMP, and cyclic AMP have been measured in the whole chick retina after stimulation. Preliminary studies on <u>frog retina</u> of <u>tricarboxylic cycle intermediates</u>, <u>glycolytic intermediates</u>, <u>cyclic nucleotides</u> and <u>high-energy phosphate compounds</u> have been made. New methods for the measurement of the <u>guanine nucleotides</u>, GTP, GDP and $5'GMP$ have been developed (10^{-12} mole sensitivity).</p>																	

Project Description:

Objectives: To determine the alterations in metabolism of the retina during "spreading depression". To determine the distribution of metabolites, putative transmitters, cyclic nucleotides in the layers of frozen-dried frog retina in normal animals and animals treated with drugs to destroy the rod outer segments. To study retina metabolism during light and dark adaptation, and metabolic changes during the shedding process of the rod outer segments. Future plans include the study of diabetic retinopathy, and ischemic injury to the retina using the gerbil model.

Methods Employed: In the case of the whole retina, the chick is decapitated, the eyes are removed and cut in half. After incubation at 35-37^o, the retina can be removed intact with a pair of forceps. The retinas are transferred to teflon racks in which depressions are drilled for experimental chambers. The retina is stimulated by touching with a fine glass filament to cause "spreading depression". At appropriate intervals the retinas are frozen in liquid nitrogen, extracted with perchloric acid, and the extracts stored until analyzed.

For the studies on retinal layers, frogs are decapitated into liquid nitrogen, the heads frozen and stored at -70^o until dissection. The eyes are dissected at -20^o and the whole eyeball removed. The eyeball is mounted on a specially designed holder, and sectioned at 10 μ m at -25^o in a cryostat. The sections are vacuum-dried at -40^o, after which they can be stored at -20^o under vacuum and for microdissection, the samples are brought to room temperature under vacuum, removed, and the retinal layers (approx 0.1 μ g) dissected with micro instruments using a low-power dissecting scope. The micro-chemical analyses are performed in oil wells using volumes of 0.05 to 0.5 μ l. Eyes from gerbils made ischemic by ligation of the carotid arteries will also be investigated. The animals will be frozen intact, in liquid nitrogen. Subsequent procedures are like that for the frog.

Major Findings: In the studies of spreading depression, ATP, and P-creatine have been found to decrease in tandem, falling to 66% of control levels 30 sec after stimulation, and reaching 50% at 3 min.

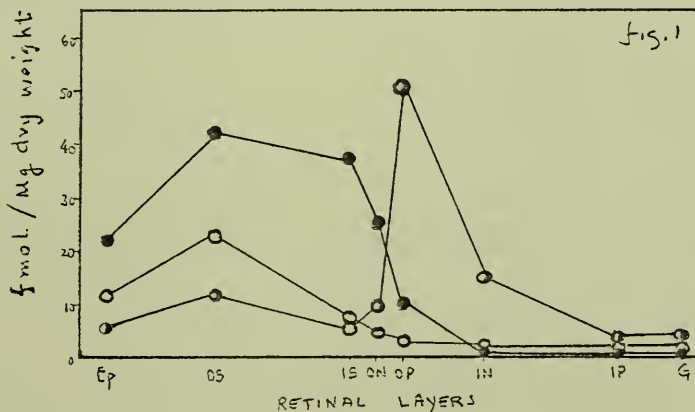
Both cyclic AMP and cyclic GMP are depressed after stimulation, the first decrease seen at 2 min after stimulation (to 66% of cAMP and 50% of cyclic GMP control values). At the latest times studied (3 min); the cyclic nucleotides are still decreasing, although slowly.

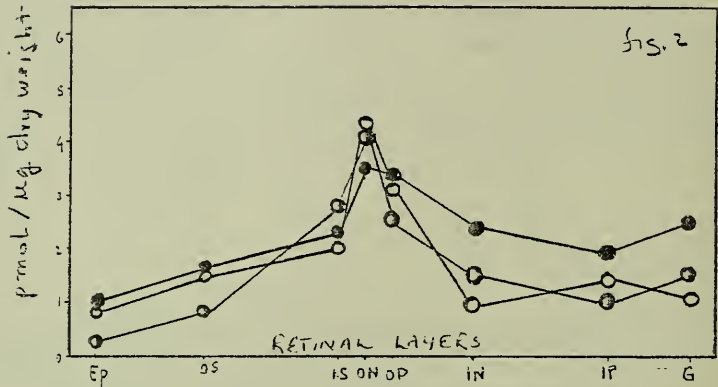
GTP, GDP and cyclic GMP were measured in the frog retinal layers and in the pigment epithelium in the following groups: dark-adapted (●), 2 min light-adapted (⊙) and 2 hours light-adapted (○). The following layers were analyzed: Ep-pigment epithelium, OS-outer segments, IS-inner segments, OP-outer plexiform, ON-outer nuclear, IP-inner plexiform, IN-inner nuclear and G-ganglion cells.

After development of methods for measurement of GTP and GDP concentrations in tissues, several tissues were analyzed for representative values.

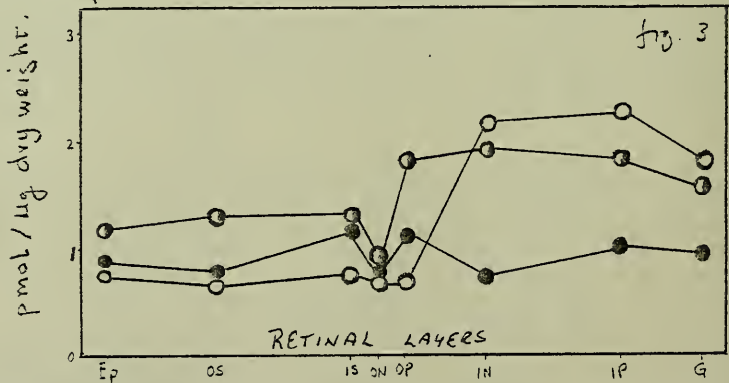
	GTP (nmoles/mg protein)	GDP (nmoles/mg protein)
Mouse		
Cortex (12)	9.84 ± 0.78	2.20 ± 0.26
Muscle (11)	6.18 ± 0.39	2.20 ± 0.25
Liver (11)	6.63 ± 0.44	2.51 ± 0.18
Heart (12)	6.59 ± 0.52	1.37 ± 0.17
Chick retina (10)	7.60 ± 0.26	1.56 ± 0.23

Cyclic GMP is predominant in photoreceptors of *in vivo* dark- and 2 hrs light-adapted frogs (Fig. 1); after 2 minutes of light exposure a transient increase in the cyclic GMP was detected in the neural retina. Specifically, the increase was observed in the inner nuclear layer and in the synaptic contacts between photoreceptors and inner nuclear cells.





The profile of the GTP concentrations within the retinal layers in the three different groups shows its peak in the outer nuclear layer (Fig. 2). Decreased GTP concentrations and a consequent increase in GDP levels (Fig. 3) were observed after exposure to light. These changes were sustained in the neural retina and were transient in the photoreceptor layers.



Significance to Biomedical Research and the Program of the Institute: Because of the layered structure of the retina and the concentration of mitochondria in the inner segment, the question of sequestration of certain metabolites of the citric acid cycle in these organelles may be approached. It is an ideal place to study whether ADP, for example, is limited exclusively to the mitochondria.

The location of key metabolites such as ATP, ADP, 5'AMP and citrate will help elucidate metabolic controls.

The function of cyclic GMP in the retina is not yet understood. It is as high as 600 pmoles/mg protein in the rod outer segments (100 x greater than the tissue with the next highest concentration, the cerebellum). The amount of cyclic GMP is increased in dark-adapted retinas as much as three-fold. Most of this work has been done in isolated rod outer segments. Our techniques provide a unique possibility to study the in vivo effects of light, to compare normal retinas and those in which the rods have been destroyed by drugs and/or congenital retinopathies. Such investigations may help elucidate the mechanism of photoactivation of the retina, and the biochemical pathology of retinopathies.

Proposed Course of Project: The preliminary work in retinal layers will be developed further. Both normal and diseased retinas will be studied in an attempt to learn the biochemical nature of retinopathies.

Publications: de Azeredo, F.A.M., Lust, W.D., and Passonneau, J.V.: Guanine nucleotide concentrations in vivo in outer segments of dark and light adapted frog retina. Biochemical and Biophysical Research Communications. 85 (1): 293-300, 1978.

de Azeredo, F.A.M., Feussner, G.K., Lust, W.D., and Passonneau, J.V.: An enzymatic method for the measurement of GTP and GDP in biological samples. Analyt. Biochem. 95 (2): 512-519, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02257-03 LNC
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Biochemistry of Experimental Seizures		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	W.D. Lust	Research Pharmacologist LNC NINCDS
OTHER:	J.V. Passonneau	Head, Sect. on Cellular Neurochem. LNC NINCDS
	Gretchen Feussner	Chemist LNC NINCDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION Section of Cellular Neurochemistry		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.5	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) A study is being made of chemically- or electrically-induced seizure activity in mouse brain. Work to date has indicated that elevated <u>cyclic GMP</u> concentrations in the <u>cerebellum</u> are associated with many types of seizures, and that when convulsions are suppressed by drugs, cyclic GMP is decreased. In addition, increased <u>GABA</u> concentrations may be involved in <u>anticonvulsive action</u> . Studies on <u>whole</u> mouse brain indicate that a major locus of action of <u>phenytoin</u> is in the cerebellum. <u>Microdissection</u> and <u>microanalytical procedures</u> have been used to analyze both <u>cerebellar</u> and <u>cortical layers</u> after <u>electroshock</u> and <u>isoniazid</u> induced seizures, and in the presence of <u>anti-convulsants</u> . In addition, <u>Purkinje cells</u> from the cerebellum and <u>Pyramidal cells</u> from the cerebral cortex were analyzed following electroshock.		

Project Description:

Objectives: To determine the effects of convulsant agents, including electroshock and isoniazid, on brain metabolism. The mechanism and/or site of action of these agents as well as anticonvulsants will be investigated.

Methods Employed: Mice are rapidly frozen in liquid nitrogen at appropriate times following treatment. The brains are removed at -25° and homogenates are made in perchloric acid for samples for direct analysis. The extracts are centrifuged, the supernatant fluid removed and neutralized and used for analyses. For microanalysis, the frozen tissue is sectioned at $20\ \mu\text{m}$, dried under vacuum at -45° , and dissected into microgram sections for analyses. The analyses are carried out in oil wells with the enzymatic cycling technique (Lowry and Passonneau, A Flexible System of Enzymatic Analysis, Academic Press, .Y., 1972).

Maximal electroshock (MES) was applied to the mice by corneal electrodes at an intensity of 50 mA for 0.2 sec. The massive shock produces a reproducible response characterized by a) a tonic extensor phase (0-15 sec) b) an intermittent clonic phase (15-30 sec), and c) a postictal depressive phase (> 30 sec).

In a separate series, isoniazid was administered in isotonic saline at a dose of 200 mg/kg SC, and mice were frozen at 30 minutes after injection, or at onset of seizures (40 ± 3 min). There was no evidence of hyperexcitability in the isoniazid-treated mice frozen at 30 minutes. The anticonvulsants phenytoin and sodium valproate were administered at doses of 25 mg/kg IP, and 400 mg/kg IP, respectively. Phenytoin pretreated mice were then subjected to MES and sacrificed at the appropriate time intervals, whereas sodium valproate was administered to isoniazid-treated mice.

Major Findings: As found by others, cyclic GMP concentrations increase in certain regions of the brain following administration of stimulants and decrease following administration of depressant drugs. In addition, both cyclic AMP and cyclic GMP increase in the cerebellum and cerebral cortex during or after electrically induced seizures. The apparent association between cyclic nucleotides and neuronal excitability is supported by physiological investigations. We have found that anticonvulsive drugs generally suppress cyclic GMP in the cerebellum, and prevent the changes in metabolites of the cerebellum induced by the convulsants. These results support the concept that the cerebellum is involved in the control of seizures. Furthermore, phenytoin inhibits the metabolite and cyclic GMP changes caused by electroshock to a greater extent in the cerebellum than in the cerebral cortex.

Of nine anticonvulsants, all but one (acetazolamide) decreased cyclic GMP in the cerebellum but not in the cerebral cortex. Two convulsant drugs tested, metrazol and isoniazid, elevated cyclic GMP in the cerebellum, and this increase was prevented by clonazepam.

MES produced marked changes in cyclic GMP, cyclic AMP, lactate, and 5'AMP in both the cerebellum and cortex. When phenytoin was given prior to MES these increases were diminished in both areas, but the predominant effect was in the cerebellum. The evidence suggests there may be a phenytoin specific locus in the pathways to the cerebellum.

We have measured the concentrations of ATP, P-creatine, lactate, glucose, glycogen, cyclic AMP and cyclic GMP in four layers of the cerebellum: molecular, Purkinje-cell-rich, granular, and white. The effects of MES have been investigated. In general, the white layer shows fewer and lesser changes; the other layers are similar. We found a significant decrease in ATP, PCr, glucose and glycogen in all 4 layers. These decreases were most significant during the excitable phase, and tended to return to normal by 10 minutes after the shock. Lactate, cyclic AMP, and cyclic GMP increased during the excitable phase following MES, and the increase in cyclic AMP temporally preceded the increase in cyclic GMP. Values had returned toward normal by 10 minutes. Pretreatment of the mice with the anticonvulsant phenytoin resulted in a bilateral clonic seizure only. In general, phenytoin pretreatment of mice resulted in a diminished response of metabolites to MES. The greatest effect was seen on the cyclic nucleotides, which were only slightly elevated as compared to values from mice receiving MES.

The convulsant isoniazid was used to produce seizures in order to study the pre-seizure state, which is not possible using MES. In this study, in addition to the 4 cerebellar layers, we examined the following layers from the cerebral cortex: plexiform (layer 1), outer small pyramidal cells (layers 2,3), inner large pyramidal cells (layers 4,5), polymorphous (layer 6), and adjacent white matter. We found that with isoniazid treatment, glucose was depressed only in the white matter of the cerebral cortex, and cerebellar glucose was unaffected. Lactate was decreased in the cortex in isoniazid treated mice in both pre-seizure animals and in those just starting to seize. In the cerebellum, lactate was decreased in the presence of sodium valproate, and unaffected by isoniazid. It appears that the metabolism of these two brain regions is not greatly compromised by isoniazid; either in the pre-seizure stage or at the onset of seizures.

Confirming previous observations on whole cerebellum, we found GABA levels decreased following isoniazid treatment, and increased following sodium valproate treatment in all cerebellar layers. By contrast, only in animals at the onset of seizures were cortical GABA levels significantly decreased, and then only in 3 of the 5 cortical layers examined. Sodium valproate however, caused significant increases in GABA in all layers of the cortex except that containing polymorphous cells. Valproate had no effect on cyclic AMP levels in either the cortex or cerebellum, nor did it have much effect on the isoniazid induced changes. Isoniazid alone caused some increases in both brain regions, and at the onset of seizures, the increases were magnified.

Sodium valproate decreased cerebellar cyclic GMP, and increased the cyclic GMP in the 2 cortical layers containing neurons. Isoniazid also elevated cyclic GMP in the cerebral cortical layers in pre-seizure mice. The decreased cyclic GMP in the cerebral cortical layers after seizures have begun may play a role in the modulation of seizure activity. The predicted effect of decreased cyclic GMP would be a decrease in the stimulus to the cortex; at the same time there is an increase in cyclic AMP, with a predicted inhibitory effect. The combination of events might influence seizure activity and ultimately result in its cessation.

The steady-state levels of ATP, P-creatine and glucose were measured in single cells and adjacent neuropil of the cerebellum and cerebral cortex. In addition, these metabolites were also examined both during and after an

electrically-induced seizure. The tissue samples weighed between 1 and 10 nanograms, necessitating metabolite measurements by enzymic cycling. The glucose levels decreased to a minimum 30 seconds after MES in all 4 tissues sampled and then increased to values 2-fold greater than control by 10 minutes after MES. The reduction of P-creatine at 10 and 30 seconds after MES was comparable in all tissues except the Purkinje cells. The recovery of P-creatine in the pyramidal cells and adjacent neuropil of the cerebral cortex was the same; whereas, the recovery in the Purkinje cells and adjacent neuropil was substantially faster. The most dramatic effect was observed in the levels of ATP. While the concentrations of ATP at 30 sec after MES decreased to about 25% of control values in the pyramidal cells and in the adjacent material from both regions, the levels in the Purkinje cells decreased only to 85% of control. Thus, the Purkinje cells were partially spared from the metabolic stress imposed by maximal electroshock. If there is a relationship between metabolism and function, then the results suggest that the activity of the Purkinje cell did not increase to the extent exhibited by the other areas. Perhaps, if the inhibitory output from the Purkinje cells were increased after MES, then the severity as well as the duration of the seizure might be lessened.

Significance to Biomedical Research and the Program of the Institute: Seizure activity is reflected by changes in the metabolite profile in both the cerebral cortex and cerebellum. The findings to date indicate that at least one major anticonvulsant, phenytoin, has a locus of action in the cerebellum. Additional support for the importance of the cerebellum in the control of seizures was indicated by the studies on single cells. The apparent sparing of Purkinje cells after MES appears to be a deleterious event that is permissive to extracerebellar paroxysmal activity. Thus, increasing cerebellar output, perhaps by pharmacological means, might serve to reduce, if not prevent, seizure activity.

Proposed Course of the Project: Future studies may include the study of cortical and cerebellar layers in other seizure models, and in the presence of various anticonvulsants. These studies will also be extended to analysis of metabolic changes in single neurons during milder forms of seizure activity.

Publications: McCandless, D.W., Lust, W.D., Feussner, G.K., Lust, W.D., and Passonneau, J.V.: Mechanism of action of phenytoin: evidence for a cerebellar locus. In Klatzo, I. and Spatz, M. (Eds.): Pathophysiology of Cerebral Energy Metabolism. Plenum Press, 1979, pp. 391-402.

McCandless, D.W., Feussner, G.K., Lust, W.D., and Passonneau, J.V.: Metabolite levels in brain following experimental seizures: the effects of maximal electroshock and phenytoin in cerebellar layers. J. Neurochem. 32: 743-753, 1979.

McCandless, D.W., Feussner, G.K., Lust, W.D., and
Passonneau, J.V.: Sparing of metabolic stress in Purkinje
cells after maximal electroshock. Proc. Natl. Acad. Sci. USA.
76: 1482-1484, 1979.

McCandless, D.W., Feussner, G.K., Lust, W.D., and
Passonneau, J.V.: Metabolite levels in brain following ex-
perimental seizures: the effects of isoniazid and sodium
valproate in cerebellar and cerebral cortical layers.
J. Neurochem. 32: 755-760, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02371-01 LNC
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Biochemical and physiological aspects of the brain during hypothermia and hibernation		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: W.D. Lust Research Pharmacologist LNC NINCDS OTHER: A. Wheaton Biological Lab Technician (Micro) LNC NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION Section of Cellular Neurochemistry		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.2	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <p>The brain has a relatively high metabolic rate which requires a continual supply of glucose and oxygen via an intact cerebral circulation. Because of the delicate balance between energy supply and energy demand, the brain is especially vulnerable to a variety of insults. A <u>hypothermia</u>-induced reduction in the metabolic rate of the brain has been shown to decrease the pathological effects resulting from brain trauma. <u>Energy metabolites</u> and <u>cyclic nucleotides</u> have been measured in brains from 1) mice artificially rendered hypothermic and 2) hamsters that were allowed to hibernate.</p> <p>In the hypothermic state, the energy status of the tissue was preserved in both groups of animals. The concentrations of ATP and P-creatine were significantly higher than in brains from normothermic animals. During <u>hibernation</u>, the levels of GABA and lactate were elevated and those of cyclic GMP were nearly depleted. These changes were not observed in brains from hypothermic animals. The metabolic events which occur during hypothermia/hibernation may offer some clues on how to more effectively treat a number of pathological conditions of the brain.</p>		

Project Description:

Objective: To determine the metabolic adaptations of the brain which occur during either hypothermia or hibernation. To correlate the electrical characteristics (EcoG) of the brain from hibernators and aroused hibernators with the observed biochemical changes. Further, to determine the extent of fixation artefact in brains from normothermic and hibernating hamsters.

Methods Employed: Experimental animals were frozen intact in liquid nitrogen and the brains were removed at -20° . The tissue was extracted in perchloric acid and the analyses were performed on the neutralized extracts.

Hypothermia. Mice were allowed to swim in a water bath for 2 min and then were placed in a cold chamber for 10 to 15 min. When the rectal temperature reached 20° , the mice were removed and kept at room temperature for 10 min prior to use.

Hibernation. Hamsters were placed in a 4° cold room and were maintained on apples and rat chow for up to 4 months. After 2 months, the hamsters were routinely observed for signs of hibernation. The non-hibernating animals in the 4° room were used as cold-adapted controls. Another group was kept at room temperature and was used as warm-adapted controls. In certain cases, the hibernators were aroused and the animals were frozen when the pouch (cheek) temperature reached either 12° or 20° .

Major Findings: Hibernation. Physiological Characteristics. The pouch temperature (PT) in hibernators was about 5° and at that temperature, the number of respirations ranged between 3 and 10 per minute. The average heart rate was approximately 15 beats/min. The heart rate and respiratory rate in a normothermic hamster ranges from 300 to 500 beats/min and from 35 to 120 resp./min, respectively. The electrocorticogram was very close to isoelectric in the hibernator.

Arousal. Heart rate increases with increasing PT and the relationship follows the equation:

$y = ax^b$, where y is heart rate, x is pouch temperature and a and b are constants, $a = 0.35$ and $b = 2.3$. Respirations increase at a rate of 9.3 respirations/min/degree pouch temperature up to 15° . The EcoG exhibits high amplitude activity almost immediately upon arousal. The pattern persists from 5° to 16° PT after which a normal EcoG becomes apparent.

Biochemical Results. In these studies, the values from the brains of warm-adapted animals served as controls. The glycogen levels in all brain regions of the hamster were 3- to 4-fold greater than those in the mouse brain. The greater stores of energy may be related to the ability of a given species to hibernate. The levels of glycogen and glucose were about the same in the hibernator and the normothermic animal; however during arousal, there was an elevated glucose and a reduced glycogen. This may reflect the stress during the arousal process. The concentrations of GABA and lactate both decreased with increasing body temperature, as did the levels of the high-energy phosphates, ATP and P-creatine. Cyclic AMP increased only slightly with increasing body temperature. The most dramatic change was in the levels of cyclic GMP which were essentially depleted in both the cerebellum and the cerebral cortex. At 12° PT, the cyclic GMP levels increased significantly in the cerebral cortex but not in the cerebellum. At 20° , the cyclic GMP levels in the

cerebellum began to increase, while the levels in the cerebral cortex dropped once again to a value close to that seen in the hibernator. In the normothermic hamster, the cyclic GMP values were back to control. Thus, the biphasic cyclic GMP response in the cerebral cortex may bear some relationship to the CNS activity during arousal. Based on previous experience on the changes to expect for a given metabolite during improper fixation, it would appear that the changes in GABA, glucose, lactate and cyclic GMP can be attributed directly to the effects of hibernation. The rest of the changes are, undoubtedly, due to fixation artefact which increases with increasing body temperature.

Significance to Biomedical Research and the Program of the Institute:

The inability of the brain to adapt may be one reason why the brain is particularly vulnerable to a wide variety of pathological insults. Hibernation is a good example of natural adaptation to adverse environmental conditions and, as such, is a good model to determine how the brain can biochemically adapt its metabolic processes and survive. There is no doubt but that hypothermia, whether induced during or after brain trauma, can reduce the deleterious effects. For example, if higher levels of GABA and lactate and lower levels of cyclic GMP would increase the survivability of the tissue, then certain pharmacological agents might be of some therapeutic value.

During ischemia and hibernation, the brains are both in an electrically quiescent state and there are biochemical features common to both conditions, such as very low levels of cyclic GMP, high levels of GABA and a low metabolic rate. A major difference between the 2 is the low energy status in the ischemic brain. Perhaps, this could explain why hibernation is readily reversible and prolonged ischemia is not.

Proposed Course of Project: The studies on hibernation/hypothermia will be continued with greater emphasis on the relationship between electrical and chemical events. In addition, regions of the brain other than the cerebellum and the cerebral cortex will also be examined both during and after hypothermia.

Publication: none

ANNUAL REPORT

October 1, 1978 through September 30, 1979

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

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ANNUAL REPORT
October 1, 1978 through September 30, 1979
Laboratory of Molecular Biology
National Institute of Neurological and Communicative
Disorders and Stroke

Ernst Freese, Chief

This year, LMB scientists made or participated in the following findings which are summarized here and detailed below: 1. Microbial differentiation is initiated by a decrease in the synthesis of guanine nucleotides. 2. In mammalian cells, butyrate induces the production of the receptors for adrenaline and cholera toxin and thereby increases the effect of agonists on the methylation phosphatidylethanolamine into phosphatidylcholine, which occurs while the phospholipid is transported to the outside of the membrane. 3. The molecular mechanism by which defective interfering virus particles are formed has been elucidated, and the role of these particles and of antibodies in persistent virus infections has been examined in neuronal tissue cultures.

1. Mechanisms controlling microbial differentiation (sporulation). Microbial differentiation generally starts when rapidly metabolizable carbon or nitrogen sources or phosphate are used up. Such a nutritional control of differentiation exists also in higher organisms but has not been studied in any detail. To determine which specific compound(s) has to decrease to allow differentiation, cells were grown in media containing excess nutrients that prevent sporulation, and different inhibitors were added or mutants were used to reveal conditions that would allow the initiation of sporulation anyhow. These studies were first performed in *Bacillus subtilis* and then in the eukaryotic yeast *Saccharomyces cerevisiae*. It was found essential to use conditions of partial growth inhibition because too much inhibition prevented the synthesis of macromolecules required for the morphological development, whereas too little inhibition allowed normal symmetric cell division. Many amino acid analogs, inhibitors of DNA, RNA, or protein synthesis, and most inhibitors of the pyrimidine pathway were unable to initiate differentiation at any concentration although they did inhibit growth. However, many purine analogs and inhibitors of purine synthesis were effective. They include compounds used in cancer chemotherapy such as amethopterin (=methotrexate, in the presence thymine) and 6-mercaptopurine. Particularly effective were specific inhibitors of guanine synthesis including decoyinine, mycophenolic acid, and psicofuranine. Specific inhibition of the AMP path by hadacidin induced sporulation in minimal medium but not in amino acid containing medium. The same results were observed with auxotrophic mutants if they were either leaky or could be supplied with slowly metabolizable purine precursors. Again, the best sporulation was observed with guanine auxotrophs whereas adenine auxotrophs showed no effect. An analysis of ³²P labeled nucleotides demonstrated that under all these and other conditions under which sporulation was initiated the concentration of GTP (and GDP) decreased, whereas the concentrations of the other nucleoside triphosphates decreased under other conditions. Thus, the decrease of guanine nucleotides was sufficient to initiate sporulation

and more generally the decrease of GTP was always correlated with the initiation of sporulation; whether this decrease is absolutely necessary to initiate sporulation remains open.

Glucose suppresses not only sporulation but also represses the synthesis of many catabolic enzymes. It had therefore been proposed that this "catabolite repression" was also responsible for the suppression of sporulation. However, the laboratory has now shown that the induction of sporulation in the presence of glucose is not correlated with a release of other enzymes from catabolite repression as demonstrated for inositol dehydrogenase and acetoin dehydrogenase.

Whereas, certain mutants blocked in the citric acid cycle or in gluconeogenesis are unable to sporulate normally, their sporulation could be induced in the presence of glucose by the addition of decoyinine. Under these conditions, glucose supplies the necessary metabolic intermediates and can regenerate ATP via glycolysis.

Using a special growth medium that contained amino acids and vitamins but no purines, the sporulation of yeast could also be induced by the general purine deficiency caused by amethopterin (in the presence of thymine) and by mycophenolic acid. The asci produced under these conditions contained mostly four spores reflecting the tetrads of meiotic nuclear segregation. We are very intrigued by this finding because it should enable us to study the initiation and control of meiosis. But this work will require more detailed metabolic studies because the differentiation of yeast is more complex than that of *B. subtilis* since glucose can still prevent sporulation even in the presence of mycophenolic acid.

2. Induction of receptor formation and coupling to AMP cyclase in mammalian cells. It had previously been reported by the laboratory that butyrate causes extensive changes in the shape of HeLa cells and induces the production of the ganglioside G_{M2} and the β -adrenergic receptor. It has now been shown that the closely related ganglioside G_{M1} , which is the receptor for cholera toxin, also increases 40-fold after 48 hr exposure of HeLa cells to 5 mM butyrate; similar increases were observed with glial and erythro-leukemic cells. Apart from the basic interest, these results provide a tool for other studies involving adenylate cyclase, because cholera toxin can maximally activate adenylate cyclase if the cell is endowed with receptors for the toxin. The coupling of adenylate cyclase to the β -adrenergic receptor (and probably other receptors) has been shown to depend on GTP since incubation in the presence of virazole which reduces the level of GTP without affecting ATP also reduces the coupling. The ability of HeLa cells to produce large amounts of β -adrenergic receptor, especially in the presence of butyrate, has been used to study the relationship between these receptors and the methylation of phospholipids. This work was performed in collaboration with the Laboratory of Clinical Science, National Institute of Mental Health. Phosphatidylethanolamine was shown to be methylated into phosphatidylcholine by the sequential action of 2 methyltransferases, using S-adenosylmethionine as methyl donor. During this methylation the phospholipids migrate from the inside to the outside of the plasma membrane. This methylation is necessary for the maintenance of β -adrenergic receptor

on the cell surface. Conversely, β -adrenergic agonists such as isoproterenol cause a 10-fold stimulation in the rate of methylation of phosphatidylethanolamine. β -adrenergic antagonists such as propranolol block this stimulation whereas α -adrenergic antagonists such as pentolamine do not. Concomitant with the stimulation of phospholipid methylation, the activity of an intracellular phospholipase increases. As a result the membrane turns over rapidly. The synthesis and degradation of phosphatidylethanolamine may be critical for the maintenance of proper amounts of cell surface receptors. An imbalance of these processes would cause oversensitization or desensitization, respectively, toward agonists of the receptors.

3. The molecular and replicative properties and mechanism of autointerference by defective interfering particles of vesicular stomatitis virus. Many members of the myxo-, paramyxo-, rhabdo-virus family can either regularly or under exceptional conditions penetrate the blood-brain barrier or directly pass into the brain through the olfactory nerve; thus they can infect the central nervous system causing encephalitis or meningitis. Despite their importance to medical neurology, very little is known about the regulation and mode of replication of these viruses in the host organism. They frequently produce defective interfering (DI) particles which are responsible for autointerference, i.e. restriction of the replication of the infectious virus, and viral persistency, i.e., maintenance of a slow but nevertheless destructive viral infection for very long times. To examine these various properties the vesicular stomatitis virus (VSV) and its DI particles are being thoroughly investigated in the laboratory. RNA sequencing has shown that the ends of the DI particle genome are complementary, a property not shared by the parent virus genome. Different DI particles have the same complementary regions for a distance of 45-48 nucleotides from the ends indicating that the particles were formed by an apparent replicative event in which the viral replicase detaches from the template and resumes synthesis at a specific recognition site, near position 45-48 from the 5' end of the viral genome. The sequence of this recognition site is 5'GGUCUU-3'. This hexamer is also part of other highly conserved terminal initiation sites of RNA polymerase. The fact that DI particles have the same attachment sites as the regular virus genome explains how they can compete for the attachment to RNA polymerase and for this reason can limit infection (less infective virus made).

The sequence studies have also revealed an interesting mechanism whereby polyA tails appear at the 3' end of each mRNA. The last seven bases of the L-protein cistron are uracil nucleotides which will be transcribed into adenine nucleotides in the messenger RNA. It is likely that the RNA polymerase chatters or slips along this stretch of 7 U's causing a repeated transcription into adenine nucleotides. Beyond the 7 uracil nucleotides a GAAA sequence, which is also found just before the beginning of the first gene of VSV, may specify a termination event of transcription.

The effects of DI particles and antibodies on the course of virus infection were studied in dissociated neuron cultures of embryonic mice. These immature neurons progressively differentiate in vitro and develop synaptic connections after 3-4 weeks. Upon infection by VSV numerous

maturation sites can be detected along the membrane of the neuron body and in postsynaptic dendrites but not in presynaptic endings. Thus, conditions for viral assembly seem to exist on the postsynaptic site of the nerve ending but not on the presynaptic one. This may have implications on the mechanism by which virus infections are spread in the nervous system. When DI particles were added together with VSV a persistent infection of the neurons was observed which lasted 2 weeks without leading to the production of infective virus. Interestingly, only mature virus neurons could thus be protected by the addition of DI particles; persistent infections of immature neurons were not obtained. Addition of viral antibodies prevented reinfection of cells and allowed the destruction of the viral antibody complex by phagocytes. However, removal of the antibodies from the cells after 3 or 4 days of treatment reactivated the viral infection, allowing maturation and release of viruses 2 to 3 days later. The chances of reactivation decreased with increasing length of antibody treatment so that no viral antigens or budding sites were detected if the antibody was removed after 1 week or longer; thus antibody interaction with virus infected neurons activates phagocytic cells, restricts maturation sites mostly to the postsynaptic areas and results in an apparent curing of infection.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01244-15 LMB
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Control Mechanisms and Differentiation		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
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	T. Endo	Visiting Fellow LMB NINCDS
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COOPERATING UNITS (if any) Department of Chemistry, New Mexico State University, Las Cruces, New Mexico		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION Developmental Biology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 6.7	PROFESSIONAL: 5.2	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The initiation of <u>sporulation</u> was analyzed in various mutants in the presence of excess glucose and ammonia, conditions under which normal sporulation remains suppressed. When <u>purine mutants</u> were fed with different concentrations of the slowly metabolizable purine precursor <u>aminoimidazole carboxymide (AICA)</u> or <u>guanine mutants</u> were fed different amounts of guanosine optimal sporulation was obtained when purine synthesis, and in particular <u>guanosine monophosphate (GMP)</u> synthesis, was partially inhibited. ³² P _i incorporation into nucleotides demonstrated that under all conditions under which sporulation occurred the concentration of GTP (and GDP) decreased by more than 50%. A number of purine nucleoside analogs such as <u>virazole</u> , <u>psicofuranine</u> , <u>mycophenolic acid</u> and others were shown to induce sporulation. The role of <u>membrane synthesis</u> was examined by the use of mutants deficient <u>branched-chain fatty acid</u> synthesis.		
5 - LMB/IRP		

Project Description:

Objectives: The differentiation of microorganisms and of certain cell types in higher organisms begins when rapidly metabolizable carbon or nitrogen sources become scarce or when phosphate has been exhausted. Conceivably, differentiation could then start either because the concentration of a particular compound that normally suppresses sporulation decreases below a critical value or because the balance of polymer synthesis is shifted so that asymmetric septation and thus sporulation is preferred. To determine which compound(s) might be involved in this control we have analyzed the effect of inhibitors and mutants on the sporulation of *Bacillus subtilis*. Furthermore, to examine the role of membranes we have investigated the sporulation properties of mutants deficient in branched chain fatty acid synthesis.

Methods Employed: *Bacillus subtilis* was grown in a synthetic sporulation medium containing excess glucose and ammonia and sometimes containing vitamin-free casein hydrolysate. The cells were aerated until the absorbency of 600 nm (A_{600}) was 0.5 and then either distributed into flasks containing different amounts of an inhibitor or first washed on Millipore filters and then suspended in medium lacking one of the compounds required by an auxotroph. Bases and nucleosides were generally dissolved in dilute KOH. The concentration of nucleotides was determined by prelabeling the cells for 2 or more generations with $^{32}P_i$, extracting them with .5 M formic acid in the cold and chromatographing them in 2 dimensions on polyethyleneimine thin-layer plates. In the first dimension potassium phosphate, pH 3.4 was used at 20 or 75 mM concentration. In the second dimension electrophoresis was used at 60 mA for 1 hr.

Major Findings: 1. Initiation of sporulation in mutants. Using a number of purine auxotrophs, it was found that the frequency of sporulation obtained 10 hr after cell transfer to a purine-free medium increased with the residual growth, i.e., the leakiness of the mutant. Stringent purine auxotrophs could also be made to sporulate well by using the purine precursor aminoimidazole carboxymide (AICA) at an optimal concentration of about 2 mM. This compound is either badly transported or slowly metabolized so that its concentration determines the rate of growth of the purine mutant. Adenine, hypoxanthine or their nucleosides could not be used for this purpose because these compounds are actively transported and metabolized into nucleotides already at μ M concentrations at which they are rapidly used up in the medium. Specific guanine auxotrophs sporulated well in the absence of guanine but did so even in its presence because guanine is only slowly taken up by *B. subtilis*. However, using different concentrations of guanosine, sporulation was improved at low concentrations but completely abolished at high guanosine concentrations. No sporulation increase was obtained with adenine auxotrophs at any adenine or adenosine concentrations. It was also found that an aspartate-requiring mutant lacking an active transport system for aspartate could sporulate well at an intermediate aspartate concentration. Other amino acid requiring mutants, with and without the relaxed (rel) mutation controlling ppGpp and RNA synthesis are now similarly tested.

2. The change of nucleotide pools during initiation of sporulation.

Many of the conditions under which sporulation could be initiated in the presence of excess glucose and ammonia, as well as normal sporulation conditions under which a carbon, nitrogen or phosphate sources are limiting, have been used to examine the change of the nucleoside triphosphate pools during the initiation of sporulation. It was found that inhibition of general purine synthesis causes a decrease in the cellular concentration of both purine nucleoside triphosphates and a slower decrease in pyrimidine triphosphates. In the presence of hadacidin, an inhibitor of AMP synthesis not only ATP but also all of the other nucleoside triphosphates decrease. However, in the presence of an amino acid mixture the decrease of GTP was avoided and (althought ATP still decreased by 90%) no sporulation was observed. Addition of decoyinine, an inhibitor of GMP synthetase, or removal of guanosine from a guanine auxotroph caused a decrease of both GTP and UTP but an increase of ATP or CTP. Transfer from a casein hydrolysate medium to one containing glutamate as sole carbon source caused a decrease of only GTP and none of the other 3 nucleoside triphosphates. These and other conditions have shown that only GTP (and GDP) decreases consistently under all sporulation conditions whereas the other nucleoside triphosphates (and diphosphates) decrease under some and increase under other sporulation conditions. Therefore, it is conceivable that the decrease of GTP is necessary for the initiation of sporulation. This is the first time that a specific metabolite has been pinpointed as the compound controlling sporulation.

3. Correlation of sporulation and catabolite repression. Many enzymes that are inducible in an amino acid containing medium can no longer be induced in the presence of glucose or rapidly metabolizable carbohydrates. Since sporulation is suppressed under the same conditions one may ask whether the initiation of sporulation by a deficiency in GMP synthesis can also release the catabolite repression of enzyme synthesis. This was investigated for the enzymes inositol dehydrogenase, inducible by inositol, acetoin dehydrogenase, inducible by acetoin, and sorbitol dehydrogenase, inducible by sorbitol. In all cases, the induction of the enzyme was prevented by glucose; a partial GTP deficiency, introduced by addition of decoyinine or by the use of a guanine auxotroph, under conditions which allowed excellent sporulation did not allow enzyme synthesis. Thus, sporulation and catabolite repression of these enzymes are subject to different control mechanisms. However, some enzymes, such as aconitase and citrate synthase increased after the generation of a GTP deficiency in the presence of glucose. These findings are now being used to examine the mechanism by which GTP deficiency causes sporulation.

4. Induction of sporulation by purine nucleoside analogs. Decoyinine is an analog of adenosine which inhibits GMP synthetase. Since it excellently induced sporulation, other inhibitors of the GMP pathway or analogs of purine nucleosides were examined for their effect on sporulation. Of many compounds tested, the inhibitors of IMP dehydrogenase, virazole and mycophenolic acid, and the inhibitor of GMP synthetase, psicofuranine, induced sporulation. These compounds were not as effective as decoyinine, probably

because it could not enter the cells as efficiently.

5. Effect of branched chain fatty acid synthesis on sporulation. The first morphologically recognizable event in sporulation is the formation of an asymmetric septum which consists mostly of two membranes and very little cell wall material between them. Reports by others had found that during the time of the septation process the composition of branched chain fatty acids change. They had therefore suggested that this change was necessary for the initiation of sporulation. We have used a mutant deficient in branched chain fatty acid synthesis which can be made to produce different branched chain fatty acids depending on the addition of the corresponding branched chain keto acids, isovalerate, 2-methylbutyrate, and isobutyrate. The results indicate that a certain minimal amount of branched chain fatty acid synthesis is necessary to allow sporulation but that it is unimportant which branched chain fatty acid is present.

Proposed Course of Project: It would be important to determine whether the partial deprivation of compounds other than purine nucleotides can also initiate sporulation and if so whether that is correlated with a decrease of GTP. Such a possibility is indicated by the fact that amino acid starvation causes an increase in ppGpp and pppGpp, compounds which in turn cause a decrease in GTP. By the use of relaxed (rel) mutants which no longer produce the highly phosphorylated nucleotides, one will be able to determine the significance of sporulation initiation by deprivation of amino acids. We have also found another highly phosphorylated nucleotide whose role in sporulation will have to be examined. Since certain enzymes increase after the decrease of GTP, including enzymes that cause turnover of RNA, the role of these enzymes or the mechanisms controlling their synthesis in the control of sporulation will be analyzed. Since membrane synthesis has to continue while cell wall synthesis has to stop during the initiation of sporulation the effect of GTP deficiency on these processes will be determined. It is expected that these studies will enable us to decide whether a particular compound has to be made or be destroyed in order to initiate sporulation or of RNA and protein versus that of membrane.

Publications:

Watabe, K., and Freese, E.: Purification and properties of the manganese-dependent phosphoglycerate mutase of B. subtilis. J. Bacteriol., 137: 773-778, 1977.

Heinze, J.E., Mitani, T., Rich, K.E., and Freese, E.: Induction of sporulation by inhibitory purines and related compounds. Biochem. Biophys. Acta, 521: 16-26, 1978.

Freese, E., and Heinze, J.E.: Control and genetics of bacterial sporulation. In Gould, G.W., Dring, J. and Hurst, A. (Eds): Bacterial Spore, Vol II, Academic Press, New York, 1979 (in press).

Freese, E., Heinze, J.E., and Galliers, E.: Partial purine deprivation causes sporulation of B. subtilis in the presence of excess ammonia, glucose and phosphate. J. Gen. Microbiol., 1979 (in press).

Freese, E., Lopez, J. and Freese, E.B.: Initiation of bacterial and yeast sporulation by partial deprivation of guanine nucleotides. In Richter, D., and Koch, G. (Eds): Regulation of Macromolecular Synthesis by Low Molecular Weight Mediators, Academic Press, New York, 1979 (in press).

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Lopez, J.M., Marks, C.L., and Freese, E.: The decrease of guanine nucleotides initiates sporulation of Bacillus subtilis. Biochem. Biophys. Acta, 1979 (in press).

Ramaley, R. Fujita, Y., and Freese, E.: Purification and properties of Bacillus subtilis inositol dehydrogenase, J. Biol. Chem., 1979 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01886-09 LMB
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Developmental Cytology		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
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	J. Kandala	Visiting Fellow
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COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION Developmental Biology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.9	3.4	0.5
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<input type="checkbox"/> (a) HUMAN SUBJECTS		
<input type="checkbox"/> (b) HUMAN TISSUES		
<input checked="" type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>The induction of differentiation by inhibitors of nucleotides synthesis was studied in <u>Bacillus subtilis</u> and in <u>yeast</u>. The following major results were obtained: 1) Several biochemical pathways that are normally needed for <u>Bacillus subtilis sporulation</u> are not required when sporulation is induced, in the presence of glucose and ammonia, by <u>decoyinine</u> an inhibitor of GMP synthetase. This was shown by the use of mutants blocked in the citric acid cycle or gluconeogenesis, or by <u>manganese</u> deficiency which prevents the function of <u>phosphoglycerate mutase</u>. 2) The yeast <u>Saccharomyces cerevisiae</u> can in appropriate purine-free growth media also be induced to undergo <u>meiosis</u> and then to sporulate by the addition of <u>amethopterin</u> (in the presence of thymine) or by <u>mycophenolic acid</u>.</p>		

Project Description:

Objectives: Microbial differentiation generally starts when all rapidly metabolizable carbon and nitrogen sources have been used up. Previous work of our laboratory has shown that the sporulation of *Bacillus subtilis*, which is prevented by excess glucose and ammonia in the growth medium, can be induced by certain inhibitors of nucleotide synthesis, decoyinine being the most effective compound. The purpose of the experiments reported here was: 1) To determine which mutants unable to sporulate under normal conditions still sporulate in the presence of glucose and decoyinine. 2) To determine whether it was possible to induce sporulation also in the eukaryote *Saccharomyces cerevisiae* in which sporulation has to be preceded by meiosis.

Methods Employed: A large number of media was investigated in order to determine under which conditions yeast sporulation could be induced in the same medium in which the cells were grown. The optimal medium found was one containing pyruvate and ammonia, vitamin-free casein hydrolysate, vitamins and mineral salts, but no purines. If a purine deficiency was to be generated by amethopterin, excess thymine and glycine were also added to the medium.

Major Findings: 1. The induction of sporulation in sporulation mutants and during manganese deficiency. Many mutant strains of *B. subtilis* have been isolated which are unable to sporulate in the usually used sporulation media. When these mutants were grown in a medium containing excess glucose and vitamin-free casein hydrolysate but no purines the induction of sporulation by decoyinine (1 mg/ml) could be measured. It was found that all citric acid cycle mutants as well as mutants blocked in gluconeogenesis (phosphoenolpyruvate-carboxykinase) could be induced by decoyinine to sporulate. This is understandable because the excess glucose in the medium supplies all compounds that are ordinarily supplied by gluconeogenesis and enables glycolysis the regeneration of ATP which is ordinarily regenerated in the citric acid cycle. Other sporulation mutants showed either no or only a very small effect of sporulation induction indicating that in these cases decoyinine could not circumvent the sporulation block. A deficiency of manganese causes the accumulation of 3-phosphoglycerate because phosphoglycerate mutase is strictly manganese-requiring. In the absence of manganese the cells require both glucose and malate for growth, and they can normally not sporulate. However, in the presence of excess glucose and malate, decoyinine (150 uM/ml) can induce sporulation if the compound is added sufficiently early so that not too much 3-phosphoglycerate has yet accumulated.

2. Sporulation in *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* ordinarily sporulates only if cells are transferred from a growth medium to potassium acetate. Addition of ammonia greatly reduces sporulation and a regular growth medium, especially one containing glucose, completely prevents it. We have found that sporulation can be induced in a growth medium containing pyruvate, ammonia, amino acids and vitamins but no purines by the addition of amethopterin (if thymine and glycine are added in excess) or by

mycophenolic acid. In the case of amethopterin the frequency of asci increased and later decreased with the amethopterin concentration whereas with mycophenolic acid the frequency increased with the concentration to a maximal value of 70-80% and then remained essentially constant. Interestingly, the frequency of asci with 3 or 4 spores increased with increasing inhibitor concentration, indicating that a partial metabolic inhibition allowed meiosis to go to completion and each of the 4 haploid nuclei to be incorporated into a separate spore. The induction of sporulation by mycophenolic acid was specifically prevented by the addition of guanosine but not by adenine or hypoxanthine.

Proposed Course of Project: We are intrigued by the fact that sporulation cannot only be induced in B. subtilis but also in yeast because the latter organism is a eukaryote and has to undergo meiosis before sporulation can take place. Consequently, these studies can be used to determine mechanisms by which meiosis can be induced. The biochemistry of this induction process as well as the reason why glucose can still prevent the induction in yeast but not in B. subtilis will be investigated. This will require the use of mutants, of special inhibitors effective in yeast and of a cell free analysis of the enzymes and transport mechanisms operative during the induction process.

Publications:

Freese, E.B., Vasantha, N., and Freese, E.: Induction of sporulation in developmental mutants of Bacillus subtilis. Molec. Gen. Genet., 170: 67-74, 1979.

Vasantha, N., and Freese, E.: The role of manganese in growth and sporulation of B. subtilis. J. Gen. Microbiol., 1979 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02026 07 LMB

PERIOD COVERED

October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Regulation of Viral Nucleic Acids Synthesis in Animal Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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L. Johnson	Chemist	LMB NINCDS
J. Keene	Staff Fellow	LMB NINCDS
M. Schubert	Staff Fellow	LMB NINCDS
M. DuBois-Dalq	Visiting Scientist	IDB NINCDS
H. McFarland	Asst. Chief	NIB NINCDS

COOPERATING UNITS (if any)

Department of Neurology, Laboratory of Neurovirology, Johns Hopkins University, School of Medicine; IDB and NIB, NINCDS

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Molecular Virology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

7.0

PROFESSIONAL:

5.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range objective of this project is the description of the component molecular events involved in the replication of the negative strand viruses (myxo, paramyxo and rabdo viruses). The topics that are currently being investigated are:

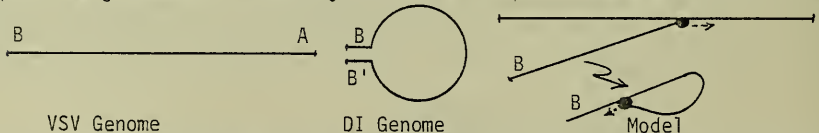
1. The origin of DI particles.
2. The mechanism of mRNA synthesis in VSV infected cells.
3. Viral infection of dissociated neuron cultures and its modulation by DI particles and antibody.
4. Synthesis and characterization of measles specific nucleic acid probe.

Project Description:

Objectives: Viral diseases of the central nervous system (CNS) usually occur as a complication rather than a normal consequence of infection. Nevertheless, many members of the myxo-, paramyxo-, rhabdovirus family, either exceptionally or as a normal consequence, infect the CNS, causing encephalitis or meningitis. Despite their importance to medical neurology, very little is known about the regulation and mode of replication of these viruses in the host organism. From what little is known, it is clear that their replication is very different from that described for polio virus or the RNA tumor viruses. Furthermore, the myxo-, paramyxo-, rhabdovirus infections also are distinguished in that they frequently elaborate defective interfering (DI) particles and exhibit evidence of autointerference and viral persistency. The description of the component molecular events involved in the replication of these viruses, the generation of defective interfering particles, autointerference and viral persistency are the subject of this project. It is anticipated that the study will delineate characteristics that can be exploited in containing and limiting viral infection to non-neural tissues or in the treatment or prevention of the viral infection.

Major Findings: Four major areas of our program have been pursued during the last year, and although interrelated, each area is discussed separately.

1. The origin of DI particles. DI particles are small viral particles that contain only a part of the viral genome (often less than one gene) but all of the viral proteins. When DI particles coinfect a cell with the homologous infectious virus, they severely restrict its replication -- a phenomenon termed interference. Early work from this laboratory demonstrated that there are many types of VSV DI particles, although most contain genetic information derived from the 5' half of the VSV genome. The terminal sequences of the DI particle genome are complementary, a property not shared by the parental virus genome. In the diagram below the genome of the infectious virus is represented by a bar with non-complementary termini, A and B. The DI genome is represented as a circular molecule to emphasize the complementarity of its termini, B and B'. It has been proposed that DI particle genomes are formed by an aberrant replicative event in which the



viral replicase detaches from the template it had been copying and resumes synthesis by copying the terminal region of its own daughter strand. This model is illustrated also in the diagram.

We have examined the lengths of the terminal complementary regions of a number of different DI particle genomes and have found them all to be very similar -- between 45 and 48 nucleotides long. The fact that these regions

The two arrows in the figure indicate the position of the sequence involved in the generation of DI particles (positions 48-43) and the end of the L protein cistron (position 60) which starts some 5000 nucleotides from this position. The sequence from position 60-66 shows that the first seven A's of the polyA tail of the L mRNA are coded in the virus genome by the stretch of seven U's. These data strongly indicate that the virus polymerase itself synthesizes the poly(A) tails on the mRNAs, perhaps by chattering or slipping on this stretch of seven U's. Recently, others have observed a stretch of seven U's at the end of the N message cistron, again corresponding to the first seven A's in its poly(A) tail. Following the stretches of U's at the end of the L and N cistrons there is the sequence AAAG. At present we do not know whether these sequences specify a RNA chain termination by the RNA polymerase or nucleolytic cleavage by a processing enzyme. Now that we are armed with the sequence we have undertaken the chemical synthesis of short RNAs that will bear the relevant sequence. We will use these in an effort to identify the processing enzyme, if it exists.

3. Viral infection of dissociated neuron cultures and its modulation by DI particles and antibody. Dissociated neuron cultures of embryonic mice contain immature neurons which progressively differentiate in vitro and develop synaptic connections after 3 to 4 weeks. The surfaces of these cells are readily available for interaction with viruses, DI particles and antibodies. Thus, these dissociated neuron cultures appear to be excellent in vitro systems for the study of a) differences in susceptibility to virus infection among various nerve cell types, b) variation in virus induced cytopathic changes with neuronal maturation, c) the effects of coinfection with defective interfering particles on a differentiated nerve cell system, d) the effects of antibody on the course of viral infection. We have examined the replication of VSV in cultured neurons and the effects of DI particles and antibodies on the course of infection using immunofluorescent microscopy, transmission and scanning electron microscopy. Our results show that sensory and immature neurons show viral replication and antigen expression faster than mature neurons. Ultrastructure studies of the infected neurons reveal numerous virus maturation sites along the neuron body membrane and in postsynaptic dendrites. In contrast, presynaptic endings show no viral budding sites although they have viral antigens inserted. Thus, conditions for viral assembly seem to exist on the postsynaptic side of the nerve endings but not on the presynaptic side. This may have implications on the mechanism of spread of infection in the nervous system.

Modulation of infection was obtained by the addition of DI particles. Under these conditions, a persistent, non-productive infection lasting 2 weeks occurred in some of the neurons. Interestingly only mature neurons could be protected by the addition of DI particles; persistent infections of immature neurons could not be obtained.

When neuron cultures were infected and subsequently fed with media containing antiviral antibodies to neutralize all free virus, the cultures could be maintained at least for two weeks. During the first days of anti-

body treatment viral buds and surface antigens were often grouped in patches and caps. In addition, phagocytic cells were seen in contact with the virus released by the neurons. Removal of antibody after three or four days of treatment resulted in a reactivation of the viral infection in more neurons than those originally infected. Complete virus maturation and release occurred only two to three days after the removal of antibody. The chances of reactivation decreased with increasing length of antibody treatment and no viral antigens or budding sites were detected after removal of the antibody if the antibody treatment had been one week or longer. Thus, antibody interactions with virus infected neurons activates phagocytic cells, restricts maturation sites mostly to the postsynaptic areas and results in an apparent curing of infection.

4. Synthesis and characterization of measles specific nucleic acid probe. Radioactive complementary DNA (cDNA) probes represent one of the most sensitive methods for the detection and analysis of viral genomes in infected cells and thus far the only way of detecting the presence of completely inactive or latent viral genetic information. This year we undertook the synthesis of radioactive cDNA probes specific for measles viral information. We will use this probe to illuminate inaccessible aspects of measles infections of biopsy and autopsy tissues and to search for measles information in tissues from infections of unknown etiology. Our early work on this project indicated that nucleic acid isolated from measles virus was grossly contaminated with host nucleic acid and was not suitable as a template for cDNA synthesis. However, now we have purified measles RNA from the cytoplasm of infected cells so that it is free of host RNA. We have successfully employed this measles RNA as a template for cDNA synthesis and are currently characterizing the cDNA probe.

Proposed course of Project: 1. To further investigate the RNA sequences of the regulatory regions on DI and infectious particle genomes. 2. To obtain specific RNA substrates for the putative viral RNA processing enzymes. In this facet of our program we will also attempt to isolate or synthesize an RNA that can be processed to yield meaningful products. Armed with substrates we will attempt to identify which proteins, viral or host, are responsible for processing events. 3. To investigate the biochemical events that underly the modulation of viral infections in neuron cultures by DI particles antibody. 4. To complete the establishment of a reliable method for probing biopsy and autopsy tissues for viral information.

Publications:

Adachi, T., and Lazzarini, R.A.: Elementary aspects of autointerference and the replication of defective interfering virus particles. Virology, 87: 152-163, 1978.

Herman, R.C., Adler, S., Lazzarini, R.A., Colonna, R.J., Banerjee, A.K., and Westphal, H.: Intervening polyadenylate sequences in RNA transcripts of vesicular stomatitis virus. Cell, 15: 587-596, 1978.

Johnson, L.D., and Lazzarini, R.A.: Synthesis of both + and - strand viral RNA in cells infected with only VSV DI particles. In Mahy, B., and Barry, R. (Eds.): Negative Strand Viruses, Academic Press, New York, 1978, pp. 555-564.

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Keene, J.D., Schubert, M.H., Lazzarini, R.A., and Rosenberg, M.: Nucleotide sequence homology at the 3' termini of RNA from vesicular stomatitis virus and its defective interfering particles. Proc. Natl. Acad. Sci. U.S.A., 75: 3225-3229, 1978.

Keene, J.D., Schubert, M.H., Rosenberg, M. and Lazzarini, R.A.: A comparative study of nucleotide sequences at the 3' termini of ribonucleic acids from vesicular stomatitis virus and its defective interfering particles. In Stevens, J., Tadaro, G. and Fox, C.F. (Eds.): Persistent Viruses, ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. III, Academic Press, New York, 1978, pp. 285-296.

Schubert, M., Keene, J.D., Lazzarini, R.A., and Emerson, S.U.: The complete sequence of a unique RNA species synthesized by a DI particle of VSV. Cell, 15: 103-112, 1978.

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Faulkner, G., DuBois-Dalq, M. Hooghe-Peters, E., McFarland, H.F., and Lazzarini, R.A.: Defective interfering particles modulate VSV infection dissociated neuron cultures. Cell, 1979 (in press).

Faulkner, G., and Lazzarini, R.A.: Autointerference by defective interfering particles. In Bishop, D.H.L. (Ed.): Rhabdoviruses, CRC Press, New York, 1979 (in press).

Johnson, L.D., and Lazzarini, R.A.: Transcriptive abilities of defective interfering particles. In Bishop, D.H.L. (Ed.): Rhabdoviruses, CRC Press, New York, 1979 (in press).

Keene, J.D., Schubert, M.H., Lazzarini, R.A.: Terminal sequences of vesicular stomatitis virus RNA are both complementary and conserved. J. Virol., 1979 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02224-04 LMB
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PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Cell Growth and Transport and its Inhibition by Lipophilic Acids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Freese	Chief, Lab. Molec. Biol.	LMB NINCDS
	R. C. Henneberry	Senior Scientist	LMB NINCDS
OTHER:	A. Bruckner	Visiting Fellow	LMB NINCDS
	P.H. Fishman	Research Biologist	DMN NINCDS

COOPERATING UNITS (if any) Laboratory of Clinical Science, NIMH; DMNB, NINCDS
Laboratory of Nutrition and Endocrinology, NIAMDD
Biological Psychiatry Branch, NIMH
Johns Hopkins University, Project No. NIH N01 5-2320

LAB/BRANCH
Laboratory of Molecular Biology

SECTION:
Developmental Biology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 3.2	PROFESSIONAL: 2.2	OTHER: 1.0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to elucidate the developmental effects of lipophilic acids and the molecular mechanisms of the propagation of biochemical signals through the cell membrane. 1) A number of lipophilic acids that potently inhibited the growth of mammalian tissue cultures were tested for their teratogenic effect in mice. Teratogenics were thymol, dichlorophene, thiopental, salicylanilid, undecylenic acid and scoparone. 2) Receptors of cholera toxin are induced by butyrate. The interaction between the β -adrenergic receptors and adenylate cyclase requires GTP. The internal methylation of phosphatidylethanolamine to phosphatidylcholine proceeds while the phospholipid migrates from the inner to the outer surface of the plasma membrane and is stimulated by isoproterenol.

Project Description:

Objectives: Lipophilic acids are used as preservatives, antiseptics, pesticides and drugs. We have found in previous studies that all these compounds inhibit the transport of amino acids into bacterial cells and they also inhibit the growth of mammalian cells. The inhibition is proportional to the partition coefficient (octanol/water) of the compounds and is also influenced by their ability to delocalize the electric charge when they are in the charged state. Since many known teratogens are lipophilic acids, we have proposed that all lipophilic acids which can strongly inhibit mammalian cell cultures are potential teratogens. This has been examined for some compounds here. We had also found previously that butyrate and other small molecular weight fatty acids have an unusual effect on mammalian cells in that they cause drastic shape changes and induce the production of large amounts of several membrane associated proteins. This induction has been useful for the investigation of those phenomena that are related to the transmission of information through the cell membrane such as the activation of adenylate cyclase via the attachment of compounds to cellular receptors. Depending on the amount of butyrate used, the production of receptors can be induced with and without coupling to adenylate cyclase. This paradigm has been employed to analyze the membrane components involved in the transmembrane signal.

Methods Employed: For teratological studies the fetuses produced by crosses of mice (C57BL/6J ϕ x DBA δ) were examined. The females were dosed from day 7 through day 11 of gestation at concentrations of the compound that were just not lethal or lethal only to a small percentage of the mice.

The number of cholera toxin receptors per cell was determined by measurement of specific binding of [¹²⁵I]-cholera toxin to suspended cells. The cholera toxin receptor, G_{M1}, was isolated from cells, separated by thin-layer chromatography on silica gel, and visualized and quantified with resorcinol reagent. β -adrenergic receptors were measured by determination of specific binding of [³H]-dihydroalprenolol. Both adenylate cyclase activity in broken-cell preparations, and cyclic AMP accumulation in whole cells were measured by determining the amount of cyclic AMP formed per unit time using a standard protein kinase binding assay. Activities of phospholipid methylating enzymes were determined in plasma membrane fractions by separating and quantifying the methylated lipids on silica gel thin-layer plates after incubation with S-adenosyl-[methyl-³H] methionine (SAM) as substrate. Phospholipid methylation in whole cells was determined by separating the lipids as above after incubation of whole cells with L-methyl-³H]-methionine equilibrated with the SAM pool.

Major Findings: 1. Teratogenicity of certain lipophilic acids. A number of lipophilic acids to which humans are frequently exposed were tested for their teratogenicity in mice. All these compounds inhibited tissue cultures at low concentrations. The results showed that the following compounds are teratogenic in mice and should, therefore, not be used during pregnancy by women: dichlorophene, thiopental, thymol, salicylanilid,

scoparone or undecylenic acid. The food additive, propylparaben showed no teratogenicity at the level of 25 mg per 10 g.b.w./day. Surprisingly, curcumin, a major ingredient of the Indian spice tumeric was also not teratogenic (or only slightly so) although it strongly inhibited tissue cultures. This compound was probably metabolized or excreted before it could reach the embryo.

2. Induction of cholera toxin receptors: with the knowledge that exposure of HeLa cells to butyrate induces the synthesis of β -adrenergic receptors and causes a striking increase in the ganglioside G_{M2} , and also knowing that the closely related ganglioside G_{M1} is the receptor for cholera toxin, it seemed reasonable to ask if butyrate induces cholera toxin receptors in HeLa cells. The results showed a 40-fold increase in cholera toxin receptors after 48 hours exposure of cells to 5 mM butyrate; this increase was reversible and occurred as well in serum-free medium. Butyrate also induced an elevation of cholera toxin receptors in rat C6 glial cells and Friend erythro-leukemic cells. A parallel increase was found in the amount of the ganglioside G_{M1} in cell membranes, as predicted from its role as the cholera toxin receptor. In addition to providing a useful model system for the study of the mechanism of action of cholera toxin, these results provide a useful tool for other studies of cell function inasmuch as cholera toxin can maximally activate a cell's adenylate cyclase when that cell is endowed with receptors for the toxin.

An *in vivo* role for GTP in the receptor-adenylate cyclase interaction was analyzed. Employing the anti-viral agent virazole at low, sub-growth inhibiting concentrations it was possible to deplete the intracellular levels of GTP to about 20% of control values without reducing ATP levels. Preliminary results indicate that when GTP levels are reduced by exposure of cells to virazole, the rate of coupling of β -adrenergic receptors to adenylate cyclase is substantially reduced. These *in vivo* results are in agreement with the *in vitro* studies of the Laboratory of Nutrition and Endocrinology, NIAMDD, who proposed such a role for GTP.

3. Relationship between β -adrenergic receptor function and phospholipid methylation. HeLa cells, like many other cell types, have an asymmetric distribution of the phospholipids phosphatidylethanolamine (PE) and phosphatidylcholine (PC) in their plasma membrane. In collaboration with Psychiatry Branch of the National Institute of Mental Health, we have recently found that HeLa cells also contain two methyltransferases which, with S-adenosyl methionine (SAM) as methyl donor, catalyze three successive N-terminal methylations of PE yielding PC; in the process of these methylations the phospholipid migrates from the inner to the outer surface of the plasma membrane. We have also described three additional important features of this system: a) Maintenance of β -adrenergic receptor number on the surface of the cell is dependent on continuing methylation of PE to PC; when inhibitors of SAM-mediated methylation such as deazaadenosine are present, methylation of PE to PC is greatly reduced and the number of β -adrenergic receptors on the cell surface declines markedly. b) Occupancy of receptors by β -adrenergic agonists such as isoproterenol causes a 10-fold stimulation in the rate of methylation of PE to PC. This stimulation is blocked by

β -adrenergic antagonists such as propranolol but not by α -adrenergic antagonists such as phentolamine. Isoproterenol-stimulation of methylation is specific for phospholipid methylation; carboxymethylation and mRNA methylation are not influenced by the presence of the hormone. The stimulation of phospholipid methylation described above depends on occupancy of the β -adrenergic receptor by agonists, but appears to be independent of the concomitant elevation of intracellular cyclic AMP levels; when adenylate cyclase is maximally activated by exposing butyrate-treated HeLa cells to cholera toxin, cyclic AMP levels in the cell increase markedly but the rate of phospholipid methylation is not affected. Preliminary results indicate that the stimulation of phospholipid methylation by agonists is not peculiar to β -adrenergic receptors. Current experiments are intended to explore the extent to which this phenomenon is general with respect to receptor function. c) Concomitant with the stimulation of phospholipid methylation by agonist occupying β -adrenergic receptors, the activity of an intracellular phospholipase is stimulated. This enzyme appears to be involved in the degradation of the PC in the cell membrane, and to be responsible for the rapid turnover of this membrane component. Current research efforts are intended to evaluate the suitability of this model system for elucidating the mechanism of desensitization; the level of PC in the immediate milieu of the receptor, as regulated by both its synthesis and its degradation, may be critical for the maintenance of cell surface receptors. If so, chronic occupancy of receptor by agonist might lead to an imbalance between synthesis and degradation of PC due to phospholipase induction, thus providing an explanation for the mechanism of desensitization.

Proposed Course of Project: The present project has been terminated but parts of it will be taken up in two new projects entitled, "Development and Teratology in Rodent Embryo Culture," and "Intracellular Communication and Transmembrane Signals".

Publications:

Freese, E.: Mechanism of growth inhibition by lipophilic acids. In Kabara, J. (Ed): Pharmacological Effect of Lipids, American Oil Chemists' Society, Champaign, Illinois, 1978, pp. 123-131.

Tallman, J.F., Smith, C.C. and Henneberry, R.C.: Properties of β -adrenergic receptors in untreated and butyrate-treated HeLa cells. Biochim Biophys. Acta, 541: 288-300, 1978.

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Fishman, P.H., and Atikkan, E.E.: Induction of cholera toxin receptors in cultured cells by butyric acid. J. Biol. Chem., 1979 (in press).

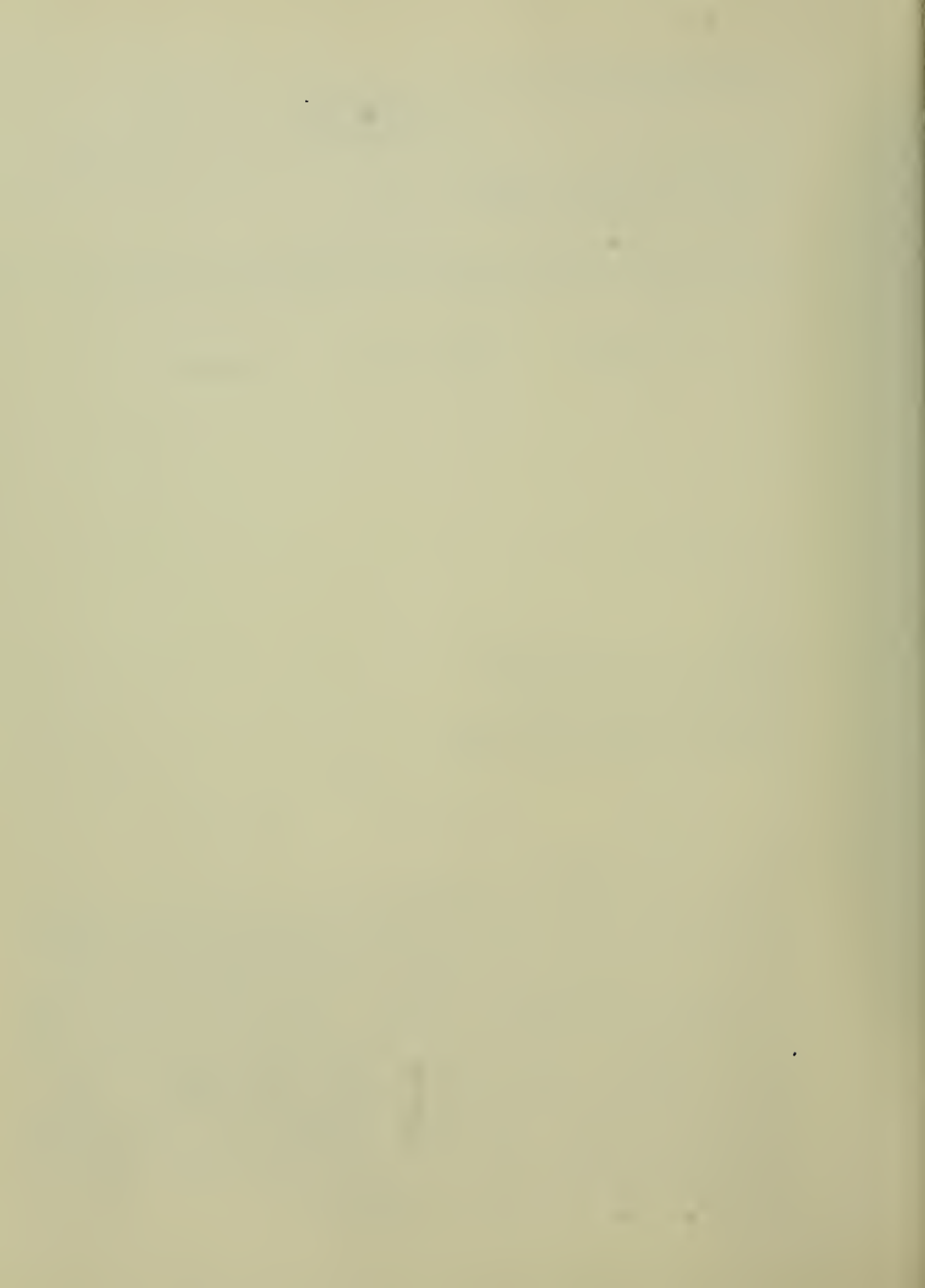
Freese, E., Levin, B., Pearce, R., Sreevalson, T., Kaufman, J.J., Koski, W.S. and Semo, N.M.: Correlation between the growth inhibitory effects, partition coefficients and teratogenic effects of lipophilic acids. Teratology, 1979 (in press).

Hirata, F., Tallman, J.F., Henneberry, R.C., Mallorga, P., Strittmatter, W.J. and Axelrod, J.: Role of phospholipid methylation in hormone receptor interactions. In Kuhar, M., and Pepeu, G. (Eds): Receptors, Neurotransmitters and Neuropeptides, Raven Press, New York, 1979 (in press).

Tallman, J.F., Henneberry, R.C., Hirata, F. and Axelrod, J.: Control of β -adrenergic receptors in HeLa cells. In Usdin, E. (Ed): Catecholamines: Basic and Clinical Frontiers, Pergammon Press, Oxford, 1979 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02364-01 LMB									
PERIOD COVERED October 1, 1978 through September 30, 1979											
TITLE OF PROJECT (80 characters or less) Development and Teratology in Rodent Embryo Culture											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 33%;">PI: E. Freese</td> <td style="width: 33%;">Chief, Lab. Molec. Biol.</td> <td style="width: 33%;">LMB NINCDS</td> </tr> <tr> <td>R.C. Henneberry</td> <td>Senior Scientist</td> <td>LMB NINCDS</td> </tr> <tr> <td> OTHER: A. Bruckner</td> <td> Visiting Fellow</td> <td> LMB NINCDS</td> </tr> </table>			PI: E. Freese	Chief, Lab. Molec. Biol.	LMB NINCDS	R.C. Henneberry	Senior Scientist	LMB NINCDS	 OTHER: A. Bruckner	 Visiting Fellow	 LMB NINCDS
PI: E. Freese	Chief, Lab. Molec. Biol.	LMB NINCDS									
R.C. Henneberry	Senior Scientist	LMB NINCDS									
 OTHER: A. Bruckner	 Visiting Fellow	 LMB NINCDS									
COOPERATING UNITS (if any) None											
LAB/BRANCH Laboratory of Molecular Biology											
SECTION Developmental Biology Section											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20205											
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) <p>The goal of this project is to evaluate a recently introduced method of <u>rodent embryo culture</u> for its suitability as a model system for basic studies in <u>developmental biology</u> and as a test system for potentially <u>teratogenic compounds</u>. This method permits <u>in vitro</u> development of <u>rat or mouse embryos</u> at normal rates for more than 48 hours, during which numerous <u>developmental changes</u> can be observed. Developmental studies will emphasize the sequence and timing of such <u>biochemical markers</u> as biosynthetic <u>enzymes</u> and cell surface <u>receptors</u> for various <u>hormones</u> and <u>neurotransmitters</u>. The efficacy of this system for the identification of <u>teratogens</u> will be examined using compounds known to cause birth defects; the approach will then be extended to suspected <u>teratogens</u>. The latter class of compounds will be compiled on the basis of certain physico-chemical properties employing the predictive knowledge gained from previous studies in this laboratory on the growth-inhibitory effects of various <u>food additives</u>, <u>preservatives</u>, <u>antiseptics</u>, and <u>drugs</u> on <u>bacteria</u> and <u>mammalian cells</u> in culture.</p>											

BIOSCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02365-01 LMB
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Intercellular Communication and Transmembrane Signals		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R.C. Henneberry Senior Scientist LMB NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION Developmental Biology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The goal of this project is to elucidate the molecular mechanisms underlying the propagation and receipt of biochemical signals by cells, with particular emphasis on the means by which certain <u>hormone</u> and <u>neurotransmitter</u> messages are communicated across the plasma <u>membrane</u> . This research direction has evolved during 6 years of studies in this Laboratory on cell surface component changes, in particular those components involved in the induction and mechanism of action of <u>β-adrenergic</u> and <u>cholera toxin receptors</u> . These studies will continue with increased emphasis on the biochemical events involved in the maintenance of cell surface receptors and in the phenomenon of <u>desensitization</u> . As an extension of this work, the role of <u>tropic hormones</u> in <u>central nervous system</u> control of <u>pituitary</u> function will be examined in cultured cells. As a specific example, the possibility of a role for <u>phospholipid methylation</u> in the response of the pituitary cell line GH3 to TRH by release of <u>prolactin</u> will be explored. Further studies on the mechanism of <u>transmembrane signalling</u> will exploit the properties of some recently derived hybrid cell lines which contain several different <u>hormone/neurotransmitter receptors</u> on a single cell.		



ANNUAL REPORT

October 1, 1978 through September 30, 1979

Laboratory of Neuro-otolarvngology
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1978 through September 30, 1979
Laboratory of Neuro-otolaryngology, IRP
National Institute of Neurological and
Communicative Disorders and Stroke

Jürgen Fex, M.D., Ph.D., Chief

The Laboratory has continued its multidisciplinary approach with the focus on the inner ear and cochlear nucleus of mammalian species, of normal animals as well as of genetically deaf animals. The two Projects of the Laboratory have been advanced, these being Project Number Z01 NS 02216 04 LNO, Inner Ear Neuronal Mechanisms: A Multidisciplinary Analysis, and Project Number Z01 NS 02217 04 LNO, Synaptic Transmission and Neuronal Connections of the Mammalian Cochlear Nucleus. In particular, during this fiscal year, the Laboratory contributed with the following new knowledge.

The refinement of methods for complete analysis of free and protein-bound amino acids at levels occurring for inner ear samples has, at least for the time being, been completed. As described in last year's report, these methods now permit total amino acid analyses, including accurate determination of cysteine and cystine, of tissue extracts and purified peptides and proteins in one run, using picomole quantities of sample. The final manuscript describing the new techniques has been accepted for publication.

As described last year, the high-resolution method for two-dimensional separation of membrane proteins has been worked out in the Laboratory. This sub-project is directed at biochemically characterizing cochlear proteins and identifying those proteins that are abnormal in genetically deaf animals. Proteins of the stria vascularis of the normal and genetically deaf waltzing guinea pig has been analyzed by one and two-dimensional acrylamide gel electrophoresis. Proteins that are exposed on the endolymphatic surface of the stria vascularis have been identified by lactoperoxidase-catalyzed iodination. No consistent changes in protein patterns of the stria vascularis from the waltzing guinea pig were detected. A manuscript on this study is in preparation.

In vitro uptake of putative neurotransmitters into the organ of Corti has been studied by autoradiography. It was intended that these studies might help to characterize the hair cell neurotransmitter. After incubation in ^3H -GABA, ^3H -glutamate and ^3H -aspartate, heavy labeling in the organ of Corti was seen over the fibers and terminals of the efferent olivocochlear bundle. That GABA, glutamate and aspartate were taken up into efferents, which are almost certainly cholinergic, suggests that high affinity uptake of these substances is not restricted to those terminals in which these substances are released as neurotransmitters. A manuscript describing this study is in press.

The last two years' study on cochlear efferents was published this year. The findings were that α -bungarotoxin reversibly blocked efferent inhibition of auditory activity in the cochlea of the cat, strengthening the hypothesis

that cochlear efferent receptors are cholinergic and suggesting that these receptors are different from cholinergic receptors at most other vertebrate synapses.

The last few years' studies on glutamate and aspartate as putative transmitters of the auditory nerve in the cochlear nucleus have been extended as follows.

Last year's submitted study on kainic acid has been published, showing that kainic acid when injected into the brainstem produces a rate and extent of degeneration that is correlated with the distribution of the primary auditory nerve fibers. This may be looked upon as evidence that glutamate is an auditory nerve transmitter.

A study is in print on the effects of DL- α -aminoadipate, a dicarboxylic amino acid antagonist, on synaptically and chemically evoked excitation of anteroventral cochlear nucleus neurons of the cat. Using microelectrode techniques with iontophoretical application of drugs it was found that in one group of neurons the action of amino acid excitants was antagonized by the aminoadipate but the synaptic excitation through auditory stimulation of these neurons was rarely affected. In another group, physiologically different from the first, the neurons were unresponsive to or suppressed by the amino acid excitants, while the aminoadipate readily blocked the synaptic, auditory excitation. These results support the hypothesis that glutamate or aspartate (or both) is (are) the neurotransmitter(s) of the auditory nerve.

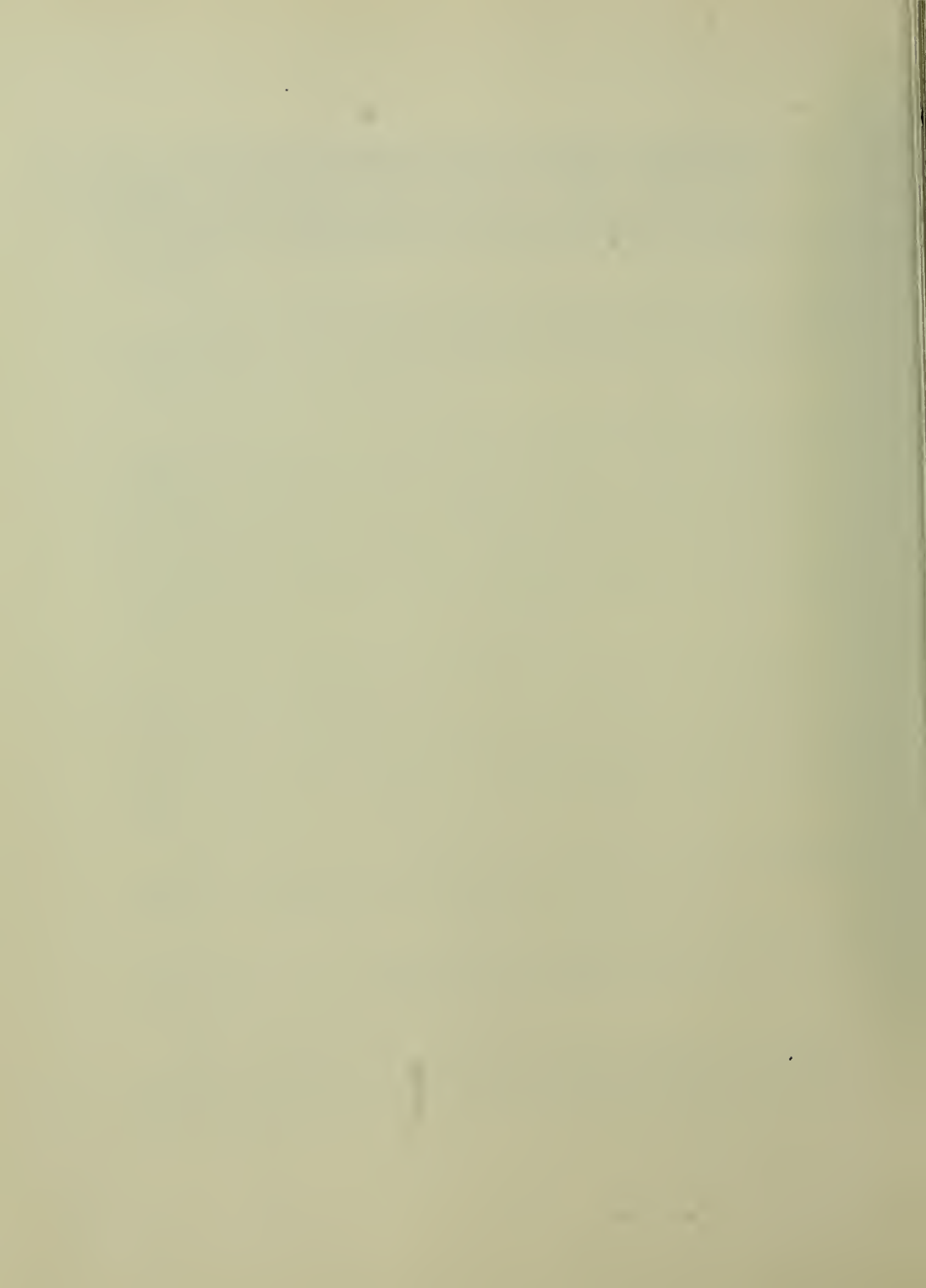
A biochemical study initiated and partly described last year has been published on calcium-dependent release with elevated potassium of amino acids from cochlear nucleus slices. It was shown that three days after auditory nerve lesion, the content of total glutamic acid is reduced 15% in the cochlear nucleus while the calcium-dependent release of glutamic acid was reduced 41%. Total aspartic acid was reduced 31% in the cochlear nucleus after auditory nerve lesion while the calcium-dependent release of aspartic acid was reduced 26%. The results suggest that glutamic acid may be a more likely candidate for the auditory nerve transmitter than aspartic acid.

A manuscript is under preparation of a biochemical study of enzymes related to the metabolism of aspartate and glutamate. The results indicate that two such enzymes, aspartate aminotransferase and glutaminase, are concentrated in both axons and terminals of the auditory nerve.

A manuscript is under preparation of a study of axonal transport in the auditory nerve. This represents the first characterization of proteins in the auditory nerve. A presynaptic membrane-associated glycoprotein with a very short half-life has been identified.

The studies of the neural connections of the cochlear nucleus have been continued. Findings described in previous annual reports have resulted in a publication in which also, and for the first time, the cytology of the nuclei of the lateral lemniscus has been described. Concerning these nuclei it has been shown, to be described in a future paper, that they contain a

certain class of cells that receive endings from octopus cells in the contralateral cochlear nucleus. The termination of the axons of the octopus cells was previously unknown. During the course of these studies, the use of a computer model for cell plotting has been extended, and the HRP technique for marking cells and a Golgi method for staining cells have been refined.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02216 04 LNO
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Inner Ear Neuronal Mechanisms: A Multidisciplinary Analysis.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Fex	Chief, LNO	LNO NINCDS
	J. C. Adams	Senior Staff Fellow	LNO NINCDS
	R. J. Wenthold	Senior Staff Fellow	LNO NINCDS

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Neuro-otology

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3	PROFESSIONAL: 1.4	OTHER: 1.6
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-range purpose of the project is to study the biochemistry, morphology, pharmacology and physiology of inner ear neurons and cells of their interactions and to describe the mechanisms of these interactions.

1. The techniques for total micro amino acid analysis of picomole quantities of sample have been refined to permit the determination also of cysteine and Cystine. 2. Two-dimensional electrofocusing/electrophoresis has been applied to the analysis of cochlear proteins. No consistent differences were seen between the normal and genetically deaf waltzing guinea pig in the protein patterns of the stria vascularis. 3. Results from application of α-bungarotoxin to cochlea strengthened the hypothesis that postsynaptic receptors of cochlear efferents are cholinergic and indicate that these receptors are different from most other kinds of cholinergic receptors at vertebrate synapses. 4. Auto₃ radiography after in vitro incubation with the labeled amino acids H-GABA, ³H-glutamate and ³H-aspartate showed heavy labeling over fibers and terminals of the efferent olivocochlear bundle.

Project Description:

Objectives: To study the biochemistry, morphology, pharmacology and physiology of inner ear neurons and cells and of their interactions and to describe the mechanisms of these interactions.

The following subprojects are now serving these objectives:

- I. Refinements of techniques for total micro amino acid analysis of picomole quantities of sample.
- II. A study of proteins of the cochlea of normal and genetically deaf animals.
- III. A physiological study of synaptic transmission in the inner ear.
- IV. A study of uptake of putative neurotransmitters in the organ of Corti.

Methods Employed:

I. The methods that have been worked out in this laboratory for total micro amino acid analysis have been described in previous annual reports. Greater detail can be found in the publication listed at the end of this section.

II. Cochlear proteins are labeled by exchanging the perilymph with a solution containing ^3H - or ^{35}S -labeled amino acids or fucose. The cochlea is then dissected under fluid and the proteins analyzed by one-dimensional gel electrophoresis or two-dimensional electrofocusing/electrophoresis. Labeled proteins are detected by fluorography. Endolymphatic surface proteins of the stria vascularis are labeled by dissecting, in buffer, strips of spiral ligament with the stria vascularis attached. The surface proteins are then labeled by lactoperoxidase-catalyzed iodination. The tissue is washed and the stria vascularis is dissected from the spiral ligament.

III. Cats were deeply anesthetized, curarized and artificially respirated. α -Bungarotoxin at concentrations of 0.2 - 10.0 μM in artificial perilymph was injected into the basal turn of the cochlea. The crossed olivocochlear fibers were stimulated electrically. Cochlear responses to sound and to stimulation of olivocochlear fibers were recorded and processed, using amplifiers, tapes and a computer.

IV. Guinea pigs weighing between 250 and 325 g were decapitated, the left temporal bone was taken out and the bulla was opened and chipped away, as was most of the bone around the cochlea. The cochlea was dissected in a phosphate-buffered saline solution containing 120 mM NaCl, 5 mM KCl, 10 mM glucose, 1.3 mM MgSO_4 , and 20 mM NaPO_4 at pH 7.4, bubbled with 95% O_2 + 5% CO_2 . The cochlear shell and part of the spiral ligament with the stria was

removed. The bony modiolus with the organ of Corti, from the end of the first turn to apex, was transferred under fluid to a conical Beem capsule for incubation and further processing.

The bony modiolus and organ of Corti were incubated for 20 minutes at 30°C in 100 μ l of the buffer with the radioactive substance at a concentration of 10^{-6} M. The ^3H -amino acids used for incubation had the following specific activities: GABA, 36.7 Ci/mmmole; glutamic acid, 23.4 Ci/mmmole; aspartic acid, 17.8 Ci/mmmole; glycine, 9.4 Ci/mmmole; leucine, 50.0 Ci/mmmole. After a brief wash in the buffered saline, the tissue was fixed for 1 hour in 4% para-formaldehyde in 0.1 M sodium cacodylate with 20 mM CaCl_2 . The spiral was dissected into turns, washed in 0.2 M sodium cacodylate with 20 mM CaCl_2 and postfixed in 2% OsO_4 in 0.1 M sodium cacodylate. The tissue was then dehydrated in methanol and embedded in Araldite. From each turn, 30 sections, 1-2 μ m thick, were cut and placed on slides. The slides were dipped in NTB-2 Kodak emulsion and stored at 4°C for 4 weeks before developing in D-19. The sections were stained with methylene blue-Azure II.

Major Findings:

I. The results concerning total micro amino acid analysis of picomole quantities of sample were described in the previous annual report and can be found in greater detail in the publication listed at the end of this section. Suffice it here to mention that with the technique worked out in this laboratory for picomole samples, adequate sensitivity has been reached for detection of mutant proteins having single cysteine substitutions.

II. Proteins of the stria vascularis were effectively labeled by replacing the perilymph with a solution containing radioactive amino acids or fucose. After ^{35}S -methionine labeling, more than 200 polypeptides could be detected with two-dimensional electrophoresis. Using labeling times of 1, 3 and 6 hours, qualitatively similar electrophoretic profiles of stria proteins were seen, but relative magnitudes of the labeled polypeptides differed. Glycoproteins were determined by labeling with fucose. No consistent differences in protein patterns of the stria vascularis were seen between the normal and the genetically deaf waltzing guinea pig after labeling with either ^{35}S -methionine, ^3H -fucose or ^3H -leucine.

Six polypeptides were labeled by lactoperoxidase-catalyzed iodination. The ^{125}I -labeled polypeptides co-migrated with relatively minor polypeptides that were labeled with ^{35}S -methionine.

III. Results of studies including the application of α -bungarotoxin to the cochlea strengthened the hypothesis that the postsynaptic receptors of the crossed cochlear efferent nerve fibers are cholinergic and that these receptors are different from most other kinds of cholinergic receptors at vertebrate synapses. See previous annual report and publication listed at the end of this section for greater detail.

IV. Autoradiography after incubation in ^3H -glycine showed that the label was heaviest over the inner hair cell, but was not confined to the synaptic region of the cell. After incubation in ^3H -GABA, ^3H -glutamate and ^3H -aspartate, heavy labeling was seen over the fibers and terminals of the efferent olivocochlear bundle. Leucine, an amino acid not thought to be a neurotransmitter, was uniformly taken up by all cochlear structures. The fact that GABA, glutamate and aspartate are taken up into efferents, which are almost certainly cholinergic, suggests that high affinity uptake of these substances is not restricted to terminals in which these substances are released as neurotransmitters.

Significance to Biomedical Research and the Program of the Institute:
This multidisciplinary study on inner ear structures and mechanisms, including the sensory cells and the neurons and their interactions, provides new knowledge on the poorly understood mechanisms of hearing. Such knowledge is of direct significance to biomedical research, will lead to better understanding of the causes of sensory deafness and nerve deafness and will most likely lead to better management of hearing disorders.

In particular, referring to subprojects:

I. The completion of these studies allows us to do total amino acid analyses, including accurate determination of cysteine and cystine, of tissue extracts and purified peptides and proteins, on a micro scale. A total micro amino acid analysis of high accuracy can now be performed in one run, using picomole quantities of sample.

II. This project is designed to investigate genetic hearing disorders at the molecular level. Although many genetic hearing disorders have been identified and studied morphologically, little work has been done to identify the protein or proteins involved in these disorders. By using animal models, this study may lead to a better understanding of genetic hearing disorders in humans. Furthermore, this study may provide new knowledge on the biochemical mechanisms involved in normal cochlear function.

III. The evidence that there may be cholinergic receptors in the vertebrate cochlea that are different from cholinergic receptors elsewhere in vertebrates is of general interest in the field of biomedical research.

IV. The implication by the findings of this subproject that high affinity uptake of certain amino acids is not restricted to terminals in which these substances are released as neurotransmitters is of general interest in the field of biomedical research.

Proposed Course:

The techniques for total micro amino acid analysis of picomole quantities of sample and for high resolution, two-dimensional separation of membrane proteins will be applied to a study of amino acids and a continued study of

proteins of the cochlea of normal and genetically deaf animals.

Publications:

Fex, J. and Adams, J. C.: α -Bungarotoxin blocks reversibly cholinergic inhibition in the cochlea. Brain Research 159: 440-444, 1978.

Gulley, R. L., Fex, J. and Wenthold, R. J.: Uptake of putative neurotransmitters in the organ of Corti. Acta Otolaryngologica (Stockholm). In press.

Lee, K. S. and Drescher, D. G.: Derivatization of cysteine and cystine for fluorescence amino-acid analysis with the o-phthaldialdehyde/2-mercaptoethanol reagent. J. Biol. Chem. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02217 04 LNO
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Synaptic Transmission and Neuronal Connections of the Mammalian Cochlear Nucleus		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	J. Fex J. C. Adams R. A. Altschuler M. R. Martin R. J. Wenthold	Chief, LNO Senior Staff Fellow Staff Fellow Staff Fellow Senior Staff Fellow LNO NINCDS LNO NINCDS LNO NINCDS LNO NINCDS LNO NINCDS
OTHER:	P. G. Nelson G. K. Bergey M. Hermes J. DeLeo D. Foxvog	Chief, LDN Research Associate Guest Worker Computer Systems Analyst Electronics Engineer LDN NICHD LDN NICHD LDN NICHD CSL DCRT CSL DCRT
COOPERATING UNITS (if any) LDN, NICHD; CSL, DCRT S.J. Bird, Dept. Pharmacol., Sch. Med., Case Western Reserve Univ., Cleveland, Ohio 44106; R.L. Gulley, Dept. Anat., Univ. Texas, San Antonio, Texas 78284; R. Lasek and M. Tytell, Dept. Anat., Case-Western Reserve, Cleveland, Ohio 44106		
LAB/BRANCH Laboratory of Neuro-otolaryngology		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 8.3	PROFESSIONAL: 3.6	OTHER: 4.7
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<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINDRS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of the project is to study the biochemistry, morphology, pharmacology and physiology of synaptic transmission and neural connections of nerve cells of the mammalian cochlear nucleus. New results from the following studies support a neurotransmitter role for glutamate and aspartate in the cochlear nucleus: a morphological study of kainic acid induced lesions in the cochlear nucleus; effects of DL- α -aminoadipate on synaptically and chemically evoked excitation of anteroventral cochlear nucleus neurons of the cat; release of endogenous glutamate, aspartate and GABA from cochlear nucleus slices; activity of enzymes associated with the metabolism of glutamate and aspartate in the cochlear nucleus. The first characterization of proteins in the auditory nerve is being undertaken through a study of axonal transport. In the continued study of connections of the cochlear nucleus the projection of the octopus cells has been determined, refinements has been made of the HRP technique and of a Golgi method, and the computer model for cell plotting has been extended.		

Project Description:

Objectives: To study the biochemistry, morphology, pharmacology and physiology of synaptic transmission and neuronal connections of nerve cells in the mammalian cochlear nucleus.

The following subprojects are now serving these objectives:

I. a. A morphological study of kainic acid induced lesions in the cochlear nucleus.

b. Morphological study of putative neurotransmitter.

II. Physiological/pharmacological studies of synaptic transmission in the cochlear nucleus.

a. Effects of DL- α -amino adipate on synaptically and chemically evoked excitation of anteroventral cochlear nucleus neurons of the cat.

b. A study of the pharmacology of dicarboxylic amino acid antagonists.

III. Biochemical studies of synaptic transmission in the cochlear nucleus:

a. Release of endogenous glutamate, aspartate and GABA from cochlear nucleus slices.

b. Activity of enzymes associated with the metabolism of glutamate and aspartate in the cochlear nucleus.

IV. Axonal transport in the auditory nerve.

V. Connections of the cochlear nucleus.

a. Ascending projections to the inferior colliculus.

b. Distribution of putative amino acid transmitters, choline acetyltransferase (CAT) and glutamate decarboxylase (GAD) in the inferior colliculus.

c. Identification of cells that contribute fibers to acoustic stria, using a stabilized tetramethyl benzidine based HRP reaction product and following its removal.

d. A fast, reliable silver-chromate Golgi method for perfusion-fixed tissue.

Methods Employed:

I. a. Guinea pigs were anesthetized and stabilized in a stereotaxic apparatus. Phosphate-buffered saline containing 1.0, 0.5, 0.15, or 0.05 mg of kainic acid per milliliter, pH 7.4, was infused into the brainstem through a 30-gauge needle, 0.2 to 0.5 mm medial to the cochlear nucleus. The volume infused was always 2 μ l, including control injections. Animals were killed at 1, 3, 6, 12, 18 and 24 hours after injection by cardiac perfusion. Slices of the brainstem containing the cochlear nuclei, both ipsilateral and contralateral to the injection site, were embedded. Sections 1 to 2 μ m thick from all regions of the cochlear nuclei were cut and examined with the light microscope. Two of the animals were injected with 2 μ g of kainic acid as described above, and after 3 hours they were killed by perfusion with 10 percent formalin. The brainstem was removed and frozen serial sections, 20 μ m thick, through the cochlear nucleus were cut in the parasagittal plane. These sections were stained with thionine.

b. The indirect immunofluorescence technique of Coons has been used on 10 micron cryostat sections to localize met-enkephalin immunoreactivity in the rat cochlear nucleus. Rats receiving an intracisternal injection of 25 μ g of colchicine in 10 μ l of phosphate buffered saline and non-treated rats were examined. Colchicine blocks axonal transport and enabled immunoreactivity to be visualized in the cell bodies of neurons in the cochlear nucleus. An absorption control to test for specificity was done on a semi-adjacent section. Nissl stains on adjacent, or the same, sections after quenching of fluorescence were used to identify cell types and regions.

The only ultrastructural description of met-enkephalin immunoreactivity (Pickel et al., Brain Res. 160: 387, 1979) described immunoreactivity in the locus coeruleus and A2 regions of the rat brain. We have modified their procedure in an investigation of immunoreactivity in the rat globus pallidus. This area was chosen because of its high met-enkephalin content. We have found that increasing the time of fixation and changing the percentage of gluteraldehyde in the fixative give an enhanced morphology without a significant decrease in immunoreactivity. A protocol was developed for immunocytochemistry. Controls, with preabsorbed primary antibody, were run side by side with experimentals. No detergents were used at any steps.

II. a. Experiments were performed in a sound chamber on anesthetized cats. Action potentials of single units were recorded extracellularly from the anterior and posterior divisions of the anteroventral cochlear nucleus using the 4M NaCl-filled center barrel of seven barrel microelectrodes. Five of the six outer barrels contained the compounds to be ejected electrophoretically using standard procedures (Curtis, 1964). These compounds included the monosodium salts of 200 or 500 mM L-glutamate and L-aspartate, 50 mM N-methyl-D-aspartate in 100 mM NaCl, 20 mM kainate in 130 mM NaCl and 200 mM DL- α -aminoadipate. Acetylcholine chloride was used in a 200 mM solution (ACh, pH 4). The sixth outer barrel contained 4M NaCl for an automatic current balancing channel. In the brainstem auditory system, those cells that receive large, calyceal auditory nerve endings show a peculiar waveform associated

with unit action potentials. Preceding each action potential by approximately 0.5 msec is a "prepotential" that has been shown to be presynaptic. The presence or absence of prepotentials were used for categorizing unit types. The rate of unit discharge was computed and displayed on a potentiometric chart recorder for on-line assessment of drug action. Averages and histograms were made on-line with a PDP-11 computer, records were kept on magnetic analog tape.

b. Fetal mouse spinal cord neurons were dissociated and cultured. After 5-10 weeks of growth, cultures were placed on a temperature and pH controlled stage of an inverted phase microscope. Intracellular recordings were made. Tetrodotoxin was added to the culture media to suppress spontaneous activity in the cultures. Current pulses of 0 to 120 nA, 100 msec in duration were used to administer glutamate, aspartate, γ -aminobutyric acid and glycine from microelectrodes close to (1-2 microns) neuronal cell membrane when eliciting responses from the cell bodies or processes. Modulation of these responses by DL- α -aminoadipate or L- α -aminadipate was tested by current application (5 to 100 nA) from a microelectrode tip immediately adjacent to the respective amino acid electrode.

III. a. A short description of methods for this study was given in last years' annual report; greater detail can be found in the publication listed at the end of this section.

b. The effects of auditory nerve lesion on the enzymes, aspartate aminotransferase (AAT), glutamate dehydrogenase (GD), glutaminase (Glnase) and glutamine synthetase (GS) in the cochlear nucleus were studied. Animals received unilateral cochlear ablations and, 3 or 14 days later, the animals were killed and both cochlear nuclei were dissected. The cochlear nuclei were divided into the ventral cochlear nucleus (VCN) and the dorsal cochlear nucleus (DCN). Enzyme activities were determined by standard fluorometric and spectrophotometric assays.

IV. Axonal transport studies were done in guinea pigs, 250-325 grams. Animals were anesthetized with urethane, the cochlea was exposed, and the perilymph was exchanged with a solution containing the radioactive precursor. Usually 120-150 μ Ci of 35 S-methionine or 200-350 μ Ci of 3 H-fucose were injected. After periods of 1h to 10 days, animals were killed and the cochlear nucleus dissected and divided into the anteroventral cochlear nucleus (AVCN), posteroventral cochlear nucleus (PVCN), dorsal cochlear nucleus (DCN), interstitial nucleus (IN) and auditory nerve (AN). Proteins were analyzed by one-dimensional gel electrophoresis and two-dimensional electrofocusing/electrophoresis. Radioactivity was localized in dried gels by fluorography.

V. a. Ascending projections to the inferior colliculus were studied following injection of horseradish peroxidase into its central nucleus. Plots of labeled cells were made using a computer, which facilitated visualization of patterns of labeling as well as cell counts. As a complement to this study, an atlas of the cells in the superior olivary complex is under construction. To make the atlas, the positions of about 35,000 cells in the olivary complex of a cat were recorded in three dimensions using the laboratory

computer. These data were sent to the Division of Computer Research and Technology where a three dimensional block model of the olivary complex is being constructed. For further detail see publication listed at end of section.

b. Adult male cats were used. Fresh brain tissue was immersed 25 seconds in isopentane at -60°C , mounted on dry ice and cut at -20°C in a cryostat. Coronal sections about $200\ \mu\text{m}$ thick were collected on dry ice and lyophilized at -60°C . The freeze dried sections were transilluminated and the inferior colliculus dissected into 10 subdivisions using an operating microscope. Enzymes were measured as described in previous annual reports and publications. Amino acids were extracted with ethanol and measured with an Aminco amino acid analyzer. Protein was determined by the method of Lowry et al. In one group of animals, the right combined dorsal acoustic stria and intermediate acoustic stria at the medial extent of the dorsal cochlear nucleus was cut. In a second group, the left auditory cortex was removed by aspirating the region including AI, AII, EP, the anterior auditory field, and adjacent structures lying beneath the sylvian fissure. Two weeks later the animals were killed and the inferior colliculus analyzed.

c. Adult cats were anesthetized and the posterior cerebellum aspirated to expose the base of the right restiform body. Just medial to the tip of the dorsal cochlear nucleus a parasagittal cut was made to sever the combined dorsal and intermediate striae. A pipette containing HRP (ca. 40% aqueous solution) was inserted into the cut and 0.2 - 0.5 μl HRP injected. The injection site was covered with 2% agar, which prevented loss of HRP by dilution with CSF. Anesthesia was maintained until the animals were killed after 24 hours. The animals were perfused intracardially, sections were cut and, immediately following sectioning, the tissue was incubated for the demonstration of the presence of HRP using either a modified diaminobenzidine procedure or the tetramethyl benzidine method of Mesulam with a modification of the stabilization procedure. To identify labeled cells also by their Nissl pattern a method was devised to remove the tetramethyl benzidine based reaction product and inspect the previously labelled cells.

d. The procedure for the adult cat brain is as follows: Intracardiac perfusion to exsanguinate with about 300 ml of 0.5% NaNO_2 in saline. Continue the perfusion with one liter of 10% formalin in saline. (If electron microscopy of the tissue is anticipated paraformaldehyde-glutaraldehyde mixtures can be substituted.) Continue the perfusion with one liter of mordant. To make the mordant, add 50 gm chloral hydrate and 50 gm potassium dichromate to 800 ml H_2O . When these are in solution add 100 ml formalin, then bring the volume to 1 liter. Remove the brain, cut the tissue into 3-5 mm blocks and place in 100-200 ml of mordant in the dark, undisturbed for 3 days. Place tissue blocks into 1% silver nitrate. Let the tissue remain in silver nitrate in the dark for 3 days. Cut 100-150 μm sections on Vibratome with 2% potassium dichromate in the tissue bath. Mount sections on subbed slides from a 2% potassium dichromate solution. Blot the sections firmly with tissue paper. After a few moments when the surface of the tissue appears dry, immerse the slide in 100% ethanol. Sections can be collected in ethanol

until cleared. Clear in methyl salicylate. This prevents loosening of section from slides by xylene. When sections are clear, rinse them briefly in xylene to remove excess methyl salicylate before removing them from the fume hood. Coverslip, using a neutral mounting medium.

Major Findings:

I. a. When injected into the brainstem, kainic acid produces a rate and extent of degeneration that is correlated with the distribution of the primary auditory nerve fibers which may be taken as evidence that glutamate is an auditory nerve transmitter.

b. Met-enkephalin fluorescent immunoreactivity was present in the small cells of the deep layer of the dorsal cochlear nucleus and immunoreactive somas of small cells were also seen in the posteroventral cochlear nucleus near the border of the dorsal cochlear nucleus. Positive fibers were seen in the dorsal, posteroventral and anteroventral cochlear nucleus and in the dorsal and intermediate acoustic striae.

We have been successful in modifying existing pre-embedding staining immunocytochemical techniques to demonstrate met-enkephalin immunoreactivity at the ultrastructural level. Good preservation of morphology has enabled the synaptic relations of met-enkephalin positive terminals to be examined. We are still in the process of analyzing the data gathered.

II. a. Non-prepotential units responded to iontophoretically applied excitants in a manner similar to units in other regions of the CNS. The amino acid action was antagonized by DL- α -amino adipate but the synaptic excitation of these units was rarely affected. Prepotential units normally either did not respond or showed depolarization block to the excitants. DL- α -amino adipate readily blocked the synaptic excitation of these units. The results support the supposition that either glutamate or aspartate (or both) is the primary afferent transmitter of the auditory nerve.

b. DL- α -amino adipate (up to 100 nA) reduces aspartate responses by 87%. The plot of antagonism is exponential with a 50% reduction occurring at 20 nA of DL- α -amino adipate. In contrast 100 nA of DL- α -amino adipate reduces the glutamate response by 24%. DL- α -amino adipate has no effect on γ -aminobutyric acid or glycine and produces no change in neuronal membrane potentials or input resistances up to currents of 200 nA. L- α -amino adipate has no discernable effects with currents up to 100 nA. These results confirm that DL- α -amino adipate, in low to moderate amounts, is an effective and specific dicarboxylic amino acid antagonist that most potently reduces the response to iontophoretically applied aspartate.

III. a. Three days after auditory nerve lesion, the content of total glutamic acid is reduced 15% in the cochlear nucleus while the calcium-dependent release of glutamic acid was reduced 41%. Total aspartic acid was reduced 31% in the cochlear nucleus after auditory nerve lesion while the calcium-dependent release of aspartic acid was reduced 26%. The results

suggest that glutamic acid may be a more likely candidate for the auditory nerve transmitter than aspartic acid.

b. Although the cochlear nucleus was not enriched in AAT or Glnase, compared to other brain regions, the auditory nerve was 2-5 times higher in these enzymes than other nerves measured (Facial, trigeminal, optic and sciatic). GD was not enriched in the auditory nerve. AAT and Glnase specific activities were consistently reduced 25-30% in the VCN 3 days after cochlear ablation and about 15% 14 days after cochlear ablation. The reduction in the magnitude of the decrease at 14 days was likely due to a decrease in protein in the cochlear nucleus. The specific activity of neither enzyme was significantly reduced in DCN. No change was seen in GD in the cochlear nucleus after cochlear ablation, while GS increased in activity in VCN. The increase in GS was likely due to glial cell proliferation in the cochlear nucleus after cochlear ablation, since a glial localization for this enzyme has been demonstrated.

The results suggest that two enzymes, aspartate aminotransferase and glutaminase, are concentrated in both axons and terminals of the auditory nerve.

IV. As has been observed in many other systems, radioactive proteins were transported down the auditory nerve at several different rates. Labeled proteins were found in the cochlear nucleus as early as 1 hour after injection, while the slowly transported proteins began to arrive about one day after injection. It was found that after ³⁵S-methionine labeling, highly labeled proteins were obtained in the cochlear nucleus with very little local synthesis taking place.

Although proteins transported at all rates have been analyzed, much of the early emphasis of this study has been placed on the rapidly transported proteins. These proteins reach the cochlear nucleus 1-3 hours after cochlear injection. Although many labeled polypeptides are present in the cochlear nucleus at this time, most of the radioactivity is present in two polypeptides with molecular weights of 25,000 and 140,000 (after cochlear injection of ³⁵S-methionine). The 140,000 polypeptide is fucosylated while the 25,000 polypeptide is not. Both are associated with membranes. The 140,000 polypeptide is especially interesting because of its very short half-life at the synaptic terminal, estimated to be about 6 hours. Studies have shown that this protein, as well as the 25,000 dalton protein, are synthesized in the cochlea and transported to the terminals, rather than arising from local synthesis in the cochlear nucleus.

V. a. A paper reporting some results of the experiments in which HRP was injected into the inferior colliculus has been published. Most of these results have been discussed in previous annual reports. Not mentioned previously is a description of the cytology of the nuclei of the lateral lemniscus. There is a peculiar class of cells in the ventral nucleus of the lateral lemniscus. A study of these cells indicated that these cells are recipients of large axon terminals that originate from octopus cells of the

contralateral cochlear nucleus. Until now, the terminations of these axons were unknown.

The computer model for cell plotting has already strikingly revealed spatial relations of cells in the olivary complex. The information gained from this model will be invaluable in future studies of the structural relationship between the olivary complex and other auditory relay stations, including the cochlear nucleus.

b. None of glutamic acid, aspartic acid, glycine, taurine, alanine, choline acetyltransferase, and glutamate decarboxylase were uniformly distributed in the colliculus. Aspartic acid had highest concentrations in central regions, glycine in ventral central regions. The distributions of glutamic acid and glutamate decarboxylase were highly correlated with both being generally high in dorsal regions. There was also a high correlation in the distributions of taurine and alanine with these substances having their highest concentrations in the posterior region and lowest concentrations in the anterior region. The concentration of choline acetyltransferase was highest in the anterior region. No consistent changes in any of the substances were seen following elimination of inputs ascending to the colliculus via the dorsal and intermediate acoustic striae. Small decreases in glutamic acid were seen in two extra-central regions following removal of the auditory cortex. A manuscript of the study has been submitted for publication.

c. Through modifications in the technique, increased sensitivity was attained with an increased number of HRP labeled cells in comparison with previous studies. Also, HRP labeled cells could be identified by their Nissl pattern after removal of the reaction product. Many cells were found in the ventral cochlear nucleus that send axons via the dorsal and intermediate striae. Labeled cells were found in the same regions in both cochlear nuclei, suggesting there are symmetrical crossed connections between these regions that course through the dorsal and intermediate striae. The number of labeled cells found in the contralateral cochlear nucleus was small but the increased numbers seen with the increased sensitivity of the TMB method suggests that still more sensitive methods may be necessary to determine the full extent of these connections. A manuscript has been submitted for publication.

d. The described method produces consistently high quality impregnations of cat brainstem and takes only one week to complete. It has an unusually high success rate for a Golgi method. No complete failures have yet been experienced with the method in over a year of use. The success rate appears to be due to the thorough and repeatable infiltration of the mordant that is made possible by the prior fixation with the aldehyde fixative and perfusion with the mordant. A manuscript has been accepted for publication.

Significance to Biomedical Research and the Program of the Institute:

The present new biochemical, morphological and physiological/pharmacological results on synaptic transmission in the cochlear nucleus (subprojects Ia, IIa, IIIa,b) support a neurotransmitter role for glutamate and aspartate in the cochlear nucleus. These new results together with previous biochemical data from this laboratory make the auditory nerve synapse in the cochlear nucleus one of the best characterized putative glutamate/aspartate synapses in the mammal. Evidence suggests that these amino acids may be major neurotransmitters in the mammalian central nervous system. This field of research has progressed slowly due to the inherent difficulty in specifying a transmitter function for these ubiquitous substances. Therefore, identifying a system in which glutamate or aspartate may function as neurotransmitters represents a major advance in biomedical research.

The Laboratory has continued to provide such new findings on connections of the cochlear nucleus that have to be considered in any penetrating analysis of the different aspects of perception of sound.

Our computer model for plotting of cells will inevitably be of future importance in studies of the grouping of cells and organization of connections of cells as soon as large numbers make direct visualization from slides difficult or impossible.

Proposed Course:

The physiology/pharmacology of cells receiving auditory nerve synapses in the cochlear nucleus will be studied with the use of electrophoretic application of putative neurotransmitters, agonists and antagonists during simultaneous intracellular recording with microelectrodes. This is to make it possible to identify the action of an exciting amino acid with that of the natural transmitter of the auditory nerve. Attempts through immunohistochemical methods will be made to determine the localization at the cellular and subcellular level of enzymes related to the metabolism of the putative neurotransmitters glutamate and aspartate. The study of axonal transport in the auditory nerve will be continued; the rapid transport and degradation of a protein under particular study suggest that this protein may play an important part in synaptic function. The study of connections of the cochlear nucleus will be continued.

Publications:

Adams, J. C.: Ascending projections to the inferior colliculus. J. Comp. Neurol. 183: 519-538, 1979.

Bird, S. J., Gulley, R. L., Wenthold, R. J. and Fex, J.: Kainic acid injections result in degeneration of cochlear nucleus cells innervated by the auditory nerve. Science 202: 1087-1089, 1978.

Martin, M. R. and Adams, J. C.: Effects of DL- α -aminoadipate on synaptically and chemically evoked excitation of anteroventral cochlear nucleus neurons of the cat. Neuroscience. In press.

Wenthold, R. J.: Release of endogenous glutamic acid, aspartic acid and GABA from cochlear nucleus slices. Brain Research 162: 338-343, 1979.

ANNUAL REPORT

October 1, 1978 through September 30, 1979

Infectious Diseases Branch

National Institute of Neurological and Communicative Diseases and Stroke

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ANNUAL REPORT

October 1, 1978 through September 30, 1979
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 five sections: 1) Immunochemistry and Clinical Investigations; 2) Experimental Pathology; 3) Neurogenetics; 4) Neurovirology; and 5) Electron Microscopy. These sections utilize the techniques of immunology, clinical investigations including human volunteers and clinical trials, experimental pathology with nonhuman primates, virology, bacteriology, mycoplasmaology, genetics, 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 large scale methods to study the relation between viral, bacterial, mycoplasma and protozoa 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 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 research and the 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 abnormal and 8,000 controls; and 3) high IgM among 30,000 children as a method to identify infected children. New highly sensitive ELISA tests are being applied to these studies.

Additional studies include high risk children and infections in relation to neonatal deaths and herpesvirus infections in pregnancy. A study is being conducted on viruses in the ovaries and uterus. Another investigation includes herpes and CMV infections in Navy personnel.

2. Acute

New ELISA tests are being used in studies of CSF from institutionalized children. Group B streptococcal meningitis infections are being studied in patients at George Washington and in experimental monkeys in our laboratories. Treatment protocols with penicillin are being investigated.

3. Chronic

Oligoclonal IgG was found in the CSF of 95% of MS as well as some patients with myasthenia gravis. Specific tests for this antibody are in progress. Special serological investigations of MS and ALS patients are in progress.

Parkinson's patients were tested but no unusual antibody levels were found in their sera or CSF.

Fundamental studies of virus induced demyelination with mouse hepatitis virus are being conducted.

B. Section on Experimental Pathology (EP)

1. Perinatal

This Section is conducting studies of experimental monkeys which develop CNS and other damage when infected in utero or in the newborn period. Current agents include CMV and VEE viruses. The value of new chemotherapeutic materials is under investigation. Experimental treatments for congenital toxoplasmosis and herpesvirus infections are being studied in monkeys.

2. Acute

Group B streptococcal meningitis is being studied in monkeys and new methods for treatment are being tested. Treatments for toxoplasmosis of the CNS is being studied in monkeys. Varicella/Flu association with Reye's syndrome is being tested in monkeys.

3. Chronic

Studies of progressive multifocal leukoencephalopathy in monkeys are in progress. Inoculation by various routes and with immunosuppression is being studied.

Human CMV is being tested in monkeys and chronic infection is being produced. Some animals are being immunosuppressed. SSPE is being studied in monkeys.

C. Section on Neurogenetics (NG)

This Section is responsible for combined genetic-infection studies of neurological diseases. These current studies include HLA-MLC studies of patient twins with MS or Parkinson's disease.

The Section is also investigating Tourette syndrome, dystonia, and families with progressive myoclonic epilepsy. CNS neoplasia are being studied for families with central neurofibromatosis.

D. Section on Neurovirology (NV)

1. Perinatal

This Section is studying new strains of CMV and mechanisms of genetic control. Cellular and humoral responses are being investigated in monkeys for both CMV and herpes.

2. Acute

Cellular immune studies are being conducted for patients with acute herpesvirus, CMV and EBV infections.

3. Chronic

Mechanisms of cellular immunity to various viruses are being tested using patient material from individuals with MS, SSPE, EBV and other neurological diseases.

The pathogenesis of PML (JC virus) are under study with the Section on Experimental Pathology. Also, possible virus etiologies of MS are under investigation using a variety of cellular immune techniques.

The immune response of monkeys infected with CMV is being studied. New antiviral drugs are being tested in vitro and in experimental animals with chronic infection.

E. Section on Electron Microscopy (EM)

This Section is using immunoelectron microscopy in studies of virus induced demyelination. Freeze-fracture, scanning and transmission EM methods are being employed with disassociated neuron cultures.

The mechanism of chronic infection in CNS tissue is being studied and the interaction between viruses and lymphocytes is being investigated. Membrane changes are being studied with measles, VSV and herpesviruses. The interaction between antibody and viruses is being tested for measles, VSV and visna viruses. The interaction between viruses and lymphoid cells is being studied with measles, EBV and VSV viruses.

IV. FINDINGS

A. Perinatal

1. New ELISA Test for Varicella Permits Identification of Susceptible Individuals (ICI)

A new ELISA test has been reported for varicella. With this test we can distinguish susceptible and immune patients. CF and most other tests are not satisfactory for this purpose. Hyperimmune sera can now be given to susceptible individuals who need this protection (patients with cancer, immunosuppression and newborns).

2. New Mumps Test Developed (ICI)

A new test for mumps antibody using ELISA was reported. This test distinguishes immune versus susceptible individuals. Most other tests do not permit this identification. We can now restrict immunization to susceptible adults at risk.

3. Congenital Hydrocephalus with Mumps in Rhesus Monkeys (EP)

Fetal rhesus monkeys inoculated with wild mumps virus developed persistent infection and hydrocephalus. This model indicates that mumps may be important in human hydrocephalus.

4. Urinary Tract Infections in Pregnancy and Abnormal Pediatric Findings (ICI)

Symptomatic urinary tract infections were found in 3.4% of women. Associated obstetrical findings included: toxemia, thrombosis, phlebitis, and anemia. Abnormal pediatric findings were: low birth weight, high rates of stillbirths, eye infections and poor motor performance. These abnormalities occurred even though the women were being treated for their infections.

5. Spontaneous Pre-eclamptic Toxemia of Pregnancy in Patas Monkeys (EP)

In pregnant patas monkeys spontaneous pre-eclampsia was found in 6% of the animals. This provides a new model for this disease.

6. ELISA Determination of Specific Micrograms of Rubella Antibody (ICI)

A micro ELISA method was perfected which permits determination of antibody in micrograms rather than titer. This permits specific determination of amounts of antibody.

7. Determination of Specific IgM Antibody by ELISA With Protein-A-Sepharose Absorption (ICI)

A new procedure for detection of specific IgM antibody was reported using protein-A-sepharose and ELISA. This permits the rapid diagnosis of recent infections.

8. Experimental Group B Streptococcal Infection in the Monkey (EP)

Group B strep infections cause serious - often fatal disease in newborns (3/1000 births). A similar disease was produced in rhesus monkeys. This provides a model for the study of treatment and vaccines.

9. Infecciones Virales en el embarazo (ICI)

The study of perinatal infections and their effect on the fetus was reported in Portuguese findings. This is an effort to provide wider dissemination.

10. Attenuated Influenza A Vaccine Virus (EP) Produces Hydrocephalus in Monkeys

Attenuated influenza A vaccine virus inoculated into fetal rhesus monkeys produced hydrocephalus. This is important to pending trials of live influenza A vaccines in humans.

11. Colposcopic Studies of Monkeys (EP) Inoculated with Herpes II Virus

Colposcopic studies confirmed cytologic abnormalities of cervical dysplasia and atypia in 84% of animals studied in this investigation of herpes and cancer.

12. Growth and Hematologic Development of the Patas Monkey to One Year of Age (EP)

Marked changes in hematocrit, hemoglobin and RBC numbers as well as leukocyte distribution were found in the first month of life in this primate. These animals are used for our studies of chronic infections of the CNS and basic information on the monkey is needed.

13. Comparison of IHA, IFA and Microneut Tests for Herpes Antibody (ICI)

The IHA test which was developed by the Infectious Diseases Branch is the most sensitive and specific indicator of primary infections.

14. Current Serological Tests for Herpes I, II, CMV and Rubella (ICI)

The IHA and ELISA tests are best for antibody to these viruses. The HI test is also important for rubella.

B. Acute

1. Human to Human Transmission of Rabies Virus by Corneal Transplant (ICI)

Previously unrecognized the human-to-human transmission of rabies by corneal transplant was demonstrated. Both patients died of rabies. Donors with neurological diseases must be reviewed carefully if their tissues are to be used in transplants.

2. Juvenile Onset Diabetes - Failure to Show Association with Coxsackie B1-6, Mumps, or RS Viruses (ICI)

Fifty women who developed diabetes early in life were studied. There was no significant increase in antibody to any of these viruses.

3. Urethral Infection of Chimpanzees by Ureaplasma Urealyticum (EP)

Human T-strain mycoplasmas were inoculated into chimpanzees and were grown in this animal. This provides a model for the study of this common infection.

C. Chronic

1. Immune Responses of SSPE patients to Measles (ICI)

SSPE patient's lymphocytes have normal responses to measles virus. Some CSF specimens have an inhibitory factor for lymphocytes. This blocking factor may be important in the disease.

2. Localization of Measles Viral Antigen in Brain Cells (EM)

Mice inoculated with measles virus showed viral antigen spread into dendritic and synaptic sites. Frequently antigen was in postsynaptic endings.

3. Measles Virus Antigens on Giant Cells (EM)

Immunoglobulins were able to induce a dramatic redistribution of viral antigens on the membrane of giant cells infected with measles virus.

4. Visna Virus - Surface Antigen Changes With Mutation of Virus (EM)

There is EM evidence for selective replication of a mutant virus when antibody treated cells are used.

5. Comparison of Two Strains of Simian Hemorrhagic Fever Viruses (ICI and EP)

The new strain of SHF cannot be cultured in vitro and has some differences from the prototype virus. This new strain was responsible for an extensive epidemic in monkeys resulting in loss of the animals.

6. EM Study of Measles Virus Infection: Fusion and Hemadsorption (EM)

Virus induced fusion and hemadsorption was studied with antibody and shifts in growth temperatures. There was inhibition by antibody at low temperatures but recovery at 37°C.

7. Brain Tumors in Owl Monkeys Inoculated With Human Polyomavirus (JC Virus) (EP and ICI)

Glioblastomas were produced in owl monkeys after a latent period of 1-2 years following inoculation with the JC virus. This is the first evidence of a solid tumor produced in a subhuman primate by a virus of man.

8. PAM Cell Test for MS Agent (NV)

The previous report of a MS agent was not confirmed.

9. In Vitro Inhibition of SSPE Virus by Ribavirin (ICI)

The antiviral drug Ribavirin blocked the SSPE measles virus in vitro. This may be of value in treating patients with SSPE.

10. Membrane Changes with Herpes 1 Infection (EM)

Major membrane changes occur with in vitro infection with Herpes 1.

11. MS Cellular and Humoral Immune Responses (ICI)

Increased levels of measles antibodies were found in MS patients. Immune responses to other viruses were normal.

12. Abnormal Immunoglobulin Bands in CSF of Myasthenia Gravis Patients (ICI)

Monoclonal or oligoclonal IgG bands in the CSF were found in 12 of 23 patients with myasthenia gravis. This may indicate a more generalized involvement in this disease than has previously been recognized.

13. EB Virus Infection Following Bone-Marrow Transplant (ICI and NV)

A 12-year-old with acute lymphoblastic leukemia received a bone-marrow transplant from a sibling and became infected with EBV. There is a need to investigate donors for EBV.

14. CSF Antibodies in ALS and Progressive Muscular Atrophy (ICI)

There was no evidence of persisting specific antibody within the CNS with the viruses studied.

15. Pathology of Measles Infection of the CNS (EM)

When measles replicate in the brain of a host with an inactive immune system,

acute cytopathic changes are seen. In adults, the infection is localized and cytolysis occurs around the focus of infection.

CONTRACT NARRATIVE

Infectious Diseases Branch, IRP, NINCDS
Fiscal Year 1978

UNIVERSITY OF FLORIDA (N01-NS-5-2318)

TITLE: The Viral Induction of Malformations in Developing Rhesus Monkeys

Contractor's Project Director: Dr. Alvin F. Moreland

Current Annual Level: \$93,208.00

Objectives: To determine if Influenza A, Mumps and Western Equine Encephalitis (WEE) viruses can be transmitted from the blood of the pregnant rhesus monkey to the fetus. Should these viruses not cross the placenta of the monkey, then intra-amniotic inoculations will be made.

Inoculations of these viruses will be given at various times of gestation: 50 and 80 days.

Major Findings:

- a. Influenza A Virus did not result in increased fetal mortality nor in fetal malformations.
- b. Mumps increased fetal mortality but the incidence of CNS or other associated malformations in surviving fetuses was very low.
- c. WEE Virus caused increased fetal mortality and a significant incidence of hydrocephalus.

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. Studies done in the Infectious Diseases Branch of NINCDS have established that the three human viral agents to be investigated under this contract are teratogenic when inoculated into non-human primate fetuses. The anomalies are mainly of the central nervous system. The work planned under this contract should establish if these viral agents are teratogens when given to the pregnant, non-human primate.

Proposed Course of the Project: This contract was terminated 15 January 1979. All rhesus monkeys were returned to the NINCDS, IDB breeding colony.

CONTRACT NARRATIVE

Infectious Diseases Branch, IRP, NINCDS
Fiscal Year 1978

MELOY LABORATORIES, SPRINGFIELD, VIRGINIA N01-NS-7-2360
(formerly N01-NS-2-2306)

TITLE: Herpes Virus Induction of Cervical Cancer in Cebus Monkeys.

Contractor's Project Director: Dr. D. Lewis Sly

Current Annual Level: \$136,154.00

Objectives: An attempt to show a relationship between infection with Herpes Simplex Virus, type II and cervical cancer in the cebus monkey. Also, an attempt to determine whether exogenous hormones may play a role with HSV-2 to produce cervical cancer.

Major Findings: Intravaginal inoculation of cebus monkeys with HSV-2 produces transient lesions and virus shedding. Infected animals produce neutralizing antibodies, and rarely undergo recurrent infection after primary infections. Reinfection after reinoculation occurs rarely, although there is evidence that each reinoculation increases the antibody titer. HSV-2 has been demonstrated from sacral ganglia by explant culture.

Mild cytological anaplastic changes were detected from virus, hormone and control inoculated animals. However, these changes were persistent in HSV-2 and hormone recipients, but not in control recipients. Cytological changes did not progress beyond class III (dysplasia).

Significance to the NINCDS Programs and Biomedical Research: The role of HSV-2 infection in perinatal and chronic neurological diseases in humans stimulated the development of a primate model to study the pathogenesis of this virus. Since the oncogenic potential of HSV-2 in cervical carcinoma of humans cannot be ascertained in humans, the cebus monkey model is used in an attempt to demonstrate its role in this disease.

Proposed Course of the Project: This study was terminated on 29 March 1979. Female cebus monkeys from the study are in use as breeders under contract N01-NS-7-2375. They will be monitored for the development of malignancies.

CONTRACT NARRATIVE
Infectious Diseases Branch, IRP, NINCDS
Fiscal Year 1979

UNIVERSITY OF ARIZONA (N01-NS-7-2364)

Title: Detection of Viral Genomes in Human Neurological Diseases

Contractor's Project Director: Dr. William J. Meinke

Current Annual Level: None

Objectives: Contractor will attempt to demonstrate the presence or absence of viral genes in cells derived from patients with brain tumors or chronic neurological diseases such as multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and Alzheimer's disease. The experimental approach is to analyze cellular nucleic acids derived from central nervous system tissues by extremely sensitive DNA-DNA and DNA-RNA molecular hybridization techniques.

Major Findings: Studies were continued to determine whether poliovirus is implicated in ALS. Complementary DNA (cDNA) has been synthesized from a poliovirus type 1 RNA template. In older probes, up to 20% of the poliovirus cDNA hybridized nonspecifically with RNA extracted from control tissues. With improved methodology, less than 1% of poliovirus cDNA now hybridizes to RNA extracted from control tissues. Poliovirus type 1 RNA sequences were not detected in RNA extracted from three ALS brains using the more specific cDNA probes.

Screening of various brain tumors for SV-40-related DNA sequences has continued. Tests on five additional brain tumors by DNA-DNA reassociation kinetics have failed to detect SV-40-related DNA sequences in any of these tumors.

Some progress was made in preparing cDNA from rubeola virus RNA. However, cDNA probes of sufficient specificity could not be prepared to evaluate whether rubeola virus genetic information is associated with tissues of MS patients.

Significance to the NINCDS Program and Biomedical Research: ALS and MS are significant human neurological diseases. The possibility of an etiologic role for poliovirus in ALS is a common theme in the neurological literature. Also, serum and spinal fluid from MS have elevated antibody titers to rubeola virus. Additionally, primate polyomaviruses have been associated in some serological and molecular hybridization studies with human tumors. The hybridization studies to be performed under this contract should provide data to elucidate whether polioviruses are involved in ALS, rubeola virus in MS, and primate polyomaviruses in human brain tumors.

Proposed Course of the Project: Contract expired November 27, 1978.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-00402-23-ID
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Perinatal Infections Causing Damage to the Child - Collaborative Perinatal Project		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	John L. Sever David L. Madden	Chief Veterinary Director
		IDB, IRP, NINCDS IDB, IRP, NINCDS
Other:	Jonas Ellenberg Anita C. Ley Renee G. Traub Dorothy M. Edmonds	Biostatistician Microbiologist Microbiologist Clinical Nurse
		OBE, OD, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS
COOPERATING UNITS (if any) Johns Hopkins University Univ. of CA, Los Angeles and Kaiser Hospital George Washington University Medical School OBE, OD, NINCDS		
LAB/BRANCH Infectious Diseases Branch		
SECTION Immunochemistry and Clinical Investigations		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	1.0	5.0
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS	<input type="checkbox"/> (b) HUMAN TISSUES	<input type="checkbox"/> (c) NEITHER
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this study is to determine insofar as possible the role of <u>perinatal infections</u> 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 <u>Collaborative Perinatal Project</u> . Now that the project is <u>complete</u> , it is possible to study <u>perinatal infections</u> with three main approaches: 1) <u>clinical infections</u> ; 2) <u>subclinical infections</u> detected <u>serologically</u> using abnormal and matched controls; and 3) <u>high risk</u> children with <u>elevated IgM</u> levels. Special investigation included the <u>epidemiology</u> of infections and the frequency of congenital <u>toxoplasmosis</u> . <u>Cooperating units</u> work with the Infectious Diseases Branch to study newborns in high risk nurseries. <u>Serum, IgM</u> volumes, plus clinical findings are being used to identify infected infants at risk for <u>perinatal damage</u> . Specific tests are then <u>developed and applied</u> for identification of the infection. Preliminary data indicates that <u>urinary tract infection</u> during pregnancy were found to increase the risk for abortion, stillbirths, neonatal deaths and prematurity.		

Project Description:

Objectives: The purposes of this study is to determine insofar as possible the role of infections and immunity in the production of abnormal pregnancy outcomes. To accomplish this, 12 collaborating institutions in the Perinatal Research Study plus two special cooperating groups in separate studies obtained specimens of blood and tissue throughout pregnancy at delivery, post partum, and at set intervals thereafter. These sera are being tested to determine the antibody responses of the patients during pregnancy and post partum and then to relate this serological information to the clinical data for the pregnancy and child. In addition, serum specimens from the children were obtained at one-year-of-age from 10,000 study pregnancies. Sera, throat swabs and urine specimens were also obtained from approximately 5,000 pregnancies. Placental specimens were obtained from 2,500 pregnancies. In special cases when congenital infection is suspected on the basis of clinical or laboratory findings, throat swabs and blood specimens were obtained from the children. Immunoglobulin determinations were performed with the cord blood specimens from the children and specific antibody determinations are also being made with these specimens.

Methods Employed: To accomplish this program, blood specimens were obtained from pregnant women at set intervals throughout pregnancy and post partum. Completeness of the sets of sera is determined at the Serum Center of the Infectious Diseases Branch. Data for the 58,000 patients in the Collaborative Perinatal Research Study show that specimens are available from 94.2% of the patients. An average of five blood specimens is available for each patient. Each specimen consists of four vials with 3 ml of serum in each. For this study then, there are approximately 300,000 serum specimens and almost a million and a half vials of sera. There are an additional 5,000 patients studied to date at the Kaiser Hospital in Los Angeles and approximately 3,000 under study at the Johns Hopkins Medical School in Baltimore, Maryland. All specimens are stored at -20°C until tested and complete filing record concerning basic patient information and the status of the serum available is maintained through a computer system by the Serum Center of the Branch.

In addition to the serum specimens, serial urine and throat specimens were also obtained on a large majority of the patients in the two special studies. These are being studied for direct virus isolation along with swabs obtained from the children at the time of birth.

To date, approximately 75 publications have resulted from the analysis of the data from these studies. The serological method most frequently employed is the complement fixation (CF) test with the use of viral antigens. The test is very versatile and can be performed rapidly and provides broad coverage for a great many of the more than 130 viruses which are known to be of importance to man. Antigens were prepared for most of these viruses and tests of specificity were conducted with animal sera. In addition to the CF method, hemagglutination inhibition (HI) tests are used for many viral serological determinations. When greater specificity is needed, enzyme-linked immunosorbent (ELISA) neutralization methods are employed. Indirect fluorescence is also

used for some of the studies. Virus isolation is conducted with tissue culture or inoculation of experimental animals.

All tests are reproduced completely and a minimum of 95% agreement within 2-fold variation is required. All sera showing significant changes in antibody, together with any sera which did not reproduce are tested the third time. We are now completing the study of reported viral, bacterial and protozoal infections in pregnant women in the study. Serological tests are used to document these reports. The data is then correlated with the pediatrics findings. Approximately 2,500 cases of reported viral infections, 3,000 bacterial infections and several hundred protozoal infections are under investigation. Clinical data is being abstracted, serological tests are being performed in order to document these infections. There are also approximately 1,200 patients identified with a positive serology for syphilis. These are being studied in detail.

A second approach involves a large scale study designed to investigate infection and immunity in relation to 8,000 abnormal children in the study and 8,000 matched controls. The print-out of abnormal patients has been obtained from the Collaborative Perinatal Research Study and this is being reviewed in detail by nurses and physicians from the IDB for more complete information.

From study records, the specific abnormalities under study include abortions, stillbirths, cataracts, congenital heart disease, neonatal deaths, low birth-weight (1,000-1,500, 1,500-2,000 grams), IQ below 50-69, enlarged liver, malformations, retarded gross motor development, retarded fine motor development, hearing deficit in both ears, visual impairment, cranial or peripheral nerve damage, cerebral palsy, delayed motor development, hypotonia with poor deep tendon reflexes, nonfebrile seizures, dyskinesia and ataxis, hearing deficit in one ear and elevated bilirubin. The specimens from the mothers of these children and from the children themselves along with carefully matched controls are being studied for antibody to 11 antigens. These antigens include Influenza A, rubeola, rubella, mumps, Coxsackie B₃, Coxsackie B₄, Varicella Zoster (VZ), toxoplasmosis, cytomegalovirus (CMV), Herpes Simplex type I and II. All of these agents are known or suspected to be responsible for damage in the perinatal period. All laboratory work is being performed under code. The data is being analyzed by Dr. Ellenberg. A second phase of this study will involve four additional antigens.

The third approach is to identify the children with elevated IgM levels in the newborn period and then correlate these findings with pregnancy outcome, clinical performance of the child and specific serological tests for IgM antibody. Almost 32,000 cord sera have been tested for IgM antibody and approximately 2,000 show elevated levels. These are now being studied in detail.

Major Findings:

The reproducibility of the HI tests for rubella antibody has been questioned and now constitutes a major problem in clinical diagnosis of this infection. New ELISA tests for rubella antibody provide an alternate method for comparison titrations.

Recent studies at NIH have shown an association between coxsackie B₄ virus and a case of fatal juvenile diabetes. In a study of 50 patients with juvenile onset diabetes, however, no association was found with coxsackie B_{1 6}, mumps or respiratory syncytial virus.

Urinary tract infection in pregnancy occurred at the rate of 3.5%. Even though the women were treated, there was an increased incidence of prematurity, abortion, stillbirths and neonatal deaths.

Significance of the Program to the Institute: The use of new serological techniques for a large group of new viruses provides an opportunity to investigate the disease caused by viruses which are either difficult to isolate or resistant to evaluation because the clinical effects are delayed until a long time after the infection has subsided. In addition, the availability of new immunologic techniques provides the unique opportunity to detect immunologic deficits and to determine the presence of intrauterine infections on the basis of immunologic response. This data can then be correlated and analyzed as in relation to the possible causes of birth defects. The application of this type of analysis has provided valuable information on infections in relation to abnormal pregnancy outcomes and is constantly giving us new insights into the causes of damage to the central nervous system and possible means of prevention of this and other damage to the developing fetus and newborn.

Proposed Course of the Project: The combined immunologic virologic program will continue during the next year. During that time we will complete the tests for the first two phases of the serological studies. Phase three testing will then be initiated using four new antigens.

The three approaches which are being emphasized now include:

1. Publication of the correlation of clinically reported infections in pregnancy with serological findings for the pregnancy, immunologic determinations and pregnancy outcome. These studies should be reported for the most part in the next fiscal year.
2. A special commitment to perform serological tests on 8,000 abnormal pregnancies and 8,000 matched controls using 11 antigens. The abnormal children have been identified and the laboratory is now approximately 80% of the way through the testing. Data analysis is being completed for the first 26 abnormal outcome categories.

3. Special test of IgM levels from 32,000 cord sera from children in the Collaborative Perinatal Research Study and in the cooperative studies. This work provides an index for identifying children with possible congenital infections so that more specific testing can then proceed. These investigations are being tested for specific antibody.

Special studies of high risk groups will be conducted for infections with cytomegaloviruses and herpesviruses.

New ELISA tests are being used for several infectious agents. These highly sensitive tests now permit testing for antibody which was previously undetectable.

Publications:

Sever, J. L.: Viral infections in pregnancy. Clin. Obstet. Gynecol. 21: 477-487, 1978.

Sever, J. L.: Infecciones virales en el embarazo. Clinicas Obstetricas y Gynecologicas 2: 509-519, 1978.

Sever, J. L.: Rubella. Perinatal Infection Newsletter 1: 1-4, 1978.

Madden, D. L., Fuccillo, D. A., Traub, R. G., Ley, A. C., Sever, J. L. and Palmer, A. E.: Juvenile onset diabetes mellitus in pregnant women: Failure to associate with coxsackie B1-6, mumps, or respiratory syncytial virus infections. J. Pediatr. 92: 959-960, 1978.

Sever, J. L., Ellenberg, J. H., Edmonds, D.: Urinary tract infections during pregnancy: Maternal and pediatric findings. In Edward H. Kass and William Brumfitt (Eds.). Infections of the Urinary Tract, The University of Chicago Press, Chicago, 19-21, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-01985-08-ID
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Presence of Viral and Nonviral Antigens or Antibodies in Perinatal and Neurological Diseases		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: David L. Madden Veterinary Director IDB, IRP, NINCDS Other: John L. Sever Chief IDB, IRP, NINCDS Mary A. Krasny Microbiologist IDB, IRP, NINCDS Aurella Krezlewicz Microbiologist IDB, IRP, NINCDS Matti Iivanainen Guest Worker IDB, IRP, NINCDS William London Veterinary Director IDB, IRP, NINCDS Maneth Gravell Research Microbiologist IDB, IRP, NINCDS William Wallen Senior Staff Fellow IDB, IRP, NINCDS		
COOPERATING UNITS (if any) Lynchburg Training School and Hospital Electronucleonics, Inc. Microbiological Associates, Inc.		
LAB/BRANCH Infectious Diseases Branch		
SECTION Immunochemistry and Clinical Investigations		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.5	PROFESSIONAL: 1.5	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Continued efforts have been made to determine the etiological agents associated with <u>Multiple Sclerosis</u> . We have continued to use the <u>direct migration inhibition, lymphocyte cytotoxicity</u> and <u>complement mediated cytotoxic</u> tests to determine the cellular immune response of MS patients and carefully matched controls. In addition, we have attempted to develop the techniques associated with <u>flow cytofluorometry</u> to measure responses in a small number of lymphocytes in an effort to determine the cellular immune response cerebrosplinal fluid cells. We have continued to attempt to develop the <u>ELISA</u> technique to measure antibody against a variety of etiological agents which may be associated with multiple sclerosis or other neurological diseases. We have found that the ELISA technique is as adequate as existing serological tests to determine antibody titers but in most cases although the antibody titers are higher the specificity of the tests are not much greater. Routine monitoring of cultures from experimental viral studies for <u>mycoplasma</u> contamination and efforts to develop new techniques to monitor tissue cultures for contamination have been continued.		

19 - IDB/IRP

Project Description:

Objectives: To isolate and identify viral and non-viral antigens and/or antibodies. To utilize these antigens and/or antibodies for more specific, rapid, sensitive identification of antigens and antibodies and/or a more accurate identification of infectious agents in diseases. To define the humoral and cellular immune response of patients with neurological disease to these antigens. To determine the relationship between specific infectious agents in pregnant women and mental retardation, congenital jaundice and postnatal jaundice.

Methods Employed: Human tissue culture lines chronically infected with sub-acute sclerosing panencephalitis (SSPE) measles virus, Herpes Simplex virus types I and II (HSV-I and II), and cytomegalovirus (CMV) have been used to determine the immunological response of SSPE, and multiple sclerosis (MS) patients and matched pal controls to these antigens. The infected cells have been labeled with ⁵¹Chromium. Target cells and various concentrations of lymphocytes or serum are reacted for 18 and 24 hours and the amount of ⁵¹Chromium specifically released is determined.

Attempts to adapt flow cytofluorometric techniques to the cellular immune response of patients with multiple sclerosis has been made. The amount of DNA in cells using the cytofluorograph has been determined by vital staining of cells in a one step method using acridine orange. The cells are stained for 30 seconds and then passed through the cytofluorograph. Analysis of the amount of DNA present in the cell is accomplished using a single phase pulse height analysis. The amount of change in DNA in specific channels is compared with the control and a stimulation index determined. The analysis takes about 2 minutes for each sample. Comparative studies using cell counts of 1×10^6 cells per milliliter to determine the amount of stimulation by cytofluorograph and conventional tridium isotope technique have been made. Attempts are currently being made to reduce the number of cells to 1×10^3 cells/milliliter thus approximating the number of cells found in cerebrospinal fluid.

Adaption of the enzyme-linked immunosorbent assay (ELISA) to detect antibody against several viruses associated with neurological diseases has been accomplished. The viral antigens are absorbed onto disposable plastic plates and the unbound antibody is then washed off. Anti-human IgG conjugated to phosphatase is reacted to the serum remaining in the plate, excess washed off, substrate added and the color which develops is proportional to the amount of antibody in the unknown sera. Correlation between existing assays are very close. Efforts are being initiated to identify viral antigens using plates coated with specific antiviral antibodies.

Virus preparations and antigens used in cellular immune studies have been examined for presence of mycoplasma. Comparison of several methods for identification of mycoplasma and tissue culture are underway. The emergence of additional strains that do not grow in liquid media makes these studies extremely important.

Major Findings: The serum antibody levels and cellular immune responses for several viruses have been determined for a large number of MS patients and matched controls. There was a significant increase in the mean titer of measles antibody in MS patients when tested with a complement fixation and hemagglutination inhibition tests; but no increase in antibodies to HSV-I, II and CMV. The increased measles antibody levels was present for each HLA type in the MS patients and no increase for the matched controls; however, no HLA type had increased levels of herpes I and II or CMV. Cellular immune studies using the direct migration inhibition technique showed no difference between MS and matched control patients with antigens to measles, CMV, HSV-I and II or vaccinia. An increase in the cellular immune response was not associated with the HLA type. Using the leukocyte-mediated cytotoxicity test, there was no difference between MS patients and matched controls using cultures infected with measles virus or CMV. Again, no significant difference associated in HLA types was observed. These studies indicate to us that HLA type is not significantly influencing the humoral or cellular immunological response of MS patients and matched controls.

Studies using the cytofluorograph to determine the stimulation response of lymphocytes with PHA have indicated that 1×10^6 cells/ml the response is similar to that found when using tridium as a marker of DNA proliferation. Response of lymphocytes at 1×10^5 cells/ml were similar to those at 1×10^6 . Cell concentrations of less than 1×10^5 do not incorporate sufficient amount of tridium to be measured accurately. DNA proliferation preliminary studies indicate that the cytofluorograph will measure cells at 1×10^4 and possibly lower. Attempts are now being made to adapt this technique to cells found in cerebrospinal fluid in order to measure the cellular immune response of cells produced within the central nervous system.

ELISA techniques have now been developed to measure antibody against many of the common viruses. Specifically mumps, measles, rubella, CMV, HSV-I and II, varicella and vaccina. It is possible to estimate the concentration of antibody using only one to three dilution of serum. However, end point titration proves to be more accurate. The ELISA technique has been developed to measure class specific IgM antibody levels. The antibody activity was measured using IgG and IgM class specific conjugates. In order to eliminate false positive IgM reaction, sera was treated with a protein A-sepharose, it was found that the nonspecific rheumatoid factor was removed, whereas the specific IgM remained. The ELISA system was also used to measure the specific amount of IgG antibody in milligrams per milliliter of sera. Negative serum samples showed specific rubella antibody levels less than one microgram per milliliter whereas levels up to

several hundred micrograms per milliliter were detected in some post infection sera. The correlation between microgram of rubella antibody and hemagglutinating-inhibition titers was not clear. The ELISA method offers a simple and sensitive antibody assay method which can be used in the laboratory for diagnosis of acute rubella infection and for evaluation of immunity.

Studies on the association of hepatitis in pregnant women and observable disease in newborns has been continued. Studies indicate that the etiology of Down's syndrome is not associated with Hepatitis A or B. However, it is now evident that Hepatitis B antigenemia persist more commonly in Down's syndrome patients than in patients with other types of mental retardation. However, the infection rate in both DS and other mentally retarded patients as measured by both antibody and antigen was similar. Data indicates that there is also no difference in the attack rates for Hepatitis A.

Studies of tissue culture cell lines, seed viruses and media for mycoplasma contamination continues. Continual monitoring of common reagents used in the Infectious Diseases Branch studies is necessary to prevent these contaminating agents from producing artifacts in the data. Several strains which do not grow on the conventional mycoplasma medias have been identified. Efforts are under way to develop new identification techniques.

Significance of the Program to the Institute: The development of more specific antigens or antibodies which measure more accurately the immunological status of an individual is needed. Highly specific antigens or antibodies may help identify the biological differences between pathogenic and nonpathogenic strains of these organisms and identify the etiology of obscure diseases.

Proposed Course of the Project: Further studies will be done to identify the antigens associated with the measles and rubella, HSV-I and II and CMV. Cellular and humoral immune studies are being expanded in an effort to detect small amounts of antigen on intact cells and immunological response differences which may account for disease.

Publications:

Fuccillo, D. A., Madden, D. L., Castellano, G. A., Uhlig, L., Traub, R. G., Mattson, J., Krezlewicz, A., Sever, J. L.: Multiple sclerosis: Cellular and humoral immune responses to several viruses. Neurology 28: 613-615, 1978.

Madden, D. L., Fuccillo, D. A., Dorosz, J. A., London, W. T., Palmer, A. E., Castellano, G. A.: Antigenic relationship of two strains of simian hemorrhagic fever virus. Lab. Anim. Sci. 28: 422-427, 1978.

Leinikki, P. O., Shekarchi, I., Dorsett, P. and Sever, J. L.: Determination of virus-specific IgM antibodies by using ELISA: Elimination of false-positive results with protein-A sepharose absorption and subsequent IgM antibody assay. J. Lab. Clin. Med. 92: 849-857, 1978.

Leinikki, P. O., Shekarchi, I., Dorsett, P. and Sever, J. L.: Enzyme-linked immunosorbent assay determination of specific rubella antibody levels in micrograms of immunoglobulin G per milliliter of serum in clinical samples. J. Clin. Microbiol. 8: 419-423, 1978.

Madden, D.L., Fuccillo, D. A., Castellano, G., Traub, R., Krezlewicz, A., Sever, J. L.: Immunological response of subacute sclerosing panencephalitis patients to measles virus. Neurologia - Neurocirugia Psiquiatria 18: 50 - 520, 1977.

Leinikki, P. O., Shekarchi, I., Tzan, N., Madden, D. L., Sever, J. L.: Evaluation of enzyme-linked immunosorbent assay (ELISA) for mumps virus antibodies (40451). Proc. Soc. Exp. Biol. Med. 160: 363-367, 1979.

Johnson, L. D., Fuccillo, D. A., Stalder, H., Oxman, M. A., Fraser, C. E. O. and Madden, D. L.: Comparison of indirect hemagglutination and indirect immunofluorescence tests with microneutralization tests for detection of type-specific herpesvirus hominis antibody. J. Clin. Microbiol. 9: 384-390, 1979.

Honors and Awards:

Meritorious Service Award - David L. Madden

Director's Award - Anita Ley

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-02038-07-ID
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Combined Clinical, Viral and Immunological Investigations of Acute and Chronic Diseases of the Central Nervous System		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	John L. Sever Sidney A. Houff	Chief Clinical Associate IDB, IRP, NINCDS IDB, IRP, NINCDS
Other:	David L. Madden Maneth Gravell Monique Dubois-Dalcq Roswell Eldridge Anita C. Ley	Veterinary Director Research Microbiologist Research Microbiologist Medical Director Microbiologist IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS
COOPERATING UNITS (if any) University of Vermont Georgetown University Medical School, Washington, D.C. Children's Hospital, Washington, D.C. VA Hospital, Washington, D.C.		
LAB/BRANCH Infectious Diseases Branch		
SECTION Immunochemistry and Clinical Investigations		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	1.5	3.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Clinical and laboratory studies</u> are conducted to determine etiology (<u>infection, immunity and/or genetics</u>) for chronic diseases of the central nervous system. Current studies include <u>Multiple Sclerosis, Progressive Multifocal Leukoencephalopathy, Subacute Sclerosing Panencephalitis, Myasthenia Gravis, Amyotrophic Lateral Sclerosis and Parkinson's Disease</u> . Combined clinical data, genetic information, HLA and MLC typing, virus serology and <u>virus isolation studies</u> are obtained for these studies. Oligoclonal IgG was found in the CSF of 90% of MS patients and about 50% of the patients with Myasthenia Gravis. Parkinson patients were tested but no unusual antibody levels or oligoclonal IgG patterns were found in their serum or cerebrospinal fluids. Previously unrecognized as a possibility the human-to-human transmission of rabies by corneal transplant was demonstrated. Donors with neurological diseases must be carefully reviewed before their tissue is used in transplantations. 25 - IDB/IRP		

Project Description:

Objectives: Clinical and laboratory studies are being conducted on chronic infections of the central nervous system (CNS). During this year, the investigations have centered primarily on multiple sclerosis (MS), progressive multifocal leukoencephalopathy (PML), subacute sclerosing panencephalitis (SSPE), myasthenia gravis (MG), amyotrophic lateral sclerosis (ALS) and Parkinson's disease. These studies have epidemiological, serological, cellular immune, viral and therapeutic components. They involve collaboration of a number of groups through the United States.

Methods Employed: MS patients and tissues are obtained from a number of collaborators throughout the world. New tissue culture methods and electron microscopic techniques are used in these studies as well as genetic and cellular immune tests.

Specimens from patients with PML, SSPE, ALS, MG and Parkinson's disease as well as several types of brain tumors are being studied virologically and immunologically. Special studies of postinfectious polyneuritis are being conducted in Virginia and Maryland. Individuals immunized with measles vaccine are being tested for antibody levels and persistence of antibody.

Major Findings: The CSF of about 90% of the MS patients have oligoclonal IgG. Monoclonal or oligoclonal bands appear in 50% of the Myasthenia Gravis patients. The occurrence of the abnormal IgG in the Myasthenia Gravis patients suggests that CNS involvement is more complex and more extensive than has been previously recognized. Serum and cerebrospinal fluid from patients with Parkinson, ALS and progressive muscle atrophy have been tested and no significant increase in the antibodies against a variety of viruses or oligoclonal IgG have been recognized.

The human to human transmission of human rabies by corneal transplant was demonstrated. The initiating source of infection was not identified although the occupational history of the original case suggests that wildlife exposure was possible. This study points out that donors with neurological disease must be carefully evaluated if their tissues are to be used for transplantation.

Significance of the Program to the Institute: Clinical and laboratory studies of MS, PML, SSPE, MG, postinfectious polyneuritis, ALS and Parkinson's disease permit direct investigation of the possible causes of these diseases and provide us with an opportunity to study unique "experiments" of nature which often provide very valuable insight into the disease process. These studies are designed to take advantage of both the epidemiology as well as the direct laboratory approaches to the problems of acute and chronic infections of the CNS.

Proposed Course of the Project: Additional studies in attempts to identify the etiology of Multiple Sclerosis, Myasthenia Gravis, Post Infectious Polyneuritis, ALS and Parkinson's Disease will be continued. Emphasis will be placed upon identifying the role the Epstein-Barr virus as a cause of neurological disease. Continued effort to identify the relationship of PML and brain tumors are being undertaken to identify the etiology of rapid diagnostic techniques and treatment of viral encephalitis with special emphases placed upon the pathogenesis of herpes simplex infections. Research to identify the cause of oligoclonal IgG bands in CSF and the cellular immune response of CNS cells is continuing.

Publications:

Houff, S. A., Burton, R. C., Wilson, R. W., Henson, T. E., London, W. T., Baer, G. M., Anderson, L. J., Winkler, W. G., Madden, D. L., Sever, J. L.: Human-to-human transmission of rabies by corneal transplant. New Eng. J. Med. 300: 603-604, 1979.

Adornato, B. T., Houff, S. A., Engel, W. K., Dalakas, M., Madden, D. L. and Sever, J. L.: Abnormal immunoglobulin bands in cerebrospinal fluid in myasthenia gravis. Lancet 2: 367-368, 1978.

Kurent, J. E., Brooks, B. R., Madden, D. L., Sever, J. L. and Engel, W. K.: CSV viral antibodies. Evaluation in amyotrophic lateral sclerosis and late-onset postpoliomyelitis progressive muscular atrophy. Arch. Neurol. 36: 269-273, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-01731-11-ID
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Isolation, Characterization and Diagnosis of Infectious Agents From Chronic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Maneth Gravell	Research Microbiologist	IDB, IRP, NINCDS
Other:	William T. London	Veterinary Director	IDB, IRP, NINCDS
	Amos E. Palmer	Research Veterinarian	IDB, IRP, NINCDS
	Marta Monzon	Visiting Fellow	IDB, IRP, NINCDS
	Olajide Agbede	Visiting Fellow	IDB, IRP, NINCDS
	Raymond H. Kiefer	Biologist	IDB, IRP, NINCDS
	Rebecca S. Hamilton	Biologist	IDB, IRP, NINCDS
	Otto Gutenson	Biologist	IDB, IRP, NINCDS
	Blanche Curfman	Biologist	IDB, IRP, NINCDS
	Robert Brown	Biological Lab Technician	IDB, IRP, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Infectious Diseases Branch

SECTION
Neurovirology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	6.0	PROFESSIONAL:	2.5	OTHER:	3.5
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Antibody titers to simian hemorrhagic fever (SHF) virus were determined by enzyme-linked immunosorbent assay in 285 patas monkeys (216 feral; 69 laboratory reared). Antibody to SHF virus was detected in 48.6% of the feral animals, but antibody was not detected in any of the laboratory reared animals. Transmission studies showed that 9 of the 216 feral animals were long-term asymptomatic chronic carriers of SHF virus. Antibody titers in the chronic carriers varied from no detectable antibody to a titer of 6000. At birth, babies of patas monkeys had the same antibody titers as their mothers. Maternally acquired antibody decreased gradually and was no longer detectable about 3 months after birth. Babies born to chronically infected mothers also lost their maternal antibody within this time frame and no evidence of in utero infection was found. These results suggest that SHF virus infection of patas monkeys is by horizontal means.

An in vitro test has been developed to detect patas monkeys chronically infected with SHF virus.

29 - IDB/IRP

Project Description:

Objectives: To use virological, biochemical and immunological techniques to study persistent viral infections and their role in chronic neurological diseases.

Methods Employed: Antibody titers of sera were determined by enzyme-linked immunosorbent assay. The indicator system for the test was p-nitrophenyl phosphate and alkaline phosphatase complexed to rabbit anti-human IgG. Optimum concentrations of purified viral antigen and enzyme-antibody conjugate for use in tests were determined by block titration.

Major Findings: Most studies of simian hemorrhagic fever (SHF) virus (a Togavirus) have concerned fatal infection of macaque monkeys. Little is known about infection of patas monkeys, a natural host of SHF virus and a source of virus for macaque infection. An enzyme-linked immunosorbent assay was used to study serum antibodies to SHF virus in a large colony of patas monkeys containing both feral and laboratory reared animals. Levels of antibody were determined in 285 animals (216 feral; 69 laboratory reared). In the feral animals 48.6% had detectable SHF virus antibodies, suggesting natural infection by SHF virus. None of the laboratory reared animals had detectable SHF virus antibodies. Transmission studies showed that 9 of the 216 feral animals were long-term asymptomatic chronic SHF virus carriers. Antibody titers in the chronic carriers varied from no detectable antibody to a titer of 6000. At birth, babies of patas monkeys had the same antibody titer as their mothers. Maternally acquired antibody decreased gradually and was no longer detectable about 3 months after birth. Babies born to chronically infected mothers also lost their maternal antibody within this time frame and no evidence of in utero infection was found. These results suggest that SHF virus infection of patas monkey is by horizontal means.

Epizootics of SHF have occurred in laboratory colonies of macaques due to accidental infection by virus from asymptotically infected patas monkeys. No rapid, simple and inexpensive technique was previously available to identify asymptotically infected animals. We have developed an in vitro tissue culture technique to identify these animals. Testing new patas monkeys for infectious virus prior to admitting them to the general colony, thus, should lessen the chance of accidental infection and loss of valuable experimental animals.

Significance of the Program to the Institute: A number of fatal neurological diseases are caused by persistent viral infections, including subacute sclerosing panencephalitis, progressive multifocal leukoencephalopathy, cytomegalovirus inclusion disease, rubella panencephalitis, etc. Usually, irreversible damage has occurred in patients with these diseases before their cause is determined and little hope remains to arrest fatal progression of the disease. Thus, emphasis must be placed on learning how persistent infections become established, evade elimination by host immunological defenses and cause pathological damage to the host. This is the long term goal of this project.

Proposed Course of the Project: We will continue to seek information on mechanisms of SHF virus persistence, the target cells involved in infection and immunological parameters associated with persistence. Future work will emphasize establishing an animal model to study how human birth defects caused by cytomegalovirus infections might be reduced or eliminated.

Publications:

Murphy, M.F.: In vitro inhibition of subacute sclerosing panencephalitis virus by the antiviral agent ribavirin: J. Infec. Dis. 138:249-251, 1978.

Gravell, M., Hamilton, R.S., Kiefer, R.H., Madden, D.L., Sever, J.L., Tourtellotte, W.W.: PAM cell assay as a test for multiple-sclerosis associated agent: Neurology 18:1050-1052, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-01983-08-ID
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Chronic Viral Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William C. Wallen	Senior Staff Fellow	IDB, IRP, NINCDS
Other:	David L. Madden	Veterinary Director	IDB, IRP, NINCDS
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	Sidney A. Houff	Clinical Associate	IDB, IRP, NINCDS
	Bernard Rentier	Visiting Scientist	IDB, IRP, NINCDS
	Renee G. Traub	Microbiologist	IDB, IRP, NINCDS
	Janet M. Mattson	Microbiologist	IDB, IRP, NINCDS

COOPERATING UNITS (if any)
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George Washington University Medical School, Washington, D.C.

LAB/BRANCH
Infectious Diseases Branch
SECTION
Neurovirology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	2.1	PROFESSIONAL:	0.6	OTHER:	1.5
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In some patients with multiple sclerosis, the suppressor T-cell response is elevated against measles virus. Non-specific suppressor cell activity is at normal levels in MS patients in stable phase of this disease. Suppressor cell response to myelin and axolemma antigens is depressed in some MS patients and most of these patients develop a positive cell-mediated immune response to these antigens.

The T_h subpopulation of lymphocytes was shown to interact with IgG, antibody and kill measles virus infected cells in an antibody dependent lymphocyte cytotoxicity assay.

Project Description:

Objectives: This project investigates clinical and biological significance of viral infections in the causation of chronic neurological diseases. The varied immunologic responses to viral infections are studied to determine which parameters control an infection and which contribute to persistence of the virus and result in disease. Particular emphasis is placed on infections by Herpesviruses, Types I and II (HSV-I, HSV-II), cytomegalovirus (CMV) and Epstein Barr virus (EBV) and polyomaviruses (JC and BK). Cell-mediated immunity appears to play a critical role in controlling these viruses. However, the relationship of immunity and its regulation to chronic, persistent infections is not understood. Therefore, we plan to study the immune response and its regulation to these viruses during chronic and acute neurologic diseases.

Methods Employed: The principal methods employed in the study of chronic viral infections of humans include: 1) virus isolation, latent genome recovery by cocultivation, chemical or mitogenic activation; 2) virus quantitation and identification by serology, host cell cytopathogenic sensitivity spectrum and fluorescent antibody techniques; 3) large scale serological surveys of materials from selected patients with specific disease entities, using fluorescent antibody assays, enzyme-linked immunosorbent assays (ELISA) and antibody dependent lymphocytotoxicity assays; 4) cell-mediated immunity (CMI) will be detected employing the lymphocyte stimulation assay for measuring effector cell functions or antigen recognition and the direct cytotoxicity or immune interferon assays for measuring effector immune mechanisms; and 5) immune regulation studies will be performed using a suppressor T-cell assay recently developed to evaluate the regulatory function during neurologic disease.

Major Findings:

a. Herpesviruses - Longitudinal immunological studies were continued on a patient with cerebellitis following infectious mononucleosis caused by EBV. Sixteen months past infection, the patient was diagnosed as having multiple sclerosis. Patient's general immunocompetence was not grossly impaired; however, there was a failure to respond to recall antigens during disease. In addition, a weak antigen specific suppressor T-cell response was detected against EBV during exacerbation of her disease. CMI response was detected to EBV antigen just after initial neurologic signs of disease. However, at the time of diagnosis of MS, no positive cellular immune response to EBV was detected. Serologic responses to EBV have returned to normal levels at this time showing past exposure to EBV but no abnormal response.

b. Papovaviruses - The inability to grow JC virus has been a major* problem. Numerous cell lines, as well as primary cell cultures, have been examined for their ability to allow JC virus replication. To date, virus growth is most permissive in primary human fetal glial cells. Primary human amnion cells are also infectable and produce JC antigens. However, these cells

are only weakly permissive. A human glioma cell line has also been found in which about 30% of the cells will produce T-antigen following infection with JC virus; however, no infectious virus is produced by these cells.

A high titered rabbit anti owl monkey IgG antiserum was prepared, purified and conjugated with phosphotase for use in an ELISA assay to evaluate humoral immunity in owl monkeys infected with JC virus. Preparation of appropriate antigens are in progress.

c. Chronic neurologic diseases - The immune regulatory response of patients with multiple sclerosis (MS) is under study. A T-cell suppressor assay was developed to study the regulations of cellular immunity in MS patients. We found that MS patients had the same level of non-specific suppressor cell activity induced by Con A as carefully matched controls when these patients were in a stable phase of disease. In addition, we found an elevated level of antigen specific suppressor activity against measles virus in stable MS patients (28%) in contrast to a uniform lack of this activity in controls. We have also demonstrated that the majority stable MS patients have specific suppressor response to brain cell associated antigen (axolemma and myelin). However, some patients even in a stable phase of disease, lack the activity and simultaneously show a positive cell mediated response to these antigens.

Seven MS patients and five controls were recently examined for a bone-marrow associated virus. Employing similar procedures to those used in a recent report of successful isolation of an agent from MS bone-marrow, we were unable to isolate an agent from our patients.

d. Mechanisms of immune response - We have initiated a study on the mechanism of antibody dependent lymphocyte cytotoxicity (ADLC). Employing a chromium-51 release assay and the scanning electron microscope, we have demonstrated that in addition to the predominant K cell activity associated with this response, a subpopulation of T-lymphocytes can also participate in this cytotoxic reaction. We have demonstrated that the T-cells bearing receptor for F ψ fragment of IgG will interact and kill viral infected target cells.

Proposed Course of the Project: We propose to

- a. continue studies of the relationship of EBV to chronic or acute neurologic diseases;
- b. continue studies on the relationship of JC virus to human disease and to determine mechanisms of immune control and continue studies of pathogenesis of JC virus in owl monkeys;
- c. continue studies on role of immunoregulatory response in patients with MS;
- d. pursue studies on the mechanisms of ADLC and the role of T γ cells in this response.

Significance of Program to the Institute: Herpesvirus and polyomavirus often establish persistent infections with neurological manifestations. The clinical spectrum of disease associated with EBV (a member of the herpesvirus family) or JC virus infections has not been completely defined. These studies are designed to examine the clinical spectrum of EBV-associated diseases of children, to describe the neurological involvement associated with these infections, and to determine whether this is an etiologic or opportunistic relationship between EBV infection and neurological abnormalities. In addition, the studies of JC virus may provide useful information regarding the spectrum of diseases which this virus can induce.

The importance of delayed hypersensitivity in several viral infections has been well documented. However, the role of CMI in chronic viral diseases with neurological complications is less well studied. These studies should help define the role of delayed hypersensitivity in herpesvirus infections particularly EBV and JC virus which are followed by neurological dysfunction.

Determination of the role of immunoregulatory responses in contributing to pathogenesis in chronic neurologic diseases would be of prime interest. Our studies in this area regarding patients with MS may help determine whether the disease is an autoimmune reaction and may help in understanding the role of infectious agents in this disease.

Publications:

Sullivan, J.L. Wallen, W.C., Johnson, F.L.: Epstein-barr virus infection following bone-marrow transplantation. Int. J. Cancer 22: 132-135, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-01984-08-ID
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Maternal Infection and Pregnancy Outcome		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	William C. Wallen Senior Staff Fellow	IDB, IRP, NINCDS
Other:	John L. Sever Chief	IDB, IRP, NINCDS
	David L. Madden Veterinary Director	IDB, IRP, NINCDS
	John H. Grossman Guest Worker	IDB, IRP, NINCDS
	Renee G. Traub Microbiologist	IDB, IRP, NINCDS
	Janet M. Mattson Microbiologist	IDB, IRP, NINCDS
	Frank J. West Biological Lab Technician	IDB, IRP, NINCDS
COOPERATING UNITS (if any) George Washington University Medical School, Washington, D.C.		
LAB/BRANCH Infectious Diseases Branch		
SECTION Neurovirology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.9	PROFESSIONAL: 0.4	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Studies regarding the <u>immune response</u> of women to <u>HSV-II infections</u> during <u>pregnancy</u> were continued. We have demonstrated the pregnancy does not compromise the general or specific immune responses. <u>Differences</u> between symptomatic pregnant and nonpregnant women regarding response to HSV-II were not significant. <u>Cellular immunity</u> appears to be <u>impaired</u> or <u>delayed</u> in some cases of <u>recurrent infections</u> since a low frequency of responses was detected during virus shedding phases compared to primary infections. The <u>ADLC</u> response was found to follow the <u>IHA</u> response but reached considerably <u>higher titers</u> when compared at peak levels. Significantly <u>elevated</u> levels of specific anti-measles antibody detected by <u>ADLC</u> and <u>ELISA</u> were found in 15% of 93 <u>matched cord-maternal serum</u> pairs. Approximately 9% of the cord samples showed a <u>concentration gradient</u> (>10-fold) for antibody (higher in cord than matched maternal sample).		
37 - IDB/IRP		

Project Description:

Objectives: Infectious diseases play a significant role in causing abnormal neurological development. We are in the process of studying several herpesviruses to determine their role in the development of birth defects and neurological diseases. Sensitive virological and immunological techniques are currently being developed and applied to the investigation of the natural course of the disease induced by these viruses during pregnancy. Particular emphasis will be placed on Herpes Simplex virus, type II (HSV-II) and cytomegalovirus (CMV) infections of pregnant women.

In addition we are studying the development of fetal immunity and its role in protection against viral infections which cause neonatal disease.

Methods Employed: HSV-II and CMV are routinely isolated employing such techniques as genome rescue by cocultivation of intact cells, cellular disruption to isolate intracellular infectious virus, as well as chemical or mitogenic activation of latent genome from infected tissues, biopsies or leukocyte populations.

Several parameters of humoral and cell-mediated immunity (CMI) are routinely employed to determine a comprehensive immunologic response patterns to viral infection. We are currently employing indirect hemagglutination (IHA), virus neutralization, fluorescent antibody assays, ELISA and the antibody dependent lymphocyte cytotoxicity (ADLC) assay for antibody to these viral antigens.

Assessment of cellular immunity include lymphocyte stimulation assay and leukocyte migration inhibition and immune interferon assay. The distribution of lymphocyte subpopulations in pregnant women and newborns will be determined employing assays for quantitation of peripheral blood T-cells and B-cells.

General immunocompetence of host lymphocytes will be performed employing the lymphocyte stimulation assay to evaluate the proliferative response to general mitogens [phytohemagglutinin (PHA), Concanavalin A (Con A) and pokeweed mitogen (PWM)] and general recall antigens (candida, mumps and staphylococcus lysate antigens). In selected cases, the ability of the individual's lymphocytes (Fc receptor cells) to participate in the ADLC assay will be measured with both autologous and known positive sera.

Major Findings: Studies regarding immunity to HSV-II infection during pregnancy have revealed:

1. Symptomatic pregnant women develop the same level of cellular immunity (lymphocyte stimulation) and humoral immunity (Indirect Hemagglutination, ADLC) and in the same frequency as symptomatic nonpregnant women;
2. Pregnancy does not compromise general immunocompetence regarding these parameters of immunity;

3. There appears to be a delay in development of CMI in recurrent infections compared to primary infections;
4. During active shedding of virus, CMI is active only in about 40% of the cases, suggesting some inhibitory factor;
5. In primary infections, ADLC response is delayed compared to IHA antibody response.

In our studies regarding passive transfer of immunity to measles virus we have found

1. A strong concordance between ADLC, ELISA, and hemagglutination inhibition antibody;
2. A lack of concordance between the levels of antibody detected by these assays;
3. Significantly elevated ADLC and ELISA antibody (>10-fold) in about 9% of cord serum samples compared to matched maternal serum;
4. Very high levels of ADLC antibody (>40,000) were detected in about 15% of the cases.

Significance of the Program to the Institute: These studies regarding the natural course of HSV-II and CMV infections in pregnant women and in newborns may help to determine the pathogenesis of these latent, persistent viruses. In addition, these studies may delineate mechanisms of immunological control of these viruses under normal conditions. It would be of importance to determine the contribution that immunity or immune deficits play in development of viral latency or persistence and to the subsequent neurological dysfunction that may occur later in life as a result of infections with these viruses.

Proposed Course of the Project: Studies regarding the natural history of HSV-I and II and CMV in pregnant women and its consequences to newborns will continue on a longitudinal basis. Immunological studies will be used to determine: 1) the role immunity plays in control of disease; 2) tests for prognostic evaluation using various viral-related antigens; and 3) the mechanisms of protection of the newborn and the contribution of immunity to latency in the newborn.

Nonhuman primates will be employed for more definitive studies regarding the natural course of CMV and herpesviruses as well as to study the parameters of immunity during gestation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-00972-08-ID
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Role of Viruses and Other Microorganisms in the Perinatal Period of Experimental Animals.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	William T. London Amos E. Palmer	Veterinary Director Veterinary Director IDB, IRP, NINCDS IDB, IRP, NINCDS
Other:	John L. Sever William C. Wallen Maneth Gravell Blanche L. Curfman Robert L. Brown Geneva M. Brown Frank J. West	Chief Senior Staff Fellow Research Microbiologist Biologist Biological Lab Technician Biological Lab Technician Biological Lab Technician IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS
COOPERATING UNITS (if any) University of Pittsburgh Presbyterian Hospital, Department of Neuropathology Pittsburgh, Pennsylvania. Meloy Laboratories, Inc., Springfield, Virginia		
LAB/BRANCH Infectious Diseases Branch		
SECTION Experimental Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 4.8	PROFESSIONAL: 0.8	OTHER: 4.0
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Dengue II</u> virus when inoculated intraamniotically into pregnant rhesus monkeys (<u>Macaca mulatta</u>) was found to be a teratogen in this species. Epidemics of Dengue fever are frequent in certain human populations. The possible teratogenic effects of this virus in humans should be investigated. <u>Venezuelan Equine Encephalitis (VEE)</u> has been implicated as a human teratogen. We are studying the pathogenesis of this infection in pregnant rhesus monkeys. The virus produces hydrocephalus 100% when inoculated intracerebrally into the fetus. We are developing a rhesus monkey <u>cytomegalovirus</u> model. This virus produces CNS lesions in the fetus when inoculated intraamniotically into CMV antibody positive pregnant rhesus monkeys.		

Project Description:

Objectives: To study the role of viruses and other microorganisms in the perinatal period, the infection of gravid and non-gravid animals of several different species by parenteral routes with various viruses and other microorganisms to determine the effects of these agents on the animals and their fetal tissues.

Attempt to recover inoculated agents from the various animals and fetal tissues and the correlation of these re-isolations with gestational age at inoculation and dosage given. Relate these findings with gross and histopathological findings. Correlate all of this information with serological findings.

Methods Employed: An investigation of the role of viruses and other microorganisms in the perinatal period by the continual use of experimental animals, tissue culture techniques, histopathological studies and serological testing. Pregnant monkeys were inoculated by various routes and times of gestation with viruses and held in isolation chambers throughout the experiment. The animals were observed and monitored by serum samples, spinal fluid, throat swabs and tissue biopsy for evidence of disease and/or effects on fetal tissue. Pregnant animals were delivered by cesarean section so all products of conception could be saved.

Major Findings:

Dengue

Dengue virus is a togavirus which is transmitted by mosquitos in temperate and tropical climates. We have observed severe destruction of the central nervous system of fetal rhesus monkeys when a Dengue II virus was inoculated intra-amniotically. The virus was inoculated at 100 days gestation into nine pregnant rhesus monkeys and an additional six animals received a similar inoculation of control fluid. At delivery (160 days) Dengue virus could not be detected in the tissues from any of the animals. The nine virus inoculated animals and their mothers developed Hemagglutination Inhibition (HI) antibody indicating that infection had occurred. Three of these animals had hydrocephalus with severe necrotic encephalopathy with total destruction of the cerebral hemispheres. Since Dengue infects as much as 6% of pregnant women in certain populations, the possible teratogenic effects of this virus for humans should be investigated.

Venezuelan Equine Encephalitis (VEE)

Rhesus monkey fetuses have been inoculated intracerebrally with VEE virus at 100 days of gestation. Fetuses are delivered sequentially every 10 days postinoculation until term (160 days). We have observed that even after 10 days, fetuses inoculated with the VEE virus show lesions in the CNS. The

lesions become more severe with time until at full term the entire cerebral hemispheres are destroyed. This virus is 100% effective in producing hydrocephalus. We previously reported cataracts in full term VEE inoculated babies. In the present studies we find that these eye lesions do not develop until some 30-40 days after inoculation of VEE virus.

Rhesus Monkey Cytomegalovirus (Rh CMV)

Rhesus Monkey Cytomegalovirus is a common viral infection of feral rhesus monkeys. About 80-90% of adult animals have had the disease and are latent carriers. This is so similar to human CMV infections that we believe the monkey model would provide a useful tool to study the disease and its effects during pregnancy. Rh CMV was inoculated intraamniotically into 50 and 80 day pregnant rhesus monkeys. We have observed that even though the inoculated monkeys had preexisting serum antibodies to Rh CMV, some of the fetuses developed CNS lesions. This is similar to what has been recently reported in humans.

Significance of the Program to the Institute:

Research for animal models for human diseases known or suspected to cause malformations of the central nervous system should provide an insight into the pathogenesis of these anomalies. Epidemiological studies have shown that there are several viral teratogens in the human populations. These could be more thoroughly studied in animal models. Environmental agents alone or in combination with infectious agents may play a role in the development of certain types of congenital malformations. Animal models would certainly be useful in the study of these conditions.

Proposed Course of the Project:

The VEE study will be completed and the abnormalities of the CNS will be examined histopathologically and reported.

The preliminary work on the Rh CMV model will be completed and additional studies will be started using the model to study the immune regulation of this disease throughout pregnancy.

We had planned to study the effects of Toxoplasma gondii trophozoites on the patas monkey (Erythrocebus patas) fetus. This has been delayed due to a shortage of pregnant patas monkeys. This problem should be resolved this year and studies will begin in this area.

Publications

Palmer, A.E., London, W.T., Sly, D.L. and Rice, J.M.: Spontaneous pre-eclamptic toxemia of pregnancy in the patas monkey (Erythrocebus patas)
Lab. Anim. Sci. 29:102-106, 1979.

Sly, D.L., London, W.T., Palmer, A.E. and Rice, J.M.: Growth and hematologic development of the patas monkey (Erythrocebus patas) to one year of age.
J. Med. Primatol. 7:156-164, 1978.

London, W.T., Kent, S.G., Palmer, A.E., Fuccillo, D.A., Houff, S.A., Saini, N. and Sever, J.L.: Induction of congenital hydrocephalus with mumps virus in rhesus monkeys. J. Infect. Dis. 139:324-328, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-01986-08-ID
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Inoculation of Animals with Tissue Culture Grown Materials from Patients with Chronic Neurological Diseases		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	William T. London Amos E. Palmer	Veterinary Director Veterinary Director IDB, IRP, NINCDS IDB, IRP, NINCDS
Other:	Maneth Gravell Sidney A. Houff John L. Sever Blanche L. Curfman Geneva M. Brown Robert L. Brown	Research Microbiologist Clinical Associate Chief Biologist Biological Lab Technician Biological Lab Technician IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS
COOPERATING UNITS (if any) Meloy Laboratories, Springfield, Virginia George Washington University School of Medicine and Health Sciences, Department of Pathology, Washington, D.C.		
LAB/BRANCH Infectious Diseases Branch		
SECTION Experimental Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.4	PROFESSIONAL: 0.4	OTHER: 1.0
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS	<input type="checkbox"/> (b) HUMAN TISSUES	<input checked="" type="checkbox"/> (c) NEITHER
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>Young cynomolgus monkeys (<u>Macaque cynomolgus</u>) that were inoculated intracerebrally (IC) with "Biken" strain of Subacute Sclerosing Panencephalitis (SSPE) virus were monitored. Animals that were immunosuppressed died in seven days with an acute encephalitis. However, monkeys that did not receive immunosuppressive drugs have developed clinical signs of neurological damage 30 months after inoculation with the SSPE virus. <u>Rubella antibody</u> titers were high in the sera and spinal fluid of the affected animals.</p> <p>In our studies with human cytomegalovirus (CMV) in monkeys, we found that it is essential to use animals that are free of monkey CMV. Owl monkeys (<u>Aotus trivirgatus</u>) and patas (<u>Erythrocebus patas</u>) are utilized. We have developed fluorescence antibody techniques (FA) to quickly identify monkeys carrying monkey CMV. We are now identifying these negative animals and they will be infected with the human strain AD169 CMV.</p>		
45 - IDB/IRP		

Project Description:

Objectives: Develop non-human primate models for the study of chronic neurological diseases (other than spongiform encephalopathies).

Methods Employed: Immunosuppressed and nonimmunosuppressed cynomolgus monkeys were inoculated intracerebrally with subacute sclerosing pan-encephalitis (SSPE) "Biken" strain virus and monitored for infection and clinical signs.

Laboratory reared patas monkeys as well as feral owl monkeys with no detectable FA antibodies to monkey CMV were inoculated with human CMV strain AD-169.

Major Findings:

a. SSPE studies - Nonimmunosuppressed cynomolgus monkeys developed clinical neurological signs (grand mal seizures) about 30 months following IC inoculation. The seizures have increased in frequency and duration. High levels of rubeola antibody as measured by enzyme-linked immunosorbent assay (ELISA) method were found in the sera and spinal fluid of affected monkeys.

Immunosuppressed monkeys developed a fatal acute encephalitis seven days after IC inoculation with "Biken" SSPE virus. No antibodies to rubeola virus could be detected in these animals with acute encephalitis.

b. Chronic cytomegalovirus studies - The owl and patas monkeys can be infected with the human strain of CMV AD-169. Human CMV studies - we experienced problems with our non-human primate model for human CMV. After preliminary studies where we demonstrated that both the owl and patas monkeys could be infected with AD-169 strain of human CMV, we could not repeat our work. This has been corrected by developing an FA antibody test for owl and patas monkey CMV to screen each animal for its homologous virus. Using only monkey CMV negative animals, we now can infect these animals with AD-169 virus.

Significance of the Program to the Institute: Chronic neurological disease represents by far the major portion of the practice of neurology in the United States. Primate models may give answers to the pathogenesis of these diseases. Pathogenic principles derived from these models may then be applied to other chronic neurological diseases, i.e., Steel-Richardson-Olchesky syndrome, Amyotrophic Lateral Sclerosis and Reye's syndrome.

Proposed Course of the Project:

a. SSPE studies - We will investigate the effects of age at the time of viral inoculation on the course of the disease in cynomolgus monkeys.

Project No. Z01-NS-01986-08-ID

Preliminary data indicates that young animals are most susceptible. Animals immunized with measles and then inoculated with "Bikin" strain of SSPE will be continued on study and monitored for clinical disease.

b. Human CMV studies - We will continue to try to produce a model to study CMV infections following immunosuppression as seen in human kidney transplant patients. This disease would then be treated with various antiviral drugs.

Publications:

Palmer, A.E., London, W.T., Sly, D.L. and Rice, J.: Spontaneous preeclamptic toxemia of pregnancy in the patas monkey (Erythrocebus patas). Lab. Anim. Sci. 29:102-106, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-02136-05-ID
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Control of Acute Infectious Diseases in Experimental Animals Using Biologicals and Chemotherapeutic Agents		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	William T. London Amos E. Palmer	Veterinary Director Veterinary Director IDB, IRP, NINCDS IDB, IRP, NINCDS
Other:	John L. Sever Maneth Gravel John W. Larsen Blanche L. Curfman Robert L. Brown Geneva M. Brown Robert M. Chanock	Medical Director, Chief Research Microbiologist Guest Worker Biologist Biological Lab Technician Biological Lab Technician Medical Director IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS LID, IRP, NIAID
COOPERATING UNITS (if any) Meloy Laboratories, Inc., Springfield, Virginia University of Wisconsin Center for Health Sciences, Department of Gynecology and Obstetrics, Madison, Wisconsin LID, IRP, NIAID		
LAB/BRANCH Infectious Diseases Branch		
SECTION Experimental Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.4	PROFESSIONAL: 0.4	OTHER: 2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Toxoplasmosis - Squirrel monkeys (<u>Saimiri sciureus</u>) are extremely susceptible to <u>Toxoplasma gondii</u> . Death from generalized infection occurs seven to nine days after inoculation. A group of these monkeys were inoculated orally with a lethal dose of toxoplasma. Treatment was administered using the antibiotic, <u>spiramycin</u> . All "spiramycin" treated animals survived. The control animals died as usual seven to nine days after inoculation. <u>Group B Streptococcus type III (GBS)</u> studies - We completed the study using prophylactic administration of penicillin to pregnant rhesus monkeys. The drug was given at the time the animals were inoculated intraamniotically with GBS. The babies from the penicillin treated mothers all lived while the babies from the control animals (not treated with penicillin) died of acute streptococcal meningitis.		

Project Description:

Objectives: To study prophylactic and therapeutic agents for the prevention and control of infectious diseases. The testing of candidate vaccines as to their immunogenicity, communicability and safety in experimental animals.

Methods Employed: New chemotherapeutic agents which show promise are studied in appropriate experimental animals. The animals are inoculated with a known infectious agent, then a therapeutic regimen is started, using the test drug. Additional animals are prophylactically treated with the drug, then challenged with the infectious agent.

Biological agents are tested for their ability to protect animals against naturally occurring and experimentally produced infectious diseases. Newly developed vaccines will be tested in susceptible experimental animals. Vaccinated animals will be exposed to susceptible sentinel animals to determine communicability. Vaccinated animals will be challenged at appropriate times to determine the immunogenicity of the vaccine.

Major Findings:

Toxoplasmosis - Toxoplasma gondii is invariably fatal when given in the cyst form to squirrel monkeys (Saimiri sciureus) orally. We have shown that squirrel monkeys infected with toxoplasma could be successfully treated with the antibiotic, spiramycin. We have determined that the optimal dosage of this antibiotic is 100 mg/Kg and the best route was oral administration for 21 days, once a day. Treatment was started 24 hours after inoculation. The control group did not receive the antibiotic and all animals died seven to nine days after inoculation. None of the monkeys treated with spiramycin developed toxoplasma antibodies in their convalescent sera. As expected, no antibodies were found in the control sera since animals died so acutely.

We found in our mouse studies that the drug had to be given initially 24 hours after the organism was inoculated; otherwise, high death losses occurred in the infected mice.

We have previously reported a primate model for Group B Streptococcus (GBS). Using this model, we have shown the efficacy of prophylactic administration of penicillin to mothers following inoculation with GBS type III. The drug was administered both intravenously and intramuscularly to each pregnant female. All babies survived from mothers treated with penicillin at the time they were inoculated intraamniotically with GBS. All babies from the control mothers that did not receive penicillin died 24 to 72 hours after inoculation with the GBS agent. We monitored the GBS infected females for several weeks after inoculation and found that they had developed a chronic cervical infection. This finding makes our primate model even more useful because of the similarity to the human problem of chronic cervical infection.

Significance of the Program to the Institute: Experimental animal studies permit the study of human diseases, their prevention and treatment with chemotherapeutic agents and biological products. Such studies provide information of efficacy, safety and side effects of these products. Information gained from experimental animal studies provides the bridge to the implementation of clinical studies in man.

Proposed Course of the Project: The treatment of toxoplasmosis in humans generally consists of sulfonamides combined with pyrimethamine. It is not highly satisfactory in preventing the spread of the organism from the infected mother to her fetus. We will compare this treatment with spiramycin using our squirrel monkey model. We intend to investigate why the squirrel monkeys treated with spiramycin did not develop toxoplasmosis antibodies in their convalescent sera. Does the drug destroy the organisms before the body can recognize the agent? Or, does the drug have some effect on the animal's immune system?

In the GBS studies we want to use various antibiotics to "clear" the chronic cervical infection in our rhesus females. This would be very useful since the very same problem exists in humans. When the commercial GBS vaccine becomes available later in 1979-80, we would like to test this vaccine in our rhesus model to determine if immunized mothers would protect their fetuses from a challenge of GBS agent.

Publications:

Larsen, J. W., Jr., London, W. T., Palmer, A. E., Tossell, J. W., Bromsteen, R. A., Daniels, M., Curfman, B. and Sever, J. L.: Experimental Group B streptococcal infection in the rhesus monkey I. Disease production in the neonate. Am. J. Obstet. Gynecol. 132: 686-690, 1978.

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Richardson, L. S., Belshe, R. B., London, W. T., Sly, D. L., Prevar, D. A., Camargo, E. and Chanock, R. M.: Evaluation of five temperature-sensitive mutants of respiratory syncytial virus in primates: I. Viral shedding, immunologic response, and associated illness. J. Med. Virol. 3: 91-100, 1978.

Belshe, R. B., Richardson, L. S., London, W. T., Sly, D. L., Camargo, E., Prevar, D. A. and Chanock, R. M.: Evaluation of five temperature-sensitive mutants of respiratory syncytial virus in primates: II. Genetic analysis of virus recovered during injection. J. Med. Virol. 3: 101-110, 1978.

Grizzard, M. B., London, W. T., Sly, D. L., Murphy, B. R., James, W. D., Parnell, W. P. and Chanock, R. M.: Experimental production of respiratory tract disease in cebus monkeys after intratracheal or intranasal infection with influenza A/victoria/3/75 or influenza A/New Jersey/76 virus. Infect. Immun. 21: 201-205, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-02271-03-ID
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Papovaviruses in Non-human Primates		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	William T. London Amos E. Palmer	Veterinary Director Veterinary Director
		IDB, IRP, NINCDS IDB, IRP, NINCDS
Other:	David L. Madden Sidney A. Houff Maneth Gravell William C. Wallen John L. Sever Blanche L. Curfman Robert L. Brown	Veterinary Director Clinical Associate Research Microbiologist Senior Staff Fellow Chief Biologist Biological Lab. Technician
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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		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.4	PROFESSIONAL: 0.4	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Seventy owl monkeys (<u>Aotus triviragatus</u>) were inoculated with JC virus, a human polyomavirus. An additional 22 monkeys were used as controls. Some of the animals were used to confirm our finding that two owl monkeys inoculated with JC virus developed brain tumors at 16 and 25 months postinoculation, respectively. Remaining animals were used in passage of the JC induced primary owl monkey tumor and variable routes of inoculations (single routes) instead of the multiple route in the original studies. The animals have been monitored for JC antibody. To date, most of the virus inoculated animals have developed hemagglutination inhibition (HI) titers to JC virus.		

Project Description:

Objectives: To study the pathogenesis of papovavirus induced tumors and disease in non-human primates.

Three serologically distinct papovaviruses have been isolated from humans. The JC and SV-40-PML strains have been isolated from patients with Progressive Multifocal Leukoencephalopathy (PML). BK virus has been isolated from the urine of renal transplant patients and at least one normal child.

Inoculation of JC, SV-40-PML and BK viruses in hamsters, rabbits, rats, mice and bovines has resulted in tumors of various types. There have been no reports of the production of tumors in subhuman primates. Lesions resembling PML have also been noticeably absent in small animals. PML in a primate host other than man has occurred spontaneously in a rhesus colony chronically treated with isoniazid. Of 400 monkey brains collected at autopsy, eight were found to have lesions similar to those described for PML. Electron microscopy of formalin fixed tissues revealed papova-like virions in six of these brains. Lymphoma, autoimmune disease and other opportunistic infections occurred in all eight animals. The development of an animal model would contribute significantly to understanding the pathogenesis and evaluating treatment.

Methods Employed: Twenty adult feral Colombian owl monkeys (Aotus trivirgatus) were inoculated intravenously (IV) and intracerebrally (IC) with JC virus in attempts to confirm the original studies. Several owl monkeys were inoculated using a single variable route. This series included: a) intracerebral; b) intraperitoneal; c) intravenous; and d) inhalation. Another group of owl monkeys has been inoculated with primary tumor cells from original owl monkey glioma. A total of 92 animals were used in these studies.

Significance of the Program to the Institute: Demyelinating diseases are a major cause of neurological disability in the United States. Multiple Sclerosis, Schilder's disease, Devic's syndrome, post vaccination encephalomyelitis as well as PML are all illnesses characterized by loss of or defective myelin. The study of a known viral induced demyelinating illness will hopefully give us the basic knowledge which is needed to understand the pathogenesis and etiology of the major white matter diseases of man.

Proposed Course of the Project: Continue to monitor the 92 owl monkeys on this study. Each monkey's general physical condition is closely followed using monthly weights, sera, antibody titers to JC virus and quarterly hemograms as parameters.

If animals develop tumors they will be killed and the following studies done:

- a. Attempts to rescue the virus from tumor using co-cultivation techniques.
- b. Hybridization between JC virus DNA and DNA extracted from tumor cells will be done to delineate portions of JC virus DNA present in the tumor genome. These attempts may define which portions of JC virus are required for tumor induction in the non-human primate.

c. Neuropathological examination of tumor tissue will be done using both light and EM microscopy. Similarities and dissimilarities to human tumors will be emphasized. If our previous experience is confirmed, the occurrence of human type tumors will provide the first primate model of glioma available for important studies in chemotherapy of brain tumors.

Publications:

London, W. T., Houff, S. A., Madden, D. L., Fuccillo, D. A., Gravel, M., Wallen, W. C., Palmer, A. E., Sever, J. L., Padgett, B. L., Walker, D. L., ZuRhein, G. M. and Ohashi, T.: Brain tumors in owl monkeys following inoculation with a human polyomavirus (JC virus). Science 201: 1246-1249, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-02034-07-ID
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Electron Microscopic Studies of Viruses of the Nervous System and of Demyelination

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Anne Claysmith	Biological Lab. Technician	IDB, IRP, NINCDS
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	William C. Wallen	Senior Staff Fellow	IDB, IRP, NINCDS
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	Jeffrey E. Greenstein	Clinical Associate	NIB, IRP, NINCDS
	Robert Lazzarini	Section Chief	LMB, IRP, NINCDS
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COOPERATING UNITS (if any)

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LAB/BRANCH

Infectious Diseases Branch

SECTION

Electron Microscopy

INSTITUTE AND LOCATION

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TOTAL MANYEARS:

6.0

PROFESSIONAL:

2.5

OTHER:

3.5

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Defective interfering particles modulate rhabdovirus infection of neurons in vitro. This modulation of viral infection is obtained more easily in differentiated neurons. Antibody interaction with vesicular stomatitis virus (VSV) infection neurons activates phagocytic cells, restricts viral maturation sites mostly to postsynaptic area and later results in apparent curing of infection. A temperature insensitive revertant of vesicular stomatitis virus causes a lower motor neuron disease in mice with selective replication in anterior horn cells of the spinal cord in the preclinical stage while neuronal vacuolization is dominant during the disease. In visna infected mouse nerve cells, a slow non-productive infection with progressive fusion of neurons occurs and virus specific proteins are expressed in the virtual absence of viral assembly. A wild measles strain can induce a slow progressive infection of mouse neurons in culture when cells are inoculated early in their differentiation. During antibody dependent lymphocyte cytotoxicity on measles virus infected cells, effector killer cells were studied by rosetting techniques in scanning electron microscopy (EM) and a subpopulation of T cells bearing FC receptors was identified.

57 - IDB/IRP

Project Description:

Objectives: To study with combined morphological and immunological techniques: 1) mechanisms of acute and chronic infection of dissociated nerve cells with a variety of viruses known to produce diseases of the central nervous system (CNS) in animals and/or man; 2) interactions between antibodies and lymphocytes and the surface of cells infected with these viruses; and 3) the brains of animals and patients with acute and chronic viral infection and/or demyelinating diseases.

Methods Employed: Tissue culture cell lines of various origin as well as mouse CNS cultures are investigated after viral infection, in transmission electron microscopy (TEM) and scanning electron microscopy (SEM), with or without labeling of viral antigen using Protein A peroxidase. New techniques have been developed for freeze fracturing monolayers of infected cells and for combining procedures with etching and rotary shadowing; a new technique for examining cells grown on formvar coated grids is presently used.

Major Findings:

Acute and Chronic Infection of Dissociated Nerve Cells With Various RNA viruses:

Defective interfering (DI) particles modulate VSV infection of dissociated neuron cultures. When dissociated neuron cultures of mice are infected with wild virus, VSV replicates selectively in neurons, producing cell death within 24 to 48 hr. Sensory and immature neurons express viral antigen most rapidly. Viral antigen and viral budding sites are detected along the neuron soma and dendrites. When large amounts of DI-T (truncated) particles are added to the wild virus inoculum, viral growth is completely suppressed in mature neurons, the cell killing effects of VSV are considerably delayed and coinfecting cultures survive 5 to 16 days. Viral antigen accumulates in cytoplasmic inclusions and on the membrane of neuron cell somas and dendrites in the virtual absence of viral assembly. Identical modulation of VSV infection in mature neuron cultures is obtained when DI-T particles are added before or after the wild virus but UV inactivation of DI's completely abolish their protective effect. Immature neurons or Vero cells cannot be protected from acute cytopathic changes by an equivalent amount of DI particles. Thus DI's interfere with replication and assembly of the wild virus and attenuate cell killing effects in mature neurons in vitro.

Antibody induced modulation of rhabdovirus infection of neurons in vitro. Dissociated neuron cultures of mice were inoculated with vesicular stomatitis virus and subsequently refed with medium containing sufficient antiviral antibody (AB) to neutralize all free virus. In contrast to acute neuronal infection which lasts 1 to 2 days, AB treated monolayers survived 1 to 2

weeks. Viral buds were often grouped in patches or caps instead of being diffused on the neuronal surface. Aggregates of released virus were seen around the neuron while large expanses of neuronal membrane were devoid of viral antigen. In addition, cells identified as macrophages directly engulfed viral particles in phagocytic vacuoles. Uncoated viruses and partly degraded viral cores were also seen in the lysosomes of these cells. In the presence of AB, nerve cell processes contained more viral antigen than in acute infection. Viral assembly occurred primarily in the vicinity of post-synaptic membrane but never through the specialized junction. Free virus present in pockets of extracellular space were seen in invaginations of the lateral membrane of presynaptic terminals or within coated vesicles forming at these sites. Thus, antiviral AB probably crosslinks membrane viral antigen on the neuron surface and this results in redistribution of viral maturation sites and clearing of antigen-AB complexes from the cell surface, partly through macrophage activation. Assembly sites are progressively restricted to perisynaptic areas where spreading of the virus from post to pre-synaptic sites seems to be favored by antibody. During the first week postinfection, removal of AB resulted in reactivation of viral infection in more neurons than those originally infected. Complete viral maturation and release occurred only 2 to 3 days after removal of AB. Chances of reactivation decreased with increasing length of AB treatment and no viral antigens or budding sites were detected during the second week of AB treatment. Thus, AB interaction with VSV infected neurons activates phagocytic cells, restricts maturation sites mostly to postsynaptic area and later results in apparent curing of infection.

Visna virus induced fusion of nerve cells in vitro. Two antigenically distinct strains of visna virus (1514 and D₁-2) were inoculated into dissociated mouse cultures containing spinal cord and ganglia neurons as well as non-neuronal cells. In contrast to the acute productive infection caused by these strains in sheep choroid plexus cells, slow and progressive cytopathic changes were observed in neuron cultures after 3 days and lasted for 2 to 4 weeks. Neurons progressively changed shape and an increasing number of multinucleated cells formed in the monolayer. With the D₁-2 strain, fusion was first observed in non-neuronal cells and later in neurons, whereas 1514-infected cultures revealed only neuronal fusion. Not more than 50% of D₁-2 multinucleated cells, but virtually all 1514 ones stained for neuron specific enolase, a neuronal marker. Staining of multinucleated neurons was consistently less intense than that of control neurons. Whereas 1514 multinucleated cells usually had several synapses present on their cell body and processes, several D₁-2 syncytia were devoid of synaptic contacts. Giant cells had peripheral nuclei and numerous cytoplasmic organelles in their center. No capsids, viral buds, or complete viruses were found by electron microscopy and no increase in infectious virus was detected in the supernatant at various times postinoculation. With immunofluorescent staining, visna core polypeptide was variably shown to be present in scattered neurons while anti-visna hyperimmune serum demonstrated viral antigen in mononucleated neurons,

nerve processes and the periphery of the largest syncytia. Thus, in visna-infected mouse nerve cells, a slow non-productive infection with progressive fusion occurs and virus specific proteins are expressed in the virtual absence of viral assembly.

A wild measles strain can induce a slow progressive infection of mouse neurons in culture. We have succeeded very recently in infecting nerve cell cultures early in their maturation with a wild type measles virus, using a high multiplicity of infection. The emergence of measles virus antigens in the infected cells as detected by immunofluorescence and immunoperoxidase occurs early after infection and lasts up to 24 days at least. This course of infection is very different from the acute infection of Vero cells (cultures of monkey kidney cells). No infectious virus has been so far recovered from these cultures and little, if any, cytopathic effect has been observed. This phenomenon indicates a host cell restriction of measles virus expression and is presently analyzed by transmission and scanning electron microscopy combined with immunoperoxidase labeling of viral antigens. In addition, the biochemical analysis of measles polypeptides in these cultures is currently performed by immunoelectrophoresis techniques in collaboration with the Neuroimmunology Branch of our Institute.

Structural Study of Interactions Between K Cells and Measles Virus Infected Cells:

During antibody dependent lymphocyte cytotoxicity (ADLC) on measles virus infected cells, structural interactions between target cells and effector (K) cells which rosette IgG coated chicken erythrocytes (CEAB) appear mostly restricted to non-T cells bearing Fc receptors (Fc^γ). Scanning and transmission electron microscopy of K cell killing showed that the first event was a recognition process occurring within 15 min. Plasma membrane and microvilli of most human peripheral blood lymphocytes (PBL) became specifically attached to virus-induced ridges over nucleocapsids and to viral buds. After 30 min., K cells changed shape and extended filopodia towards target cells which in turn showed long villi contacting the K cells. At 4 hrs., when cytotoxicity was maximum (20 to 25% of ⁵¹Chromium releases), K cells had flattened and numerous blebs and ruffles formed on their surface. K cell alterations varied in intensity with the type of measles infected target cell and sometimes K cells appeared irreversibly damaged. Besides the K cells just described, a subpopulation of T cells, which bind sheep erythrocytes, were found to be also involved in ADLC. These Tfc⁺ cells were purified by successive rosettings with Se and CEAB and identified by their double surface label. They extended one long uropod on the target cell but did not show intense cellular alterations, suggesting that they might remain functional after interaction.

Animal and Cell Studies on Viral Infection and Demyelinating Diseases:

Infection of the central nervous system produced by R₁ vesicular stomatitis virus (in collaboration with J. Greenstein and H. McFarland). We studied the disease produced by R₁VSV, a temperature insensitive revertant derived from the TS T1026. In vitro R₁VSV replicates to high titers while allowing normal host protein synthesis. While intracerebral inoculation of BALB/c mice with wtVSV produces encephalitis and death in 2-3 days, inoculation of R₁VSV produces a paralytic disease on day 5 post inoculation and death on days 7-8. Immunofluorescent staining demonstrated viral antigen throughout the CNS but predominantly in the anterior horn cells. These were rarely necrotic showing extensive dilation of the endoplasmic reticulum in the absence of viral nucleoprotein accumulation with moderate spongy change in the surrounding neuropil. In R₁VSV, unlike tsVSV infections, the neurons were surrounded by large numbers of inflammatory cells. Perivascular cuffing by mononuclear cells were prominent throughout the spinal cord. Large amounts of virus were present in brain and spinal cord by day 3 but declined coincident with the onset of clinical disease, corresponding with the infrequent finding of viral budding demonstrated by electron microscopy. The neuronal pathology and inflammatory response seem related to the characteristics of R₁VSV and appear to represent an intermediate step between wtVSV and essentially non-productive tsVSV infections.

Cultures of myelin forming cells are presently developed in our section. Suckling mouse brain cultures enriched in astrocytes or oligodendrocytes have been established. In these cultures, fibroblast growth was inhibited using FudR or synthetic media. The identification of specific nerve cell types in these cultures are presently attempted with specific markers for neurons (by specific enolase or tetanus toxin) for oligodendrocytes (anti-galactocerebroside and glycerol phosphate dehydrogenase) and for astrocytes (gliofibrillary acidic protein).

Significance of the Program to the Institute: Measles in men, visna and VSV in animals are all involved in chronic infections of the CNS. Basic knowledge on how these viruses modify the cell membrane, first in simple cell system and second in nerve cells will help to understand the pathogenesis of disease. Recent development of high resolution SEM allows detailed analysis of cell surfaces with the same level of resolution as in thin section.

Furthermore, the chronic stage of disease might often result from the modification of the infected cell membrane by specific antibody. Details of that interaction should thus be analyzed as well as the selective replication of virus in one type of nerve cells which might result in specific dysfunction. Any study aiming at a better recognition and understanding of glial cell susceptibility to viral infection and of myelin vulnerability seems meaningful to multiple sclerosis research.

Proposed Course of the Project: Since measles infection of dissociated neuron cultures has been obtained, it is now possible to study 1) the surface changes induced by the virus using SEM combined with immunolabeling of viral antigens; 2) how the surface viral components are modified in the presence of specific antibody; and 3) intramembrane changes in these cells using new techniques that allow freeze-fracture of monolayers. We also want to study the effect of antimeasles virus antibody on the outcome of infection of nerve cells and determine whether the continuous presence of this antibody in the culture medium can favor a more persistent infection and modulate the course of infection as demonstrated with other viruses such as vesicular stomatitis virus. Antibodies can control the level of virus production and act as a mediator for the antibody-dependent lymphocyte cytotoxicity (ADLC), a cell-killing activity effected by a subpopulation of lymphocytes carrying Fc receptors for IgG. The techniques we have developed for the observation of ADLC on infected cell cultures should be applied to dissociated neuron cultures.

The dissociated neuron cultures present many advantages compared to other types of nervous system cultures, mainly an accessibility of the cells to homogenous viral infection, to antibody treatment, to immunolabeling and to surface observation with the scanning electron microscope. Aggregated neuron cultures are much too thick for these purposes and organotypic explants are covered by a continuous membrane separating them from the medium. However, a disadvantage of the dissociated neuron cultures is that they rarely form myelin and, if any, never enough to compare with an *in vivo* situation. We would attempt to produce myelination by grafting oligodendrocytes or Schwann cells to the dissociated neuron cultures during axon sprouting. The rat oligodendrocytes are known to induce myelination of axons *in vitro*. The myelin obtained would thus be in direct contact with the medium and this system would allow the study of the process of demyelination during infection by various RNA viruses and during exposure to antiviral or antimyelin antibodies and lymphocytes.

An interesting virus for the study of demyelinating diseases is mouse hepatitis virus. We would like to localize viral antigens *in vitro* and *in vivo* following infection with wild type and temperature-sensitive mutants of this virus in collaboration with scientists at the Scripps Clinic, San Diego. Mouse hepatitis virus type 4 (JHM) produces an acute fatal encephalomyelitis with involvement of both grey and white matter. The surviving animals occasionally exhibit primary demyelination. Recently temperature-sensitive (ts) mutants have been isolated that are highly attenuated ($LD_{50} < 10,000$ PFU) and yet produce demyelination with high frequency. The purpose of this study would be to determine whether ts mutants have altered cell tropism in the central nervous system compared to the wild type (wt) virus. Further,

viral expression in nerve cells may be attenuated. We plan to investigate this problem using immunolabeling methods for specific viral antigens both in vitro and in vivo at the light and electron microscopic levels.

In addition, new techniques are being developed in our Section on Electron Microscopy. A scanning transmission electron microscope will be used for study of whole cells and other thick specimens and for high resolution secondary electron imaging. A freeze-fracturing apparatus equipped with 2 electron beam guns and a rotary table allow us to view intramembrane and surface changes as well as cytoplasm organization when combined with deep etching and rotary shadowing (in collaboration with Dr. T.S. Reese). We have set up recently a video intensified microscopy (VIM) system for continuous observation of living cells with the phase microscope. This system allows long term observation of cells. Because of the high sensitivity of the TV camera, low light levels are required and cells are not damaged by illumination. A time-lapse recorder accelerates the actual motions during playback and permits a better understanding of cell behavior in culture, including cytoplasmic motion, neurite extension, synapse formation and all recognition phenomena, as well as modifications and abnormal function of virus infected cells, like cell fusion for instance. The VIM set up will also be very useful in the observation of lymphocyte cytotoxicity, for numeration of active lymphocytes, time course study and observation of possible recycling of the lymphoid cells.

Publications:

Dubois-Dalcq, M.: Pathology of measles virus infections of the nervous system. Comparison with multiple sclerosis. Int. Rev. Exp. Pathol. 19: 101-135, 1978.

Rentier, B., Hooghe-Peters, E.L. and Dubois-Dalcq, M.: Electron microscopic study of measles virus infection: Cell fusion and hemadsorption. J. Virol. 28: 567-577, 1978.

Van Pottelsberghe, C., Rammohan, K.W., McFarland, H.F. and Dubois-Dalcq, M.: Selective neuronal, dendritic and postsynaptic localization of viral antigen in measles infected mice. Lab. Invest. 40: 99-108, 1979.

Dubois-Dalcq, M., Narayan, O. and Griffin, D.: Cell surface changes associated with mutation of visna virus in antibody treated cell cultures. Virology 92: 353-366, 1979.

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Faulkner, G., Dubois-Dalcq, M., Hooghe-Peters, E., McFarland, H.F. and Lazzarini, R.A.: Defective interfering particles modulate VSV infection of dissociated neuron cultures. Cell, 1979. (in press).

Hooghe-Peters, E., Dubois-Dalcq, M. and Schmechel, D.: Visna virus induced fusion of nerve cells in vitro. Laboratory Investigation, 1979. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-01924-09-ID
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Genetic Studies of the Torsion Dystonias, Tourette Syndrome and Other Disorders of Movement

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Stanley Fahn	H. Houston Merritt Professor of Neurology	College of P&S, N.Y.
	Donald Riker		Yale University

COOPERATING UNITS (if any) Neurological Institute, New York, New York
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LAB/BRANCH
Infectious Diseases Branch

SECTION
Neurogenetics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.75	PROFESSIONAL: 0.25	OTHER: 0.5
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this project we seek to clarify and expand the nosology of the hereditary movement disorders, contribute to the understanding of their underlying biochemical basis, determine the most effective treatment for each and suggest guidelines for counseling individuals at risk. General syndromes under study include the dystonias, tic disorders including Tourette syndrome, Huntington's chorea 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.

Low levels of biopterin, the hydroxylase cofactor for tyrosine, tryptophan, and phenylalanine in CSF of four patients with familial dystonia suggest a promising area for study.

Project Description:

Objectives: Included among the disorders of movement such as the choreas and dystonias and tic syndromes are a number of discrete diseases which are due to a single gene mutation. Examples of mutations producing autosomal dominant traits are Huntington's chorea and one form of torsion dystonia. Examples of mutations leading to autosomal recessive traits are Lafora type myoclonic epilepsy and the type of torsion dystonia responsible for most cases in the Jewish population.

In this project we seek to uncover additional specific diseases within general movement disorder syndromes, contribute to the understanding of their underlying biochemical basis, determine the most effective treatment for each and suggest guidelines for counseling individual family members.

Methods Employed: Initially families with members exhibiting a particular syndrome undergo detailed clinical evaluation. Extensive genetic data is then analyzed in conjunction with clinical observations and relevant laboratory studies. A nosologic classification is prepared. Promising biochemical leads are explored in collaboration with established investigators. Simultaneously, existing treatment programs are evaluated, and where indicated, there are therapeutic trials of new agents.

Major Findings: During the past year, comprehensive reviews have been prepared concerning genetic epidemiology of Gilles de la Tourette syndrome and the autosomal recessive form of torsion dystonia. The former review was based on a study of 21 selected families in New York City and the available 14 families in Minneapolis with Tourette syndrome. Together these studies indicated: a familial concentration of cases in those of Jewish or other East European ancestry; history of frequent transient motor and vocal tics in female relatives of Tourette patients who are predominantly male; and an abnormal behavior component which is seen in some with this trait.

The dystonia review, based on our experience with over 230 families, documents the uniform clinical picture and natural history and the diffuse European ancestral origins of Ashkenazic families. The association between recessive dystonia and increased IQ is documented and the role this association plays in explaining the unusual gene frequency and its possible biochemical basis is discussed.

Significance to Biochemical Research and the Program of the Institute: Individually these disorders are uncommon but collectively the hereditary disorders of movement represent one of the major public health problems. In addition, information gained from analysis of these discrete genetic traits may contribute understanding to the cause and treatment of more common problems, such as Parkinsonism, in which the genetic constitution may be only one of several contributing factors.

Proposed Course: Continue search for distinct entities within movement disorders syndromes seeking their biochemical basis, specific therapy and prevention. Paroxysmal choreo-athetosis and progressive myoclonus epilepsy are among those other traits which may be specifically investigated with collaborators in the near future.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-01927-09-ID
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Clinical, Genetic, Pathophysiologic Study of Hereditary Nervous System Tumors		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	Roswell Eldridge	Medical Director IDB, IRP, NINCDS
Other:	Thelma Koerber	Technical Reference Assistant IDB, IRP, NINCDS
	William Kanter	Research Assistant IDB, IRP, NINCDS
	George Todaro	Chief LVC, NCI
	Robert Fabricant	Staff Associate LVC, NCI
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COOPERATING UNITS (if any) Department of Neurology, Mount Sinai College of Medicine, New York LVC, NCI SN, NINCDS		
LAB/BRANCH Infectious Diseases Branch		
SECTION Neurogenetics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.25	0.5
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS	<input checked="" type="checkbox"/> (b) HUMAN TISSUES	<input type="checkbox"/> (c) NEITHER
<input type="checkbox"/> (a1) MINORS	<input type="checkbox"/> (a2) INTERVIEWS	
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>In this project we seek: to define and classify <u>hereditary tumors</u> of the <u>nervous system</u> in addition to the eight such diseases already recognized; to add to the <u>clinical description</u> and <u>natural history</u> of these diseases; to suggest methods for <u>early diagnosis</u>; <u>evaluate present modes of treatment</u>; and develop methods for <u>preclinical detection</u> and <u>screening</u>.</p> <p>We have documented a <u>central form</u> of <u>neurofibromatosis</u>. In cooperation with several collaborators, we have demonstrated distinct alteration in <u>nerve growth factor</u> in blood in affected individuals and approximately half of those <u>at risk</u>.</p>		
69 - IDB/IRP		

Project Description

Objectives: There are at least eight genetically determined syndromes which include as one of their chief manifestations, tumors of the nervous system. Central neurofibromatosis and tuberous sclerosis are among the more common examples. It is the objective of this project to document additional hereditary traits which can cause such neoplasms; add to the clinical description and natural history of such traits; suggest effective means of early diagnosis; evaluate various modes of treatment and develop methods of preclinical detection and screening.

Methods Employed: In families with two or more individuals affected with the same rare tumor of the nervous system, members undergo clinical, genealogic and radiologic evaluation. Appropriate physiologic and biochemical studies are carried out in collaboration with laboratory investigators.

Major Findings: We have documented the existence of "Central Neurofibromatosis" the hallmark of which is the presence of Bilateral Acoustic Neuroma. Recently, we have reported on clinical and genetic findings in over 130 individuals with this trait.

In collaboration with the Department of Medicine, Johns Hopkins Hospital; Laboratory of Viral Carcinogenesis, NCI; and Department of Neurology, Mount Sinai College of Medicine, we are evaluating the usefulness of nerve growth factor in serum as a means of preclinical detection. To date, nerve growth factor has been evaluated in 30 affected individuals and their relatives from three kindreds previously studied by us. A major advantage of the nerve growth factor assay currently employed is its reproducibility, even at low concentrations.

Significance to Biochemical Research and the Program of the Institute: Hereditary tumors of the central nervous system are generally treatable if diagnosed early. Radiologic and physiologic techniques permitting early diagnosis would be of great use. Since many of these hereditary tumors are autosomal dominant with onset during or after the childbearing years, there are individuals who carry a 50 percent risk of developing the trait. Such individuals would gain immediate benefit and relief if a reliable, noninvasive, predictive test were developed. Also, knowledge gained in the course of this development should contribute to understanding the mechanisms of tumor development.

Proposed Course: A report is in press summarizing our clinical and genetic findings in 130 affected individuals with central neurofibromatosis. The utility of nerve growth factor as a preclinical detector in individuals at risk should be clarified.

When sufficient professional personnel and financial resources are available to this Section, comprehensive screening programs for neurofibromatosis, von Hippel-Lindau syndrome and other hereditary nervous system tumors could be undertaken.

Publications:

Kanter, W. and Eldridge, R.: Maternal effect in central neurofibromatosis. The Lancet, 2:8095, 903, 1978.

Fabricant, R.N., Todaro, G.J., and Eldridge, R.: Increased levels of a nerve-growth factor cross-reacting protein in "central" neurofibromatosis. The Lancet 1: 8106, 4-7, 1979

Honors and Awards:

Invitation to speak at the initial meeting of the Metropolitan Washington Chapter of the National Neurofibromatosis Foundation.

Invitation to serve on the Medical Advisory Board of the National Tuberous Sclerosis Association, Laguna Beach, California.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-02167-05-ID
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Infectious, Immunogenetic, Epidemiologic and Clinical Studies in Multiple Sclerosis, Parkinsonism and Other Multifactorial Neurologic Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Roswell Eldridge	Medical Director	IDB, IRP, NINCDS
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COOPERATING UNITS (if any)
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LAB/BRANCH
Infectious Diseases Branch

SECTION
Neurogenetics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.5	OTHER: 1.0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this project we are coupling genetic study of selected families and twin pairs with epidemiologic, immunologic, serologic and neurochemical studies of disorders due to multiple factors such as multiple sclerosis and Parkinsonism. This approach should clarify the etiology of these diseases, indicate individuals or populations at high risk and suggest mechanisms for prevention and treatment.

To date, 18 presumptive "Multiple Sclerosis" families and 97 twin pairs with this condition have been ascertained. Over 15 twin pairs with Parkinsonism have been ascertained.

Project Description

Objectives: As the genetic control of immune response becomes clarified, new avenues of exploring diseases such as multiple sclerosis (MS) are suggested. Improved understanding of the chemical and cellular changes underlying Parkinsonism also permits new approaches to its study. Our objective is to clarify disease mechanism, indicate high-risk individuals and populations and suggest possible means for prevention and treatment.

Methods Employed: Form techniques of clinical genetics, neurochemistry, serology, and immunology will be merged. Selected populations including families with multiple members of twin pairs affected with the disease will be studied in depth. Unaffected family members, unaffected twins and spouses will serve as controls. Specific investigations may include: detailed history and neurologic examination, computerized axial tomography; dermatoglyphic analysis; genotyping of blood for red cell antigens, serum proteins and the A, B, and D loci of the major histocompatibility complex; serum studies of viral antibody, immunoglobulin levels and complement levels; spinal fluid examination for routine elements, plus determination of immunoglobulin content, oligoclonal banding, presence of myelin basic protein; and cellular study of migration inhibition and mixed lymphocyte culture response and genotyping of "B" lymphocyte.

Major Findings: Most impressive has been the difficulty in ascertaining bonafide MS families, and twins with either MS or Parkinsonism. Given the frequency of twinning and the frequency of MS and Parkinsonism, over 1,000 twin pairs with each disorder would be predicted in the United States. Utilizing a variety of ascertainment techniques, including patient and physician contact, notices in medical and lay publications, and base twin registries, less than one-tenth of a predicted number of twin pairs have been found for each condition.

Our detailed study of 14 MS families and 30 MS twin pairs has resulted in the following preliminary conclusions. In half of the families, and several of the twins, diagnosis of MS could not be confirmed clinically. Thus, careful clinical documentation is an essential prerequisite in any patient-based study of this disorder. One monozygotic twin pair over 50 years of age was discordant for MS, although concordant for the HLA-DW₂ antigen which is associated with MS in an unusual frequency. No consistent segregation of HLA type was noted between affected and unaffected family member. Thus, there is not a single, major gene within the HLA complex whose presence is sufficient or necessary for the development of MS. Oligoclonal IgG bands were found in the CSF of all 9 of the unaffected dizygotic twins and 2 of the 6 unaffected monozygotic twins.

Significance to Biomedical Research and the Program of the Institute: Disorders in which both genetic and environmental factors contribute such as MS and Parkinsonism, comprise a major neurologic public health problem. Ample evidence from data based on populations already indicates genetic factors have a role in their causation. By coupling existing knowledge of genetics, the immune response, and neurochemistry, understanding of this groups of disorders should be advanced, methods for prevention and treatment suggested and the risk for these diseases of close relatives assigned more accurately.

Proposed Course: Ascertainment of MS families, MS twin pairs, and Parkinson twin pairs continues. The first phase of the MS family and twin studies is nearing completion. A presentation of the clinical and laboratory observations based on 30 MS twin pairs is in preparation. Genetic and epidemiologic reports on 56 twin pairs will follow.

A preliminary report based on 12 monozygotic twin pairs discordant for Parkinsonism has been presented. Affected twins smoked less and had more introverted personalities than unaffected individuals.

The second phase of these studies will focus on appropriate epidemiology in laboratory studies in selected genetic groups in which the MS or Parkinson phenotype can be assigned more definitely.

Publications:

Eldridge, R., McFarland, H., Sever, J.: Reply to Dr. Karis' letter. Annals of Neurology 4, 1978.

Williams, A., McFarland, H., Eldridge, R., Houff, S., Krebs, H., and McFarlin, D.: Clinical and immunologic studies on selected twins with multiple sclerosis. Neurology 29:573-574, 1979. [Abstract]

Duvoisin, R.C., Eldridge, R., Williams A.: A twin study of Parkinson disease. Neurology 29:578-579, 1979. [Abstract]



ANNUAL REPORT

October 1, 1978 through September 30, 1979

Experimental Therapeutics Branch
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1978 through September 30, 1979
Experimental Therapeutics Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke
Donald B. Calne, D.M., F.R.C.P., Chief

Research has continued on the pharmacology of the central nervous system and the etiology and treatment of neurological disease. There has been no major change in the resources available to the Branch. A program in neuroendocrinology is being started within the Pharmacology Section.

Therapeutics Section

1. Inhibition of Monoamine Oxidase B

Deprenyl has been studied in Parkinsonism, to investigate the effects of inhibition of monoamine oxidase B on transmitter concentrations, and evaluate possible therapeutic efficacy. A fall in the level of plasma epinephrine was detected, together with a rise in the concentration of dopamine in the cerebrospinal fluid. The concentration of catecholamine metabolites in the urine was reduced (homovanillic acid and methoxy-hydroxyphenylglycol). In a double blind study, deprenyl failed to elicit any significant therapeutic response other than elevation of mood.

2. Studies with Bromocriptine

Clinical experience with bromocriptine continues to support the role of this agent as an adjuvant to levodopa therapy. Bromocriptine has also been employed as a tool to study the dopamine receptors concerned with inhibition of prolactin release. We have found that in Parkinsonism there is no impairment of the response of these receptors, assayed as either reduction of spontaneous plasma prolactin levels, or blockade of the increase in prolactin elicited by thyrotropin releasing hormone. This work was performed in collaboration with the University of Virginia, Charlottesville.

A new observation in patients receiving bromocriptine has emerged from biopsy studies of the skin. In subjects who develop an erythromelalgic syndrome while receiving bromocriptine, the red, tender, warm, edematous extremities are associated with mononuclear infiltration of the walls of the dermal blood vessels.

3. Parkinsonism in Twins

Twelve pairs of monozygotic twins have been studied, in whom one is known to have Parkinson's disease. Full neurological evaluation has failed to demonstrate any evidence of Parkinsonism in the second twin. Further twins will be investigated, but the current total discordance is unexpected, and suggests that exposure to environmental factors, after children leave home, may contribute to the etiology of Parkinsonism. This study is being performed in collaboration with Mount Sinai School of Medicine and the Infectious Diseases Branch, IRP, NINCDS.

4. Tetrahydrobiopterin

Last year it was found that the concentration of tetrahydrobiopterin (THB) in the cerebrospinal fluid declines with advancing age, but in Parkinsonism the level is reduced

further (beyond age matched controls). Examination of the cerebrospinal fluid from other neurological patients has shown decreased THB in several disorders, some of which have Parkinsonian features (such as the Shy-Drager syndrome) but also in diseases quite distinct from Parkinsonism (such as torsion dystonia). This work was performed in collaboration with the National Heart, Lung, and Blood Institute.

5. Quantification of Neurological Deficits

In collaboration with the National Institute of Mental Health, techniques are being developed for precise quantification of neurological deficits such as tremor, clumsiness, and disturbances of gait. Computerized methods are being employed for data storage, analysis, and retrieval. These studies will be applied to the evaluation of therapy, and the investigation of pathophysiology. Over the last year this approach has yielded evidence that the rigidity and augmented long loop reflex of Parkinsonism can also be found in elderly subjects without neurological symptoms. This finding may provide an important link between Parkinsonism and the normal process of aging of the nervous system.

6. Oligoclonal Bands in the Cerebrospinal Fluid in Postencephalitic Parkinsonism

In collaboration with the Neuroimmunology Branch, IRP, NINCDS, it has been found that patients with postencephalitic Parkinsonism have oligoclonal bands of immunoglobulin in their CSF, in contrast to patients with idiopathic Parkinsonism. This observation, if confirmed and extended, could provide the basis for a laboratory test to distinguish between postencephalitic and idiopathic Parkinsonism.

Pharmacology Section

1. Stable Isotope Studies of Transmitter Metabolism

Investigations in this Section continue to explore the relation between dysfunction in a specific neurotransmitter system and the appearance of a particular neurologic syndrome. One promising biochemical approach involves the use of a non-radioactive isotope of oxygen, $^{18}\text{O}_2$, to evaluate central monoamine metabolism. Following inhalation of a breathing mixture containing 79% nitrogen and 21% oxygen (95% $^{18}\text{O}_2$), labeling of the major metabolites of dopamine, norepinephrine, and serotonin is readily detectable in plasma, urine, and cerebrospinal fluid (CSF). The time course for the appearance of labeled metabolites in spinal fluid suggests a relatively rapid central turnover for the parent amines. Comparison of results from patients with Parkinson's disease and Huntington's chorea appears to indicate that the former disorder is associated with a relatively small pool of dopamine which is turning over rapidly. Conceivably, the degeneration of dopamine neurons which occurs in parkinsonian patients may be accompanied by a compensatory hyperactivity of residual dopaminergic cells.

2. Substance P in Human CSF

The application of sensitive radioimmune assay to the study of substance P in CSF also continues to yield interesting results. Since no appreciable concentration gradient for this undecapeptide has been found in lumbar spinal fluid, most substance P obtained by lumbar puncture probably derives from spinal cord or dorsal root ganglia rather than

from supraspinal structures. Consistent with this view is the finding of significantly reduced substance P values in patients with Shy-Drager syndrome (multiple system atrophy), but not in those with Parkinson's disease, Huntington's chorea, or various other extrapyramidal disorders (with neuronal degeneration confined to the brain). Remarkably low substance P levels have also been found in patients with peripheral neuropathies, especially in those with predominantly sensory dysfunction. This latter observation is not unexpected in view of preclinical data implicating substance P as a major transmitter in primary afferent pathways.

3. GABA in Human CSF

Studies of the GABA content of lumbar spinal fluid have also remained active during the past year. Reduced CSF GABA values have now been found in patients with Shy-Drager syndrome, progressive supranuclear palsy, and segmental dystonia. These observations, together with earlier results, suggest that brain GABA levels may be diminished in a broad variety of central nervous system disorders, possibly due to degeneration or hypofunction of GABA-containing neurons.

4. Tetrahydrobiopterin in Human CSF

Spinal fluid levels of tetrahydrobiopterin (BH_4), a cofactor for rate limiting enzymes mediating catecholamine and serotonin synthesis, may provide an index to the functional capacity of monoaminergic pathways in the central nervous system of man. Recent CSF studies indicate that Huntington's chorea, amyotrophic lateral sclerosis, and familial dystonia are associated with significantly diminished BH_4 values. These observations, with their implications regarding monoamine system dysfunction, are of particular interest in dystonic patients.

5. Dopamine Agonist Therapy of Neuropsychiatric Disease

The discovery of multiple subtypes of central dopaminergic receptors, with differing pharmacologic characteristics, should enable more focused pharmacotherapeutic interventions in disorders involving the dopamine system. For example, considerable preclinical evidence suggests that drugs which inhibit dopaminergic transmission through preferential stimulation of dopamine autoreceptors might ameliorate certain hyperkinetic extrapyramidal disorders or schizophrenia. During the past year, clinical trials have been completed with two ergot type dopamine agonists, selected on the basis of their pharmacologic profile in the experimental animal as well as because of their differing antiparkinsonian efficacy in man. Bromocriptine, at relatively low dose levels, failed to influence either involuntary movements or psychotic behavior in otherwise untreated patients with chronic schizophrenia and tardive dyskinesia. Another ergot dopaminergic agonist, CF25-397, also had no consistent therapeutic effect in this patient group. On the other hand, preliminary results with a non-ergot dopamine agonist, pibedil, appear more favorable. Until additional preclinical data are available concerning the detailed pharmacologic characteristics of these agents and more thorough dose-finding studies have been completed in man, the status of our hypothesis that dopamine autoreceptor stimulation will alleviate symptoms of some hyperdopaminergic states remains indeterminate.

6. Cholinergic Agonist Therapy of Neuropsychiatric Disease

Anatomic and pharmacologic studies in the experimental animal suggest that augmentation of striatal cholinergic transmission should improve patients with tardive dyskinesia. Modestly beneficial results with acetylcholine precursor therapy support this theoretical rationale. Nevertheless, a recently completed study of arecoline, a potent and specific muscarinic agonist, failed to significantly suppress hyperkinetic symptoms, and an exploration of factors which might account for the differences between cholinergic precursor and cholinergic agonist therapy are now underway. Since the reduction in brain choline acetyltransferase activity in Alzheimer's disease may indicate cholinergic hypofunction contributes to the cognitive defect, studies of arecoline therapy have also been initiated in patients with various senile and presenile dementias. Preliminary indications suggest that a dose-dependent improvement in memory does occur in a subgroup of these patients.

7. Stiff-Man Syndrome

Four of five patients studied with this rare disorder have been found to have associated autoimmune dysfunction and all had an HLA haplotype (BW44). This result may indicate that an autoantibody directed against spinal cord inhibitory neurons contributes to the pathogenesis of stiff-man syndrome. CSF studies of these patients revealed an increase in norepinephrine and a decrease in GABA levels. Although attempts to correct possible GABA system hypofunction with a GABA agonist (muscimol), or to counteract possible hyperadrenergic function with a beta adrenergic blocker (propranolol) were ineffective, thymoxamine (an alpha adrenergic antagonist) alleviated symptoms in some patients.

Clinical Epilepsy Section

1. Diagnostic and Therapeutic Reevaluation of Patients with Intractable Epilepsy

Many patients, especially those with complex partial seizures, are incapacitated by their disorder in spite of recent advances in the therapy of epilepsy. The Clinical Epilepsy Section has been interested in improvement of seizure control, reduction of drug-induced side effects, and improved potential for rehabilitation by the utilization of newly developed intensive monitoring techniques. These include simultaneous video recording of seizures, long-term telemetering of EEGs, and daily determinations of antiepileptic drug concentrations.

A long neglected area that has been investigated by intensive monitoring is the psychogenic (hysterical) seizure, a major diagnostic problem. Intensive monitoring of patients with intractable seizures has uncovered a previously unsuspected frequency of psychogenic attacks. In our own series of 78 patients who have undergone intensive monitoring, six (8%) have unequivocal psychogenic attacks. It is important to establish and evaluate diagnostic criteria because: (1) these patients are a relatively common problem; (2) diagnosis can be quite difficult; (3) although they are often treated with antiepileptic drugs for their non-epileptic seizures, they require, obviously, a different therapeutic approach.

The Clinical Epilepsy Section is constantly making technical advances in intensive monitoring, both at the electronic and the pharmacologic level. The intensive monitoring unit at the Clinical Center continues to be a model for investigators and clinicians interested in the epilepsies. The NIH is expected to provide leadership in the technical

and scientific aspects of intensive monitoring; the Section has continued to provide this.

2. Clinical Pharmacology of Antiepileptic Drugs

In addition to the relatively wide scope of the intensive monitoring protocol above, the Clinical Epilepsy Section is interested in the clinical pharmacology of old and new antiepileptic drugs. Pharmacologic projects are underway and are described in the following paragraphs.

As an integral part of the protocol on intensive monitoring of patients with intractable epilepsy, the Clinical Epilepsy Section has been evaluating the efficacy of a single drug as an alternative to polypharmacy in the treatment of seizures. This is in response to reports which suggest that the use of multiple drugs may actually be detrimental to seizure control in some patients. This concept has not previously been applied to a population of highly intractable patients. We treated nine hospitalized patients with severe, intractable complex partial seizures with phenytoin alone; all were tried on two or more different phenytoin dosages. We evaluated 32 steady-state phenytoin regimens, using dosages of 225-450 mg qd. The phenytoin monotherapy regimens were compared with multidrug regimens. Using all monotherapy regimens, average seizure frequency was unchanged in one patient and worse in eight ($p < 0.01$). Using the most effective monotherapy regimen, two patients were improved and seven were worse; this difference was not significant. These preliminary findings suggest that most patients with intractable seizures are more effectively treated with a multiple drug regimen than with phenytoin alone.

Valproic acid is a new drug which is structurally unrelated to conventional anticonvulsant drugs. However, when valproic acid is administered in conjunction with phenobarbital, it potentiates side effects of the latter. We investigated the mechanisms for the alteration by valproate in the serum level of phenobarbital by mass spectroscopy. The data suggest a primary effect at the site of metabolism of phenobarbital with inhibition of hydroxylation, rather than by increased renal absorption or changes in the volume of distribution.

The Clinical Epilepsy Section is training a gradually increasing number of neurologists in the diagnosis and therapy of the epilepsies; this year's clinical associate will start an epilepsy program at Temple University in Philadelphia. In recognition of the potential for training investigators and clinicians, the epilepsy program at NINCDS has been designated a World Health Organization Neuroscience Center for senior international fellowships in epilepsy. The first fellow will come from Nigeria in the Fall of 1979; the second fellow will come from Sri Lanka in 1980.

The video-taped seizures at the Clinical Epilepsy Section have formed the basis of an unparalleled library of seizures for teaching and analysis. For example, tapes of atypical absence, unilateral, and unclassified attacks were utilized in the "Third Workshop on Classification and Terminology of the International League Against Epilepsy", May 6, 7, and 8, in a continuing effort to improve our understanding of the empirical data on epileptic seizures. The video-taped seizures also form the integral part of an education film, "Differential Diagnosis of Complex Partial Seizures", now being completed.

Finally, the Clinical Epilepsy Section has given advice to a large number of physicians who send their patients with uncontrolled seizures to the outpatient clinic. Although no patients is seen unless there is some hope of acceptance into an inpatient protocol, and although only a very few patients can be formally admitted, all patients receive an

extensive outpatient evaluation with detailed therapeutic recommendations to the referring physician.

Biochemical Pharmacology Unit

Biochemical Studies of Dopaminergic and Beta-Adrenergic Receptors in Brain

1. Multiple Categories of Dopamine Receptors

A variety of evidence suggests the existence of pharmacologically distinct categories of dopamine receptors. The D-1 dopamine receptors are linked to the enzyme adenylate cyclase so that agonists increase the activity of this enzyme. The bovine parathyroid provides a prototypic example of this category of receptor. The second category of dopamine receptor, designated D-2, does not stimulate adenylate cyclase. During the past year, we have focused attention on the D-2 dopamine receptor in the intermediate lobe of the rat pituitary. In this tissue, cyclic AMP enhances the release of alpha-melanocyte stimulating hormone (alpha-MSH). A beta-adrenoceptor stimulates adenylate cyclase activity and enhances alpha-MSH release. Dopamine inhibits alpha-MSH release and antagonizes both the physiological and biochemical effects of stimulation of the beta-adrenoceptor. Investigations of the pharmacology and biochemistry of the dopamine receptor are continuing.

2. Endogenous Guanyl Nucleotides and Hormone Responsive Adenylate Cyclase

The demonstration of hormone sensitivity of the adenylate cyclase activity in brain requires the presence of GTP. There is sufficient GTP in the homogenates of the striatum or cerebellum to permit maximal responsiveness to dopamine or beta-adrenergic agonists, respectively. Endogenous GDP represents another constituent of the brain which can confer hormone sensitivity to adenylate cyclase. However, the activity of this second guanyl nucleotide appears to arise as a consequence of its conversion to GTP during the assay of adenylate cyclase activity. When this conversion is blocked by the use of an appropriate ATP analogue as the substrate for the adenylate cyclase assay, GDP functions as an antagonist of the hormone sensitivity which has been restored by the addition of GTP.

3. Biochemical Pharmacology of the Dopaminergic Ergots

The dopaminergic ergots continue to be of use in identifying different categories of dopamine receptors. Thus, compounds such as lisuride or lergotril, which are antagonists of the D-1 dopamine receptor, are among the most potent agonists upon the D-2 dopamine receptor which inhibits the release of alpha-MSH from the intermediate lobe of the rat pituitary. Furthermore, these drugs display a high affinity for specific binding sites identified with either [^3H]-spiroperidol or [^3H]-dihydroergocryptine in cell free homogenates of bovine intermediate lobe. This suggests that these binding sites may be related to the dopamine receptors with which these drugs interact. The high

affinity of the ergot bromocriptine for the [^3H]-spiroperidol binding sites has been exploited to permit the development of a saturation assay for this compound. This assay is currently being used to measure levels of bromocriptine in serum from patients receiving the drug for the treatment of the symptoms of Parkinsonism.

Physiological Neuropharmacology Unit

1. Dopamine Autoreceptors and the Striatonigral "Feedback Loop" in Regulation of Dopamine Activity

The most commonly quoted hypothesis regarding the role of the striatal afferents to the substantia nigra proposes that this pathway regulates the activity of the nigrostriatal dopamine neurons, altering the activity of the dopamine cells to compensate for changes in the amount of stimulation received by the postsynaptic side of the dopamine synapse in the striatum. Previously, we found that systemically administered apomorphine, a dopamine agonist which normally depresses the firing of dopamine neurons, still effectively inhibits the activity of the dopamine cells after kainic acid-induced lesion of the striatonigral pathway. This observation suggested that dopamine agonists might act directly at dopamine autoreceptors to inhibit dopamine activity and raised questions about whether the striatonigral pathway in fact had any effect on the nigrostriatal dopamine neuron. Now we have found that the ability of the dopaminergic ergot, lisuride, to inhibit dopamine activity is attenuated in rats with lesions of the striatonigral pathway. In a control series of experiments, the ability of lisuride to inhibit the activity of the serotonergic neurons of the dorsal raphe was unaffected by the striatal lesions. These results indicate that some neuronal system in the striatum susceptible to the effects of kainic acid does appear to play a role in regulation of dopamine activity. The results also suggest that there may be a significant difference between the dopaminergic effects of lisuride and the more classic dopamine agonist, apomorphine.

2. Time Dependent Changes in Dopamine Receptor Function

Tachyphylaxis develops rapidly to the effects of dopamine agonists on dopamine single unit activity. A similar resistance to the effects of agonists is not observed in other catecholamine systems. We examined the possibility that this development of resistance might be occurring at the level of the dopamine autoreceptors on the dopamine cell bodies by iontophoresing dopamine onto dopamine cells before, during and after a series of i.v. injections of apomorphine. It was found that the dopamine cells initially inhibited by iontophoresis of dopamine became resistant to the effects of this transmitter as the cells developed resistance to the effects of systemic apomorphine. Other studies have indicated that dopamine itself can also induce changes in autoreceptor sensitivity. These results suggest that a rapid change may be occurring in sensitivity to the agonists or transmitter as a normal consequence of dopamine autoreceptor stimulation and/or subsequent membrane events leading to a change in firing rate.

3. The Role of GABA and Effects of GABA-mimetics in the Substantia Nigra

The striatal afferents to the substantia nigra are, in part, GABA containing processes. This has led to the expectation that GABAergic mechanisms are involved in the striatonigral feedback regulation of dopamine activity. However, when the effects of systemically and iontophoretically administered GABA-active agents were examined, we found that the non-dopaminergic neurons of the pars reticulata have a greater capacity to be affected by GABA and GABA-mimetics than do the pars compacta dopamine cells. The results also suggest that if the dopamine cells are regulated by GABAergic neurons, specifically the striatonigral GABA pathway, this interaction is predominately indirect and may involve a second inhibitory neuron within the nigra. We also found that lesions of the striatonigral pathway did not significantly attenuate the stimulatory responses of either type of nigral neuron to systemic administration of picrotoxin, a GABA antag-

onist. Thus, either picrotoxin stimulates the nigral neurons by a non-specific excitatory mechanism or another GABA projection to the substantia nigra, other than that destroyed by striatal kainic acid lesions, may, either indirectly or directly, inhibit these cells.

We have used the rats with kainic acid-induced striatal lesions to try to gain insight into the failure of muscimol, a GABA agonist, to alleviate the symptoms of Huntington's disease. Our inability to demonstrate development of supersensitivity to muscimol in the substantia nigra, after the kainic acid lesions of the striatonigral GABAergic pathway, has suggested that the effects of this GABA-mimetic may not be facilitated in the substantia nigra or other regions where GABA neurons are lost in Huntington's disease. Lack of any specificity of action at the denervated sites might account for the development of toxic effects seen with this drug in the absence of therapeutic actions. We are currently examining the effect as THIP, 4,5,6,7-tetrahydroisoxazolo- 4,5-c - pyridin-3-01, a new GABA agonist, which to date appears to be less potent but similar in action to muscimol, in hopes that it will have a therapeutic advantage over muscimol.

Behavioral Neuropharmacology Unit

I. Animal Models of Neurological Disease

A. Basal Ganglia Lesions and Neurobehavioral Function

The Unit has studied the neurochemical and behavioral effects of specific lesions in the basal ganglia of rats. Using the excitotoxins kainic acid and ibotenic acid, the effects of destroying the striatonigral GABAergic pathway and the striatal cholinergic interneurons have been explored. The results showed (1) after substantial disruption of striatal cholinergic function (using indices of presynaptic and postsynaptic neurochemistry), responses to cholinergic drugs are unexpectedly potentiated. The results may be consistent with reports that cholinomimetics are of little therapeutic value in treating HD, because of the comparisons drawn between HD and effects of kainic acid intrastrially; the effects of kainic acid and ibotenic acid on motor behavior are significant, and can be clearly distinguished from the effects of another neurotoxin, 6-OHDA, which was injected intrastrially to lesion the nigrostriatal dopamine pathway.

B. Interactions of Transmitters in the Basal Ganglia

Substance P, dopamine, and GABA occur in relatively high amounts in the basal ganglia, and there is evidence to suggest important interactions among pathways utilizing these compounds for neurotransmission. The Unit has investigated aspects of neurotransmitter interaction by exposing synaptosomes prepared from caudate and substantia nigra to dopamine, substance P and GABA. The results demonstrated, first, that nigral tissue can accumulate dopamine by a sodium dependent, high affinity system, and that this dopamine is releasable in a K⁺-stimulated, Ca²⁺-dependent manner. Second, it was found that nigral dopamine cycling is significantly different from that in caudate in its response to GABA and substance P.

Recent neuroendocrinological research has focused on the control of estrogen/prolactin by central monoamines. The Unit has studied the effects of estrogenization of male rats on dopaminergic neurochemistry and behavior. The results show that one-time administration of estradiol potentiates behavioral responses to apomorphine and amphetamine, which appear to be correlated with an increase in spiroperidol binding. The results

indicate that the interactions of CNS transmitters and sex hormones are bidirectional.

C. Ergot Drugs

Many new ergot derivatives have been synthesized recently and proposed to have therapeutic potential in the treatment of neuroendocrine disorders and basal ganglia disease. In coordination with the Therapeutics Section, the Unit has attempted to develop comprehensive information on the neurochemical and behavioral effects of the most promising ergot drugs. The results have shown that these compounds have a wide spectrum of activity on dopaminergic, serotonergic, and noradrenergic function. The results are also of interest in that they suggest that some models of antiparkinsonian activity (such as the unilaterally lesioned rat) may not be predictive of activity of ergot drugs.

D. Neurotoxic Compounds

Red dye no. 3 (erythrosin B) has been implicated in causing, or aggravating, behavioral disorders in children. However, little evidence beyond the anecdotal has been available. The Unit has studied the possible neuroactive properties of erythrosin B and found that this compound can act noncompetitively to inhibit reuptake of dopamine by synaptosomes in vitro. This effect is consistent with a stimulatory property, since it is analogous to the mechanism of action of amphetamine and cocaine; however, the extrapolation of these findings to clinical situations requires considerable further investigation.

Studies have continued on the actions of lead on CNS neurotransmission; these studies have expanded to include investigation of the effects of porphyrin precursors on neurochemistry. The results show that lead exposure, acutely or chronically, inhibits GABA release and increases GABA receptor binding (postsynaptic supersensitivity). However, these effects cannot be produced by lead in vitro. For these reasons, the unit has studied the possibility that lead exposure produces a metabolite which has neurotoxic properties on GABAergic neurons.

2. Analytic Electron Microscopy

The Unit has continued developing methods for the application of new techniques of analytic electron microscopy. In continuation of earlier studies on heavy metal localization, it was found that lead can enter capillary endothelial cells rapidly and is deposited in calcium-binding sites within mitochondria, similar to its localization within neurons. Recent work has concentrated on the feasibility of study receptor ligand binding with the morphologic resolution of scanning-transmission electron microscopy. Preliminary studies indicated that an irreversibly binding ligand was necessary, in order to prepare tissue by routine methods of fixation, osmication, and embedding. We have begun studies of the localization of α -bungarotoxin binding, a nicotonic cholinergic receptor ligand, after characterizing its binding properties in CNS tissue.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZOI-NS 02258-03 ET
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PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Therapeutic Studies in Parkinsonism and Other Movement Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any) Infectious Diseases Branch, NINCDS, Laboratory of Clinical Science, NIMH; Laboratory of Neurophysiology, NIMH; Biochemical Pharmacology Section, HE, NHLBI; Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville; Virginia, Department of Neurology, Mount Sinai School of Medicine, New

LAB/BRANCH

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Experimental Therapeutics Branch

SECTION

Therapeutics Section

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TOTAL MANYEARS:

4.5

PROFESSIONAL:

4.5

OTHER:

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to investigate the possible efficacy and safety of new compounds applied to the treatment of certain disorders of movement, and to employ drugs as tools to analyze the physiological and pharmacological mechanisms mediating various motor deficits.

The major conclusions deriving from observations over the last year are:-(1) deprenyl has no significant therapeutic action in Parkinson's disease; paradoxically, for an inhibitor of monoamine oxidase, this induces a reduction in the plasma concentration of norepinephrine and epinephrine, without altering dopamine; (2) bromocriptine elicits a normal reduction of dopamine receptors in the anterior pituitary; (3) the erythromelalgia, produced as an adverse reaction to bromocriptine, is associated with mononuclear infiltration of the wall of dermal blood vessels; (4) the reduction of tetrahydrobiopterin in the cerebrospinal fluid, previously described in patients with Parkinson's disease, has also been recorded in other neurological diseases such as the Shy-Drager syndrome; (5) in twelve monozygotic twin pairs, one twin had Parkinson's disease without any evidence of neuropathology in the other twin. This finding implies an important role for an environmental factor in the etiology of this disorder.

Project Description:

Objectives:

This project is designed to improve treatment and elucidate the pharmacological, physiological and biochemical abnormalities occurring at synaptic level in certain neurological diseases.

Methods:

Inpatients and outpatients are studied. Specimens of body fluids (including CSF) are taken for assay of transmitters, their metabolites, drugs, and routine biochemical and haematological indices of pharmacotoxicity. Motor control is studied by measuring velocity and force of movement, integrated electromyographic activity, and by conventional clinical scoring techniques that involve careful history taking and physical examination. Where possible the patient is used as his own control by making observations during different (blind) therapeutic regimens, and by studying asymmetric motor deficits.

Major Findings and Significance to Biomedical Research and the Program of the Institute:

1) Inhibition of Monoamine Oxidase B

Deprenyl has been studied in Parkinsonism, to investigate the effects of inhibition of monoamine oxidase B on transmitter concentrations, and evaluate possible therapeutic efficacy. A paradoxical fall in the level of plasma epinephrine and norepinephrine was detected as the major biochemical action of deprenyl; no significant change in the level of dopamine was found. In a double blind study, deprenyl failed to elicit any significant therapeutic response, in contrast to previous published experience of other workers.

2) Studies with Bromocriptine

Clinical experience with bromocriptine continues to support the role of this agent as an adjuvant to levodopa therapy. Bromocriptine has also been employed as a tool to study the dopamine receptors concerned with inhibition of prolactin release. We have found that in Parkinsonism there is no impairment of the response of these receptors, assayed as either reduction of spontaneous plasma prolactin levels, or blockade of the increase in prolactin elicited by thyrotropin releasing hormone. This work was performed in collaboration with the University of Virginia, Charlottesville.

A new observation in patients receiving bromocriptine has emerged from biopsy studies of the skin. In subjects who develop an erythromelalgic syndrome while receiving bromocriptine, the red, tender, warm, edematous extremities are associated with mononuclear infiltration of the walls of the dermal blood vessels.

3) Parkinsonism in Twins

Twelve pairs of monozygotic twins have been studied, in whom one is known to have Parkinson's disease. Full neurological evaluation has failed to demonstrate

any evidence of Parkinsonism in the second twin. Further twins will be investigated, but the current total discordance is unexpected, and suggests that exposure to environmental factors, after children leave home, may contribute to the etiology of Parkinsonism. This study is being performed in collaboration with Mount Sinai School of Medicine and the Infectious Diseases Branch, IRP, NINCDS.

4) Tetrahydrobiopterin

Last year it was found that the concentration of tetrahydrobiopterin (THB) in the cerebrospinal fluid declines with advancing age, but in Parkinsonism the level is reduced further (beyond age matched controls). Examination of the cerebrospinal fluid from other neurological patients has shown decreased THB in several disorders, some of which have Parkinsonian features (such as the Shy-Drager syndrome) but also in diseases quite distinct from Parkinsonism (such as torsion dystonia). This work was performed in collaboration with the National Heart, Lung, and Blood Institute.

5) Quantification of Neurological Deficits

In collaboration with the National Institute of Mental Health, techniques are being developed for precise quantification of neurological deficits such as tremor, clumsiness, and disturbances of gait. Computerized methods are being employed for data storage, analysis, and retrieval. These studies will be applied to the evaluation of therapy, and the investigation of pathophysiology. Over the last year this approach has yielded evidence that the rigidity and augmented long loop reflex of Parkinsonism can also be found in elderly subjects without neurological symptoms. This finding may provide an important link between Parkinsonism and the normal process of aging of the nervous system.

6) Oligoclonal Bands in the Cerebrospinal Fluid in Postencephalitic Parkinsonism

In collaboration with the Neuroimmunology Branch, IRP, NINCDS, it has been found that patients with postencephalitic Parkinsonism have oligoclonal bands of immunoglobulin in their CSF, in contrast to patients with idiopathic Parkinsonism. This observation, if confirmed and extended, could provide the basis for a laboratory test to distinguish between postencephalitic and idiopathic Parkinsonism.

Proposed Course

1) The possible therapeutic value of lithium will be investigated in patients with severe dyskinesia.

2) Two new dopaminergic ergot derivatives will be studied in Parkinsonism, lisuride and pergolide. These compounds have the advantage of much lower cost than bromocriptine; they also have somewhat different profiles of pharmacological activity.

3) More precise methods will be developed to quantify neurological deficits, in particular tremor, precision of finger movement, and disturbances of gait.

4) An attempt will be made to study elderly normal black subjects, and compare various physiological (long loop reflexes) and biochemical (CSF tetrahydrobiopterin) measurements with corresponding values in whites. Since these indices are abnormal in Parkinsonism, it is important to ascertain whether there are differences between normal blacks and whites, which might be correlated with the much higher prevalence of Parkinsonism in whites.

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CT No. 76-N-206

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02265-03 ET	
PERIOD COVERED October 1, 1978 to September 30, 1979					
TITLE OF PROJECT (80 characters or less) Pharmacology, Biochemistry and Physiology of Central Neurotransmitters.					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
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Other:		Adrian Williams		Clinical Associate ETB NINCDS	
		W.M. Lovenberg		HEB NHLBI	
		C.A. Tamminga		BPB NIMH	
COOPERATING UNITS (if any) B.L. Beasley, U.S.P.H.S. Hospital, Staten Island; S.E. Leeman, Harvard Medical School; G. Sedvall, Karolinska Institute, Stockholm; D. Samuel, Weizmann Institute, Rehovot; HEB, NHLBI; BPB, NIMH.					
LAB/BRANCH Experimental Therapeutics Branch					
SECTION Pharmacology Section					
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205					
TOTAL MANYEARS: 1.2		PROFESSIONAL: 1.0		OTHER: 0.2	
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input checked="" type="checkbox"/> (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to develop improved drug therapies for nervous system disease. Towards this end investigations seek to determine the relationship between <u>dysfunction in a specific neurotransmitter system</u> and the appearance of a <u>particular neurologic syndrome</u> . In addition, the ability of novel pharmacologic agents to modify the activity of specific transmitter systems in brain and spinal cord are investigated. Major topics under current study include:					
1) Use of a stable <u>isotope of oxygen</u> to evaluate central monoamine metabolism in man.					
2) Relation of monoamine- and peptide- containing neural system activity to specific motor, sensory, behavioral, or endocrinologic functions.					
3) Ability of selective agonists and antagonists of receptors in systems mediated by dopamine, serotonin, acetylcholine, or gamma aminobutyric acid to influence neurologic function.					

Objectives and Methods Employed:

The Section continues to direct its principal research efforts towards the rational development of improved drug treatments for neurologic disease. First, using biochemical methods, attempts are made to link dysfunction of a particular transmitter system with the presence of a specific motor, sensory, or behavioral disorder. Second, using drugs as tools, alterations in neurologic function are observed during the administration of pharmacologic agents believed to act selectively on a single transmitter system or subsystem. Together, these approaches provide information to help elucidate pathogenetic mechanisms and rational therapies in patients with nervous system disease.

Major Findings and Proposed Course:

1. Stable isotope studies of transmitter metabolism in man.

Studies using a non-radioactive isotope of oxygen, $^{18}\text{O}_2$, to evaluate central transmitter metabolism have continued in collaboration with the Karolinska Institute of Sweden and the Weizmann Institute of Israel. Following inhalation of a breathing mixture containing 79% nitrogen and 21% oxygen (95% $^{18}\text{O}_2$), labeling of the major metabolites of dopamine, norepinephrine, and serotonin is readily detectable in plasma, urine, and cerebrospinal fluid (CSF). The time course for the appearance of labeled metabolites in spinal fluid suggests a relatively rapid central turnover for the parent amines. Comparison of results from patients with Parkinson's disease and Huntington's chorea appears to indicate that the former disorder is associated with a relatively small pool of dopamine which is turning over very rapidly. Conceivably, the degeneration of dopamine neurons which occurs in parkinsonian patients may be accompanied by a compensatory hyperactivity of residual dopaminergic cells. These studies will be extended to the evaluation of other neurologic disorders as well as to the effects of drugs on central monoamine metabolism.

2. Substance P in human CSF.

The application of a sensitive radioimmune assay to the study of substance P in CSF continues in collaboration with the Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School. Since no appreciable concentration gradient for this undecapeptide has been found in lumbar spinal fluid, most substance P obtained by lumbar puncture probably derives from spinal cord or dorsal root ganglia rather than from supraspinal structures. Consistent with this view is the finding of significantly reduced substance P values in patients with Shy-Drager syndrome (multiple system atrophy), but not in those with Parkinson's disease, Huntington's chorea, or various other extrapyramidal disorders (with neuronal degeneration confined to the brain). Remarkably low substance P levels have also been found in patients with peripheral neuropathies, especially in those with predominantly sensory dysfunction. This latter observation is not unexpected in view of preclinical data

implicating substance P as a major transmitter in primary afferent pathways and will receive further experimental attention.

3. GABA in human CSF.

Studies of the GABA content of lumbar spinal fluid have also remained active during the past year. Reduced CSF GABA values have now been found in patients with Shy Drager syndrome, progressive supranuclear palsy, and segmental dystonia. These observations suggest that brain GABA levels may be diminished in these disorders, possibly due to degeneration or hypofunction of some GABA containing neurons. Future investigations will address the possible therapeutic implications of these findings as well as evaluate by pharmacologic means the relation between GABA values in lumbar CSF and the functional state of central GABA neurons.

4. Tetrahydrobiopterin in human CSF

Since tetrahydrobiopterin (BH_4) serves as a cofactor for rate limiting enzymes mediating catecholamine and serotonin synthesis, CSF levels of this substance may provide an index to the functional capacity of monoaminergic pathways in the central neurons system. Investigations previously conducted by the Therapeutics Section of this Laboratory demonstrated reduced BH_4 levels in patients with Parkinson's disease. Recent studies in collaboration with the Section on Biochemical of Pharmacology, NHLBI, now indicate that Huntington's chorea, amyotrophic lateral sclerosis, and familial dystonia are also associated with significantly diminished BH_4 values. These observations, possibly implicating monoamine system dysfunction, are of particular interest in dystonic patients and will be the subject of further study during the coming months.

5. Dopamine agonist therapy of neuropsychiatric disease

The discovery of multiple subtypes of central dopaminergic receptors, with differing pharmacologic characteristics, should enable more focused pharmacotherapeutic interventions in disorders involving the dopamine system. For example, considerable preclinical evidence suggests that drugs which inhibit dopaminergic transmission through preferential stimulation of dopamine autoreceptors might ameliorate certain hyperkinetic extrapyramidal disorders or schizophrenia. In collaboration with Mantino State Hospital, double-blind, placebo-control trials of two ergot type dopamine agonists have been completed during the past year. These agents were selected on the basis of their pharmacologic profile in the experimental animal, as well as their differing antiparkinsonian efficacy in man. Bromocriptine, at relatively low dose levels, failed to influence either involuntary movements or psychotic behavior in otherwise untreated patients with chronic schizophrenia and tardive dyskinesia. CF25-397 also had no consistent therapeutic effect in this patient group. On the other hand, preliminary results with a non-ergot

dopamine agonist, piribedil, appear more favorable. Until more preclinical data are available concerning the detailed pharmacologic characteristics of these agents and more thorough dose finding studies have been completed in man, the status of our hypothesis that dopamine autoreceptor stimulation will alleviate symptoms of some hyperdopaminergic states remains indeterminate. Additional studies of piribedil and other newly developed dopamine agonists are planned.

6. Cholinergic agonist therapy of neuropsychiatric disease.

Anatomical and pharmacological studies in the experimental animal suggest that stimulation of striatal cholinergic transmission should improve patients with tardive dyskinesia. Modestly beneficial results with acetylcholine precursor therapy support this theoretical rationale. Nevertheless, recently completed double-blind, placebo-controlled trials of arecoline, a potent and specific muscarinic agonist, failed to significantly suppress hyperkinetic symptoms. An exploration of factors which might account for the apparent differences between cholinergic precursor and cholinergic agonist therapy is anticipated during the coming year. Since the reduction in brain choline acetyltransferase activity in Alzheimer's disease may indicate that cholinergic hypofunction contributes to the cognitive defect, studies of arecoline therapy have now been initiated in patients with various senile and presenile dementias. Preliminary indications suggest that a dose-dependent improvement in memory may occur in a subgroup of these patients.

7. Stiff-Man Syndrome

Four of five patients studied at the NIH Clinical Center with this rare disorder were found to have associated autoimmune dysfunction and all had an HLA haplotype (BW44). This result may indicate that an autoantibody directed against spinal cord inhibitory neurons contributes to the pathogenesis of stiff man syndrome. CSF studies of these patients have revealed an increase in norepinephrine and a decrease in GABA levels. Attempts to correct possible GABA system hypofunction with muscimol, a potent GABA agonist, produced no therapeutic response. Although propranolol (a beta adrenergic blocker) also was ineffective, thymoxamine (an alpha adrenergic antagonist) improved symptoms in two patients. Further exploration of a possible immunologic factor in the etiology of stiff-man syndrome as well as further attempts at symptomatic therapy by means of alpha adrenergic blockade are anticipated.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02236-04 ET
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Diagnostic and Therapeutic Reevaluation of Patients with Intractable Epilepsy		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
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W. C. Whitehouse	Video Engineer	OAM CC NIH
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LAB/BRANCH Experimental Therapeutics Branch		
SECTION Clinical Epilepsy Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS	<input type="checkbox"/> (b) HUMAN TISSUES	<input type="checkbox"/> (c) NEITHER
<input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>Despite recent advances in the therapy of <u>epilepsy</u>, many patients, especially those with <u>complex partial seizures</u> are incapacitated by their disorder. We have been investigating improvement of seizure control and reduction of medication side effects through the application of newly developed intensive monitoring techniques including simultaneous video recording of <u>seizures</u>, long-term telemetering of EEGs and frequent determinations of antiepileptic drug concentrations. Patients with very long histories of uncontrolled seizures are admitted for a complete evaluation, including all basic neurologic studies and daily objective toxicity battery. <u>Video recording</u> and long-term <u>telemetered EEGs</u> establish a seizure diagnosis, a concept which has not been adequately emphasized in the management of patients with intractable seizure disorders. Efforts are then made, based on this seizure diagnosis, to "tailor make" a regimen which is appropriate for each patient. This includes use of newer anti-epileptic medications which have decreased side effects in conjunction with <u>blood concentrations</u> which allow maximum therapeutic levels with minimal toxicity.</p>		

Project Description:

Objectives:

It is the hypothesis of this study that utilization of new techniques of intensive monitoring of patients with intractable seizures can improve clinical control in many patients with refractory seizure problems and can aid in the diagnosis of patients with disorders of unknown type such as psychogenic seizures. The fundamental method of therapy is medical, and modification of therapy will be dependent on collected information from all sources, including detailed history and examination, as well as routine and special laboratory studies, as indicated.

Methods:

Patients with intractable seizures are admitted to the Clinical Center according to the following criteria: 1) Primary consideration will be given to patients with complex partial seizures. Patients with other types of seizure disorders will be considered at a later date. A limited number of patients with suspected psychogenic seizures are also admitted for study. 2) The history of uncontrolled seizures should preferably be a continuous pattern of attacks during the few months prior to admission at the very least, and preferably for many years. 3) Seizure frequency by history must be sufficient to make video monitoring effective. Priority is being given to patients whose seizures occur once or more per day. 4) Patients must be able and willing to cooperate with the entire experimental protocol, including intensive monitoring studies.

Video recordings are made of patients in two separate rooms, which are completely equipped for closed-circuit television studies. Video recordings are made in six-hour periods from 0900 to 1500. Each patient has a minimum of one recording on every new regimen after drug steady state level has been reached. This is compared with the baseline recording for evaluation of the new regimen.

EEG telemetry is performed using either a four or an eight-channel FM-FM transmitter and receiver. The transmitter is worn by the patient and the signal is picked up by a dual diversity antenna and receiver system; the information is integrated into the television format so that a simultaneous display of patient and the electroencephalogram can be obtained.

Blood levels of antiepileptic drugs are determined by gas-liquid chromatography and by immunoassay in the pharmacology laboratory of the Epilepsy Branch.

Initially baseline studies are performed. The seizure frequency and seizure type is characterized by the intensive monitoring techniques and correlation of this information is obtained with blood levels of the anti-epileptic drugs. The patient subsequently begins new regimens which are based on the seizure diagnosis. Repeat studies of the patient on each new

regimen demonstrates the efficacy of this regimen and whether any change in seizure type or frequency occurs. Each regimen is instituted with the aid of frequent antiepileptic drug levels to assure that each drug is used to maximal benefit with minimal toxicity.

A specific protocol has also been designed to investigate the etiologies of selected patients with epilepsy and progressive neurologic deterioration. This study, which involves a multidisciplinary team of investigators, is capable of analyzing neurologic, electroencephalographic, radiologic, pathologic (including brain biopsy when indicated), metabolic, and virologic data in an attempt to delineate some of the causes of seizure disorders.

Major Findings:

The Clinical Epilepsy Section has become a referral center for patients with intractable seizures, although only a very small number can be accepted into the program. A long-term follow-up of patients admitted to this program is continuing and it appears that the success of this diagnostic and therapeutic venture will be similar to that obtained in the first 23 patients. These data demonstrated that 70% of the patients had a decreased seizure frequency, that 83% of the patients had decreased medication toxicity, and that 48% had improved in their social adjustment.

During the past year, special emphasis has been placed on the psychogenic (hysterical seizure). Six patients had 42 psychogenic seizures in 6 to 24 hours of recording. Diagnosis was determined by assessment of four major criteria: deviation of seizures from characteristics of known seizure types, absence of epileptiform activity in the ictal EEG, absence of slowing in the postictal EEG, and relation of seizure frequency to decreasing plasma concentrations of antiepileptic drugs. The clinical characteristics of psychogenic attacks were compared with generalized tonic-clonic and complex partial seizures. Although a 1-year seizure-free period without medication is likely the absolute criterion for psychogenic seizures, intensive monitoring greatly increases the accuracy of the diagnosis, using the criteria developed in this study.

Significance to Biomedical Research and the Program of the Institute:

Intensive monitoring techniques of patients with intractable seizures can improve seizure control, decrease medication toxicity, and have a positive effect on the patient's work and social status. The need for reevaluation of patients with uncontrolled seizures is emphasized and the importance of these specialized techniques in obtaining these goals is documented. Furthermore, the Clinical Epilepsy Section continues to be a model for investigators and clinicians interested in setting up such intensive monitoring units throughout the world. The NIH is expected to provide leadership in the technical aspects and scientific uses of intensive monitoring and the Section has continued to provide this.

As experience is gained with intensive monitoring units, it is apparent that much more is needed than demonstration projects which show the usefulness of the technique. Rather the technique is proving to be a powerful investigative tool in a) evolving the proper, objective classification of epileptic seizures, b) controlling seizure types for antiepileptic drug studies, and c) furthering our knowledge of response of various specific seizure types to specific drugs.

Another, long neglected area that has been investigated by intensive monitoring is the psychogenic (hysterical) seizure, a major diagnostic problem that frequently confronts the neurologist. Intensive monitoring of patients with intractable seizures has uncovered a previously unsuspected frequency of psychogenic attacks. Although these patients tend to be refractory to therapy, causing them to gravitate to specialized centers and making estimates of incidence difficult, several centers using monitoring techniques report a high incidence of patients with such seizures, either with or without associated epileptic attacks. In a recent large series of patients, the incidence was estimated to be 15% and others have emphasized the coexistence of "real" and "hysterical" seizures. In our own series of 78 patients who have undergone intensive monitoring, six (8%) have unequivocal psychogenic attacks. It is important to establish and evaluate diagnostic criteria because (1) these patients are a relatively common problem; (2) diagnosis can be quite difficult; (3) although they are often treated with antiepileptic drugs for their non-epileptic seizures, they require, obviously, a different therapeutic approach. Although the problem is very complex and the patients very heterogeneous, the concepts introduced by these studies will be useful both to physicians confronted with problem patients and to investigators who wish to increase our understanding in this area.

Proposed Course:

The Clinical Epilepsy Section continues to make technical advances in intensive monitoring both at the electronic and at the pharmacologic level. There are many further refinements which are indicated, and these have received considerable priority in the Section. Because of the remarkable heterogeneity of patients with intractable seizures, and because more information is gradually being obtained about the value of intensive monitoring as well as information on various seizure types, patients will continue to be accepted into this study. The program will continue to be active in technology transfer in intensive monitoring, both from technical and scientific standpoints.

Publications:

Penry, J.K., Porter, R.J.: Epilepsy: Mechanisms and Therapy.
Med Clin North Am, 1979 (in press).

C.T. No. 75-N-124
C.T. No. 77-N-195
C.T. No. 78-N-158

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02318-02 ET
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) Clinical Pharmacology of Antiepileptic Drugs		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
P.I.: J. K. Penry Chief, Clinical Epilepsy Section ET NINCDS		
Other: R. J. Porter Neurologist ET NINCDS		
H. J. Kupferberg Pharmacologist EB NDP NINCDS		
E. Schulman Clinical Associate EB NDP NINCDS		
COOPERATING UNITS (if any) Epilepsy Branch, NDP, NINCDS		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Clinical Epilepsy Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20205		
TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.8	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The Clinical Epilepsy Section has been studying the <u>clinical pharmacology</u> of old and new <u>antiepileptic drugs</u> . In spite of the fact that many such medications have been marketed for many years, there is still a great deal to be learned about the proper use of the drugs which are already in our armamentarium. Advances in pharmacology in the past 20 years have allowed the use of new approaches and techniques to gain insight into the proper use of our currently available drugs. In addition, the Clinical Epilepsy Section is involved in <u>clinical trials</u> of new antiepileptic drugs, such as sodium valproate which was recently marketed in the United States. The pharmacologic evaluation of these drugs is coupled with efficacy studies which are carried out by <u>intensive monitoring techniques</u> including videotape analysis of epileptic seizures with simultaneous electroencephalographic recording and long-term telemetered EEG recording. The daily determination of antiepileptic drug levels is an integral part of the on-going studies. Recent emphasis has been on the efficacy of single drug therapy in patients with complex partial seizures, and on mechanisms for valproic acid-phenobarbital interaction.		

Project Description:

Objectives:

This project includes a large number of independent pharmacologic studies in the investigation of the clinical pharmacology of antiepileptic drugs. The object of each study is different, but each may include obtaining such pharmacologic data as (1) single dose plasma half-lives, (2) relative plasma levels of parent drugs and metabolites with chronic administration, (3) relationships of the efficacy of parent drugs and/or metabolite to plasma levels, (4) efficacy of various compounds against different seizure types as correlated with intensive monitoring techniques, (5) evaluation of efficacy and toxicity of new antiepileptic agents, such as sodium valproate, (6) measurement of rate of biotransformation of various antiepileptic medications, and (7) determination of the clinical consequences of withdrawal of antiepileptic drugs.

Methods:

Patients with uncontrolled seizures, especially complex partial seizures, are accepted for study. Such patients usually have a detailed seizure calendar available prior to entering the study, and enter a week-long period of baseline determination of seizure frequency and blood levels of antiepileptic drugs while in the hospital. Each pharmacologic protocol varies, but all require modification of the antiepileptic regimen and addition of the medication under study. This may be done in single dose or chronic administration studies depending upon the particular protocol in question. As a rule, plasma levels are drawn at least daily, and on occasion, much more frequently for specific studies. Following the completion of the pharmacologic protocol, the patient is placed on a regimen which is best suited for the seizure type which has been identified by videotape/telemetered EEG analysis. This regimen is stabilized prior to discharge of the patient.

Major Findings:

1) In response to several recent reports which suggest the use of multiple drugs may not only be unnecessary, but actually may be detrimental to seizure control in some patients, the Clinical Epilepsy Section has been investigating the efficacy of single drugs as an alternative to polypharmacy in the treatment of seizures. Although this has been tested to some extent in some patients not previously treated with antiepileptic medications, it has not been applied to the population of highly intractable patients, such as the population at the Clinical Center, NIH. For this reason, patients have been admitted to the Clinical Center with intractable complex partial seizures for evaluation of phenytoin as a single drug as their sole mode of therapy. We treated nine patients with severe, intractable complex partial seizures with phenytoin alone; all were tried on two or more different phenytoin dosages. Patients were hospitalized during each steady-state period of monotherapy. We evaluated 32 steady-state phenytoin regimens, using dosages of 225-450 mg qd. Blood phenytoin levels ranged from 16-52 $\mu\text{g/ml}$ (mean, 34 $\mu\text{g/ml}$). The steady-state periods averaged 9 days each, and the average ranges of the

steady-state phenytoin levels varied from 3.8-6.7 $\mu\text{g/ml}$. Only one steady-state period was seizure-free; an average of 33 seizures per week occurred in the other steady-state periods. Before the patients were discharged, the phenytoin monotherapy regimens were compared with multidrug regimens of phenytoin and carbamazepine (6 patients), phenytoin and valproate (2 patients), and all three drugs (one patient). Using all monotherapy regimens, average seizure frequency was unchanged in one patient and worse in eight ($p < 0.01$). Using the most effective monotherapy regimen, two patients were improved and seven were worse; this difference was not significant. The time to reach steady-state was quite long in some patients, consistent with recent models of phenytoin kinetics which suggest that many weeks may ensue before the level is stable.

These preliminary findings suggest that most patients with intractable seizures are more effectively treated with a multiple drug regimen than with phenytoin alone. Monotherapy in a minority of such patients, however, may decrease side effects of drug therapy and perhaps even improve seizure frequency.

2) Valproic acid (VPA, Depakene) was recently marketed in the U.S. for the treatment of epileptic seizures. It has been reported to cause a significant elevation of plasma phenobarbital levels. The mechanism of this drug-drug interaction was not previously elucidated. Three possible mechanisms of interaction were investigated: (A) inhibition of phenobarbital metabolism, (B) increased renal reabsorption of phenobarbital via urinary pH effect, and (C) change in volume of distribution. Valproic acid was added to the phenobarbital regimens. Pharmacokinetic parameters were obtained by determining the area under the curve of a single pulse dose of [$1,3\text{-}^{15}\text{N}$, $2\text{-}^{13}\text{C}$]phenobarbital before and after the administration of valproic acid. Plasma levels of labelled phenobarbital were determined by gas chromatography-mass spectrometry. Urinary pH, urinary phenobarbital levels, and urinary p-OHPB levels (free and total) were also quantitated. The volume of distribution and urinary pH did not change during the entire experimental period, thus eliminating mechanisms (B) and (C). Plasma phenobarbital increased approximately 25%, with a concomitant decrease in total body phenobarbital clearance and decrease in excretion of total p-OHPB. Therefore, these results support mechanism (A).

3) Noncompliance with a prescribed dosage regimen of antiepileptic drugs is a well-known cause of subtherapeutic serum antiepileptic drug levels and of uncontrolled seizures. A brief study was performed on a patient who purposefully failed to take the prescribed medication.

Significance to Biomedical Research and the Program of the Institute:

1) Although the use of single antiepileptic drugs is to be encouraged in all patients where such therapy has a likelihood of success, these studies demonstrate that patients with intractable seizures may require more than one medication for optimal seizure control. This adds a new dimension to the concept of a single drug therapy in the treatment of the epilepsies, since some

investigators have recently suggested that most patients, if not all, might do best on monotherapy. This study suggests that such is not the case for most patients with intractable seizures.

2) Most of the drugs currently employed in the treatment of epileptic disorders have a relatively narrow therapeutic ratio and when used in conjunction with other antiepileptics or other drugs, they frequently become involved in drug-drug interactions. The propensity of these agents for drug-drug interaction complicates effective and safe management of seizures. A new antiepileptic drug is structurally unrelated to conventional anticonvulsant drugs. It has a broad spectrum of antiepileptic action and is remarkably free of toxic side effects. However, when valproic acid is administered in conjunction with other antiepileptics, most notably phenobarbital, it potentiates side effects of the latter. This results in increased sedation, and even coma in some patients. It is important to understand the mechanism of this interaction, so that the physician can be aware of which organ systems are involved and what can most effectively be done to overcome this problem. For the future of antiepileptic drug development, this kind of pharmacologic information is valuable in the consideration of the design of new drugs. In general, it has been well demonstrated that the more we understand about the pharmacology and mechanism of actions of drugs, the more effectively we can utilize these to the benefit of the patients involved.

3) Although noncompliance with a drug regimen of patients with epilepsy is a well-known and well-documented phenomenon, the concept of active non-compliance by a hospitalized patient has rarely been followed carefully and documented on a day-to-day basis. Documentation of this particular problem should alert physicians to the possibility that clinical studies may be altered by such behavior and that physicians should be continuously aware that their patients may not be taking their medications even when hospitalized.

Publications:

Porter, R.J., Penry, J.K., Lacy, J.R., Newmark, M.E., Kupferberg, H.J.: Plasma concentrations of phensuximide, methsuximide and their metabolites in relation to clinical efficacy. Neurology, 1979 (in press).

Kapetanovic, I.M., Kupferberg, H.J., Porter, R.J., Penry, J.K.: Valproic acid-phenobarbital interaction: a systematic study using stable isotopically labelled phenobarbital in an epileptic patient. Proceedings of WODADIBOF IV, Voksenasen, Norway, June 7-9, 1979. Raven Press, New York, 1979 (in press).

Desai, B., Riley, T., Porter, R.J. and Penry, J.K.: Active noncompliance as a cause of uncontrolled seizures. Epilepsia 19:447-452, 1978.

C.T. No. 76-N-344
C.T. No. 77-N-92
C.T. No. 78-N-171
C.T. No. 79-N-04

Project Description:

Objectives:

1. Study the coupling of the beta-adrenoceptor and the D-1 dopamine receptor with the enzyme adenylate cyclase.
2. Identify, classify and characterize the D-2 dopamine receptor.
3. Identify biochemical and pharmacological effects of the dopaminergic ergot derivative.

Methods:

1. Coupling of the Receptors with Adenylate Cyclase.

A. Studies of the beta-adrenoceptor involve the measurement of adenylate cyclase activity in tissue homogenates. The biochemical sign of receptor occupancy by a beta-adrenergic agonist is an increase in the rate of formation of cyclic AMP. Utilizing this system, it is possible to quantitate the affinity of the receptor which regulates the activity of this enzyme for both agonists and antagonists. In addition the beta-adrenoceptor can be studied with beta-adrenergic antagonists radiolabeled to high specific activity. With these compounds, [³H]-dihydroalprenolol and [¹²⁵I]-monoiodohydroxybenzylpindolol, it is possible to identify specific binding sites with properties similar to the receptor which regulates adenylate cyclase activity.

B. Both the beta-adrenoceptor and the D-1 dopamine receptor regulate adenylate cyclase activity in cell free homogenates of mammalian brain. However, following repeated centrifugation, of the initial homogenate, the hormone sensitivity of these brain enzymes is no longer evident. The hormone sensitivity can be restored by the addition of either soluble components of the brain or exogenous compounds. In addition, in the case of the beta-adrenoceptor the properties of the receptor can be directly studied (see above). Thus, the washed particulate material from the rat caudate nucleus and the rabbit cerebellum provide systems in which it is possible to assay the endogenous materials in brain which confer hormone sensitivity to adenylate cyclase activity.

2. Identification of the D-2 Dopamine Receptor

The intermediate lobe of the rat pituitary gland provides a system which contains both cyclase linked (beta-adrenergic) and cyclase-independent (D-2) receptors. A variety of experimental procedures permit the identification and characterization of these receptors. Dispersed cells can be harvested from the neurointermediate lobe of the pituitary. These cells release alpha-melanocyte stimulating hormone (alpha-MSH), which can be assayed by a sensitive, specific radioimmunoassay. The functional activity of the beta-adrenoceptor can be studied in several different procedures: 1) stimulation of alpha-MSH release; 2) stimulation of the formation of cyclic AMP; and 3) direct binding assays utilizing monoiodohydroxybenzylpindolol. In addition, dopamine inhibits the release of alpha-MSH and antagonizes the accumulation

of cyclic AMP and the release of alpha-MSH release which are stimulated by l-isoproterenol. The properties of the dopamine receptor can be inferred from these types of studies.

Major Findings (during fiscal Year 1979) and Their Significance to Bio-medical Research and the Program of the Institute

1. Coupling of Receptors with Adenylate Cyclase Activity.

A beta-adrenoceptor regulates adenylate cyclase activity in cell-free homogenates of the rabbit cerebellum. The demonstration in vitro of the "coupling" between the beta-adrenoceptor and adenylate cyclase activity requires the presence of either guanosine 5'-triphosphate (GTP) or guanosine 5'-diphosphate (GDP). Thus, repeated washing of the particulate material in homogenates of the rabbit cerebellum abolishes the sensitivity of the adenylate cyclase activity to beta-adrenergic agonists. The addition to the particulate cerebellar material of either the soluble constituents of the cerebellar homogenate or the exogenous guanyl nucleotides, GTP or GDP, restores the sensitivity to beta-adrenergic agonists. Utilizing high pressure liquid chromatography (HPLC) the amount of GTP and GDP in the soluble components of the cerebellar homogenate can be measured; these two guanyl nucleotides can account for the restoration of the sensitivity to beta-adrenergic agonists. The endogenous nucleotides in the cerebellum were isolated with HPLC. Only the endogenous GTP and GDP were capable of restoring the coupling between the beta-adrenoceptor and adenylate cyclase activity; none of the other compounds isolated with HPLC were active. The effectiveness of GDP may reflect its conversion to GTP during the assay of adenylate cyclase activity. If ATP is used as substrate, approximately 75% of the exogenous [14-C]-GDP is recovered as [14-C]-GTP at the end of the assay of adenylate cyclase activity. However, if AMP-P(NH)P is the substrate such conversion is negligible; under these latter conditions GTP, but not GDP, can restore sensitivity to beta-adrenergic agonists. The beta-adrenergic antagonists, [3-H]-dihydroalprenolol (DHA) identifies specific binding sites similar to the beta-adrenoceptor which regulates adenylate cyclase activity. Exogenous GTP does not affect either the number of DHA binding sites or the affinity of these sites for l-isoproterenol. Furthermore, GTP does not cause a shift in the activation affinity of the adenylate cyclase activity for l-isoproterenol. In conclusion, the guanyl nucleotides GTP and GDP are endogenous constituents of the rabbit cerebellum which are essential for the functional "coupling" of the beta-adrenoceptor and adenylate cyclase but which do not affect the receptor per se: the activity of GDP occurs as a consequence of its conversion into GTP; The activity of GTP may reflect a direct effect of this compound.

Repeated washing of the particulate material from rat striatum abolishes the dopamine-sensitivity of the adenylate cyclase activity. Readdition of the soluble fraction of the caudate homogenate restores the dopamine-sensitivity to the enzyme activity. Fractions of these soluble components which have been prepared with thin layer chromatography (and which can be shown with high-pressure liquid chromatography to contain endogenous GTP or GDP) also restore

dopamine-sensitivity to striatal adenylate cyclase activity. The effectiveness of GDP in restoring coupling of the dopamine receptor and adenylate cyclase results from its conversion to GTP during the assay of enzyme activity. When this conversion is eliminated, GDP can specifically block the coupling between the dopamine receptor and adenylate cyclase. The inhibitory action of GDP differs from the action of drugs which compete with dopamine for the receptor linked to adenylate cyclase. GDP inhibits dopamine-sensitivity by causing a decrease in the maximal response to dopamine; the nucleotide does not affect the apparent affinity of the receptor for dopamine (which is inferred from the ability of dopamine to stimulate adenylate cyclase). In contrast to the inhibitory mechanism of GDP, neuroleptic drugs produce an apparent decrease in the potency of dopamine without affecting the maximal response to the amine. Thus, the inhibitory action of GDP may arise from its ability to compete with GTP for a regulatory site distinct from the dopamine receptor regulating adenylate cyclase. The role of guanyl nucleotides in the physiology of dopaminergic transmission in the striatum remains unknown. However, since the striatal dopamine-sensitive adenylate cyclase is located on neurons intrinsic to the striatum, it is possible to conclude: 1) GTP is an endogenous constituent of the striatum which is a regulatory component of a postsynaptic neuronal dopamine receptor; and 2) GDP is a specific, endogenous inhibitor of the coupling effect of GTP upon a neuronal dopamine receptor. Either or both of these guanyl nucleotides may contribute to the initiation or termination of an intracellular response to the neurotransmitter, dopamine.

2. Identification, Classification and Characterization of the D-2 Dopamine Receptor.

The D-2 dopamine receptor can be studied in the intermediate lobe of the rat pituitary gland. Since this receptor does not function through a cyclic AMP mechanism, it is necessary to initially establish the role of this nucleotide in the functioning of the intermediate lobe of the pituitary. The cells of the intermediate lobe contain beta-adrenoceptors which promote an enhancement of the release of alpha-melanocyte stimulating hormone (alpha-MSH) through a cyclic AMP system. Thus, treatment of these cells with the beta-adrenergic agonist, l-isoproterenol, promptly enhances the release of alpha-MSH by as much as 5.5-fold. The effect of l-isoproterenol can be mimicked by exogenous cyclic AMP, derivatives of cyclic AMP and the phosphodiesterase inhibitor, theophylline. Using the radiolabeled ligand [¹²⁵I]-monoiodohydroxybenzylpindolol, the beta-adrenoceptor in the neurointermediate lobe can be directly characterized. Furthermore, the beta-adrenoceptor can also be characterized by its ability to stimulate adenylate cyclase activity in cell-free homogenates of the intermediate lobe. The properties of the beta-adrenoceptor inferred from adenylate cyclase studies, ligand binding studies, and the direct measurement of l-isoproterenol-stimulated cyclic AMP accumulation are in good agreement. However, the l-isoproterenol-stimulated release of alpha-MSH is some 30-fold more sensitive than might be anticipated from a consideration of the properties of the beta-adrenoceptor per se. An explanation of this phenomenon is that submaximal accumulations of cyclic AMP are sufficient to yield the maximal physiological response from the tissue. These

studies are consistent with models of the intermediate lobe in which a beta-adrenoceptor regulates the enzyme adenylate cyclase; stimulation of the beta-adrenoceptor leads to an enhanced formation of cyclic AMP which in turn initiates intracellular events which ultimately lead to the release of alpha-MSH. The effects of dopamine upon the intermediate lobe appear not to be mediated by a stimulation of adenylate cyclase activity. Thus, treatment of the spontaneous release of alpha-MSH (this contrasts with the stimulatory effect of the beta-adrenergic agonist, l-isoproterenol). Dopamine does not increase the content of cyclic AMP; indeed, dopamine antagonizes both the release of alpha-MSH and the accumulation of cyclic AMP which are stimulated by l-isoproterenol. Thus, it appears that dopamine does not achieve its physiological effects upon the intermediate lobe by causing an enhanced synthesis of cyclic AMP. Other investigators have proposed that a specific dopamine receptor exists on the intermediate lobe which regulate alpha-MSH release. Our working hypothesis is that a D-2 dopamine receptor occurs in this tissue. We feel that this system will permit a detailed biochemical, physiological and pharmacological characterization of the mechanisms which underlie the activity of the D-2 dopamine receptor.

3. Identification of Biochemical and Pharmacological Actions of the Dopaminergic Ergots.

The dopaminergic ergot drugs have been investigated in several of the biochemical and physiological test systems which are available in the laboratory. Among the results obtained were the following: 1) Lisuride was found to be unique among a number of ergolines in that it is a beta-adrenergic antagonist which is one-tenth as potent as the classically recognized beta-antagonist, propranolol; 2) Lisuride and bromocriptine were found to be the most potent compounds in the inhibition of alpha-MSH release from cells of the intermediate lobe of the rat pituitary. These compounds will be of use in characterizing the D-2 dopamine receptor in this tissue (see #2, above); and 3) The high affinity of the ergots for the [³H]-spiroperidol binding sites in membranes of the bovine anterior pituitary has permitted the development of a sensitive assay for ergot drugs such as bromocriptine. This assay procedure is currently being used to measure the level of bromocriptine in the serum of patients receiving the drug for the treatment of the symptoms of Parkinsonism.

Publications:

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6. Chen, T.C., Cote, T.E. and Kebabian, J.W.: Guanyl nucleotides are endogenous components of the brain which couple catecholamine receptors to adenylate cyclase. Proceedings of 4th International Catecholamine Symposium, Asilomar, California, Sept. 17-22, 1978, Pacific Grove, California. New York, Pergamon Press, 1979. In press.
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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02139-05 ET
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (60 characters or less) Pharmacology and Physiology of Central Neurotransmitters		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J.R. Walters Head, Physiological Neuropharmacology Unit, ETB, NINCDS Others: C.A. Tamminga IPA BPB NIMH B.L. Waszczak Staff Fellow ETB NINCDS		
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LAB/BRANCH Experimental Therapeutics Branch		
SECTION Physiological Neuropharmacology Unit		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
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CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to improve our understanding of ways in which drugs may alter centrally mediated <u>neurotransmission</u> and to develop better <u>pharmacotherapies for neurological disorders</u> . Topics currently under investigation include: (1) the role of the <u>striatonigral pathway</u> , <u>dopaminergic auto-receptors</u> and <u>substantia nigra pars reticulata neurons</u> in regulation of the activity of <u>nigrostriatal dopamine neurons</u> ; (2) the function γ aminobutyric acid (GABA) afferents to the <u>substantia nigra</u> ; (3) effects of potential <u>dopamine agonists</u> and <u>GABA-mimetic agents</u> on the function of identified neurons in the <u>basal ganglia</u> ; (4) mechanisms involved in endogenous regulation of <u>glutamic acid decarboxylase (GAD)</u> .		

Project Description:

Objectives:

This project involves investigation of the role of specific neurotransmitters in regulating neuronal activity in extrapyramidal systems. The specific goal is to establish improved pharmacological treatment to compensate for apparent neuronal degeneration and dysfunctions which occur in the basal ganglia in a variety of neurological disorders. Parkinsonism, for example, is associated with loss of dopamine-containing nigrostriatal neurons. Profound loss of GABA, substance P and in some cases, acetyl choline-containing striatal and pallidal neurons attends Huntington's chorea. It is hoped that pharmacological replacement of, or compensation for, the factors lost with these cellular degenerations will alleviate the behavioral and psychological malfunctions of the disorders. Thus current studies of this Unit are dedicated toward understanding the normal processes regulating information flow in these brain regions and the various ways in which drugs may interact with these processes and compensate for specific neuronal degeneration.

I. Regulation of Neuronal Activity in the Substantia Nigra

Methods:

These studies utilize: (1) determination of single-unit activity of identified brain cells in anesthetized rats, some with neurotoxin-induced lesions of specific brain regions, (2) investigation of effects of microiontophoresed drugs or neurotransmitter substances on single unit activity, and (3) estimation of drug-induced changes in the apparent *in vivo* synthesis rate of dopamine by measurement of dopamine precursor and metabolite levels in brain homogenates.

Major Findings and Significance to Biomedical Research and the Program of the Institute:

The dopamine-containing neurons of the substantia nigra degenerate in parkinsonism, while the striatal afferents to this region are lost in Huntington's chorea. Thus, the substantia nigra is an important pharmacological target site in these disorders. This area may also be significant in the etiology of many other types of neurological and psychiatric disorders as well, since many drugs, such as lithium, neuroleptics, reserpine, amphetamine, cholinomimetics, as well as the dopamine and GABA agonists, which have significant positive or negative effects on these disease states, also have significant effects on neuronal function in this region.

In order to obtain a better understanding of factors regulating neuronal activity in the substantia nigra and the consequences of pharmacological intervention, our current investigations have focused on 3 areas: (1) the potential role of dopamine autoreceptors and the striatonigral "feed-

back loop" in regulation of dopamine activity and mediation of dopamine agonist effects; (2) time dependent changes in dopamine receptor function; and (3) the role of the GABA afferents to the substantia nigra and consequences of GABA agonist administration.

(1) Dopamine Autoreceptors and the Striatonigral Feedback Loop in Regulation of Dopamine Activity

Early hypothesis about the regulation of the nigrostriatal dopamine pathway by a striatonigral "feedback loop" predicted that the activity of the nigral dopamine neurons would be inhibited by dopamine receptor stimulating agents. In fact, previous studies have demonstrated that drugs which increase the stimulation of dopamine receptors, either by increasing dopamine levels (L-dopa), by increasing the release of dopamine (amphetamine) or by mimicking the action of dopamine (apomorphine), depress the activity of substantia nigra pars compacta dopamine cells when administered systemically. We have more recently shown that lergotrile and lisuride, ergot derivatives which have dopamine agonist properties and antiparkinson efficacy, as well as the ergot LSD, also inhibit the activity of dopamine neurons. Lisuride has a potency close to that of apomorphine while lergotrile and LSD are less potent.

These results are consistent with the "feedback loop" hypothesis which predicts that in response to a dopamine agonist's effects on postsynaptic dopamine receptors in the neostriatum, the dopamine cells in the substantia nigra will receive greater net inhibitory input from the striatonigral pathway. Alterations in the activity of dopamine cells and changes in the levels of dopamine metabolites have been thought to reflect the amount of stimulation received by the postsynaptic side of the dopamine synapse in the striatum. Consequently dopamine metabolite levels in human CSF have been used as an index of postsynaptic dopamine receptor stimulation.

However, other observations raised the possibility that changes in firing rates of dopamine neurons and changes in dopamine synthesis rates and metabolite levels may not reflect postsynaptic changes in dopamine receptor stimulation at all, but rather may be the consequence of direct interaction of drugs with dopamine receptors on the dopamine cell bodies (autoreceptors). It has been shown that dopamine or apomorphine iontophoresed into the vicinity of a dopamine cell body can directly inhibit the activity of the dopamine neurons, presumably by interacting with dopamine autoreceptors. We have recently confirmed this observation. Using either 5 or 6 barrel micropipettes (the latter consisting of a single barrel electrode with optimum recording characteristics glued to a 5 barrel micropipette with optimum iontophoresis characteristics) we have demonstrated that the dopamine neurons of the pars compacta substantia nigra are inhibited by iontophored dopamine while the non-dopaminergic neurons of the pars reticulata are not inhibited by this neurotransmitter. These observations caution against using a change in dopamine cell firing or dopamine metabolite levels as an index of alterations in the activity of neostriatal neurons.

Although the iontophoretic studies suggested that dopamine agonists (and perhaps endogenous dopamine itself released from dopamine dendrites) may directly affect the activity of dopamine neurons, they gave no indication of the relative importance of this mechanism in regulating dopamine activity and mediating dopamine agonist effects. We have been able to investigate the relative contributions of the dopamine autoreceptors and the striatonigral feedback loop to effects of systemically administered dopamine agonists on dopamine activity by the use of kainic acid injections into the rat neostriatum. Kainic acid is thought to lesion neuronal cell bodies, leaving incoming axons intact. Following a neostriatal kainic acid injection, the output pathway from the neostriatum, including the striatonigral fibers believed to function as part of a feedback loop from the striatum to the substantia nigra, are removed, while the dopamine cells projecting to the neostriatum remain intact.

We reported previously that apomorphine still effectively inhibits the activity of the substantia nigra dopamine cells after the kainic acid-induced striatal lesion. More recently, we have recorded the responses of dopamine cells contralateral to the kainic acid injection to investigate the possibility that asymmetry in striatonigral output might produce asymmetrical changes in dopamine cell responses, in view of a report in the literature of reciprocal variations in nigral dopamine release on opposite sides of the brain. The results showed that kainic acid-induced lesion of one striatum does not significantly affect the ability of the contralateral dopamine cells to respond to apomorphine. These observations raised questions about whether the striatonigral pathway in fact plays any role in regulating dopamine cell activity, an idea supported by work with GABAergic agents, described below.

Since the effects of systemic administration of the ergot derivatives on dopaminergic activity were similar in many ways to those of apomorphine, we were interested in determining whether the ergots were also exerting a direct dopaminergic effect on the dopamine autoreceptors and inhibiting dopaminergic activity in a manner independent of the striatonigral feedback loop. When we investigated the effects of systemically administered lisuride in animals with kainic acid-induced striatonigral lesions, we were quite surprised to find that lisuride was less effective at inhibiting dopamine activity in the lesioned rats than in the control rats. Thus, although apomorphine appears to affect dopaminergic activity independent of the striatonigral feedback loop, lisuride, which produces changes in dopaminergic activity in normal rats similar to those of apomorphine, appears to be inducing these changes, at least in part, through a site which is susceptible to the consequences of intrastriatal kainic acid injections. This observation raised anew the question of the role of the striatonigral pathway in regulation of dopaminergic activity and suggested that there may be a significant difference between the dopaminergic effects of at least one ergot derivative and the dopamine agonist, apomorphine. We have tried to explore this difference in the following ways.

Since the effects of amphetamine have recently been shown to be attenuated, like those of lisuride, in rats with kainic acid-induced lesions of the striatonigral pathway, we wondered if lisuride might be acting more like amphetamine, causing the release of dopamine, than like the direct acting agonist, apomorphine. We examined the effects of lisuride in rats pretreated with α -methylparatyrosine and reserpine to block dopamine synthesis and storage. This treatment completely eliminates the effects of amphetamine on dopaminergic activity but had no effect on lisuride's actions.

Since lisuride is known to be a potent serotonin agonist and may also interact with other neurotransmitter systems, it also seemed possible that the effects of lisuride which were lost in the kainic acid lesioned rats might be mediated through serotonin receptors or some other mechanism compromised by the kainic acid injections, rather than through the postsynaptic dopamine receptors. As one approach to this question, we have examined the effects of lisuride on the serotonin-containing neurons of the dorsal raphe in control rats and in rats with striatonigral lesions. Lisuride was found to be very potent at inhibiting the serotonin neurons and this effect was unaltered in the kainic acid lesioned rats.

We have also made several attempts to iontophorese lergotriole and lisuride onto the dopamine neurons to determine whether the ergots do have direct effects on dopamine unit activity like those of apomorphine and dopamine, but to date, the results have been inconclusive because of problems with keeping the drugs in solution and getting solutions which are sufficiently concentrated. We hope the use of the 6-barrel micropipettes will facilitate this approach.

We will continue to explore the mechanisms behind the differences between lisuride and apomorphine's effects since these differences raise important questions about the role of the striatonigral pathway, the mechanisms regulating the activity of substantia nigra dopamine neurons and the pharmacology of the ergot derivatives.

(2) Time-dependent Changes in Dopamine Receptor Function

The observation that dopamine agonists may induce significant effects on dopaminergic activity by interacting with dopamine autoreceptors on the dopamine neurons also suggests that dopamine agonists may have some paradoxical behavioral effects, acting more like dopamine antagonists, if the firing rates of dopamine cells and the release of dopamine are reduced at doses lower than those which cause significant stimulation at some or all subcategories of postsynaptic dopamine receptors. We plan to compare the effects of the agonists at the dopamine autoreceptors with their actions at sites postsynaptic to the dopamine neurons. In addition, we felt these observations would provide more insight into the role of the autoreceptor as an endogenous regulator of dopamine activity and would be more relevant to other behavioral and pharmacological studies if we also investigated the rate of desensitization and time dependent changes in sensitivity of the various dopamine receptor systems.

To this end, we have been exploring the development of tolerance at the level of the dopamine autoreceptor. In previous studies, a rapid desensitization to the inhibitory effects of systemically administered apomorphine has been observed. Moreover, cells which have become resistant to the effects of apomorphine are also no longer inhibited by either lergotrile, lisuride or other dopamine agonists. The studies with apomorphine in rats with kainic acid-induced lesions of the striatonigral pathway suggested that the events responsible for this rapid development of resistance might be occurring at the level of the dopamine autoreceptors on the dopamine cell bodies. To investigate this, we examined the iontophoretic effects of dopamine on the single unit activity of dopamine cells before, during and after a series of i.v. injections of apomorphine. It was found that the dopamine cells initially inhibited by iontophoresis of dopamine became resistant to the effects of this transmitter as the cells developed resistance to the effects of systemic apomorphine.

In addition, we observed that cells recovering from i.v. administration of amphetamine (believed to act totally indirectly by releasing dopamine) showed diminished responses to apomorphine, suggesting that dopamine itself may also induce changes in autoreceptor sensitivity. This observation and the fact that apomorphine can initially inhibit dopamine cells completely before resistance develops, suggests that a rapid change may be occurring in sensitivity to the agonists or transmitter as a normal consequence of dopamine autoreceptor stimulation and/or subsequent membrane events leading to a change in firing rate. We plan to investigate L-dopa's ability to induce such a change and to determine the time course of this agonist-induced subsensitivity. We hope to eventually study the time dependent changes in response of postsynaptic dopamine receptors as well, and are currently collaborating with Dr. Carol Tamminga, Neuroendocrinology Unit (see Project Z01 NS 02265-03 ET) to develop techniques for investigating dopaminergic mechanisms and the development of tolerance to dopamine agonist effects in neuroendocrine systems. The issue of whether different subcategories of dopamine receptors respond differently to tonic transmitter or agonist application is currently unexplored and especially significant in regard to the development of more specific and effective pharmacological agents for the treatment of parkinsonism and other neuroendocrine and neuropsychiatric disorders.

(3) The Role of GABA and Effects of GABA-mimetics in the Substantia Nigra

The striatal afferents to the substantia nigra are, in part, GABA containing processes. This has led to the expectation that GABAergic mechanisms are involved in the striatonigral "feedback loop" regulation of dopaminergic activity. However, just as the apomorphine studies raised some doubt about the significance of the "feedback loop" in regulation of dopamine activity, our previous studies on the interactions between GABA-active agents and the dopamine system also have not supported the expectation that GABA-mediated mechanisms tonically inhibit dopamine activity. While GABA-active agents do have some effects on dopamine synthesis, these effects are not consistent with the feedback hypothesis and do not appear mediated through changes in firing rates of dopamine neurons.

Our ongoing investigations with muscimol, a GABA agonist, and picrotoxin, a GABA antagonist, have continued to raise doubts about how much potential the dopamine neurons have for significant direct modulation by GABAergic processes. We have compared the responses of the dopamine neurons to those of the non-dopaminergic cells of the substantia nigra pars reticulata during systemic administration of increasing doses of muscimol and picrotoxin and during iontophoresis of muscimol and GABA. Confirming our previous results, we observed that i.v. administration of muscimol caused dose dependent increases in the unit activity of the dopamine neurons and an inhibition of nigral pars reticulata cells. We also found that the depressant effects of the drug upon reticulata neurons were reversible by administration of GABA antagonists, picrotoxin and bicuculline HCl, but the stimulatory effects of i.v. muscimol upon dopamine neurons was not abolished by these agents. In contrast to the systemic effects of muscimol, microiontophoresis of GABA and muscimol inhibited the activity of both pars compacta and pars reticulata cells, although the reticulata neurons were much more sensitive to the inhibitory actions of these agents than the dopamine neurons. These results show that a population of neurons in the substantia nigra pars reticulata have a greater capacity to be affected by GABA terminals in the nigra than do the pars compacta dopamine neurons. They also suggest that if the dopamine cells are regulated by GABAergic neurons, specifically the striatonigral GABA pathway, this interaction is predominantly indirect and may involve a second inhibitory neuron within the nigra. Alternatively, it may be possible that muscimol (or GABA) exerts an unconventional excitatory effect on dopaminergic activity if the dopamine dendrites are exposed to the drug, as they would be during systemic administration but not during iontophoretic application. GABA appears to exert such an effect on some hippocampal neurons.

The i.v. administration of picrotoxin alone caused only moderate increases in dopaminergic activity but markedly stimulated the activity of the pars reticulata cells. This further supported the idea the cells of substantia nigra pars reticulata are more tonically inhibited by GABAergic mechanisms than are the dopamine neurons of the pars compacta. To investigate the possibility that the picrotoxin-induced increases in nigral activity might be due to a blockade by picrotoxin of tonically active GABA neurons in the striatonigral pathway, we examined the effect of picrotoxin on activity of nigral cells in rats after kainic acid lesions of the striatonigral pathway. We found no changes in the responses of the dopamine neurons to i.v. picrotoxin in the lesioned animals and only a slight but insignificant attenuation of the stimulatory effect of picrotoxin upon reticulata cells. These findings suggest that (1) picrotoxin may stimulate nigral neurons by a non-specific excitatory mechanism unrelated to GABA receptor blockade, or (2) another GABA projection to the substantia nigra, other than that destroyed by striatal kainic acid lesions, may, either indirectly or directly, inhibit cells. This latter possibility is supported by biochemical evidence that approximately 30% of the control level of GAD activity consistently remains in the substantia nigra after striatal kainic acid lesion. We are currently examining the effects of iontophoreted GABA uptake blockers and GABA receptor blockers in an attempt to gain more insight into the role of the GABA-

ergic afferents to the nigra neurons. We hope that one result of these investigations will be a better understanding of how to compensate for the degeneration of the nigrostriatal GABA pathway which occurs in Huntington's chorea.

In view of the questions being raised about the role of the striatonigral pathway as a feedback loop, tonically and directly regulating dopaminergic activity, some investigators have proposed that the striatonigral pathway may instead be an important output system carrying messages from the striatum to the pars reticulata neurons which, in turn, project to the thalamus. Thus, the behavioral effects of dopamine agonists (and, perhaps the clinically therapeutic effects of these drugs) may be mediated, in part, through the pars reticulata neurons. In this respect the pars reticulata of the substantia nigra may function in a manner comparable to the globus pallidus. We plan to investigate this hypothesis by examining carefully the effects of dopamine agonist administration on the activity of the pars reticulata cells.

Other studies have been directed toward the questions of why muscimol has not proven an effective agent in the treatment of Huntington's chorea. It was hoped that administration of a centrally active GABA-mimetic agent would compensate for the GABA-containing neurons which are lost in this disease. Our inability to demonstrate development of supersensitivity to muscimol in the substantia nigra after kainic acid lesion of the striatonigral GABAergic pathway has suggested that the effects of this GABA-mimetic may not be facilitated in the substantia nigra or other regions where GABA neurons die in Huntington's. Lack of any specificity of action at the denervated sites might account for the development of the toxic effects seen with this drug in the absence of therapeutic actions. In addition, the studies with picrotoxin suggest that the striatonigral pathway may not provide tonic inhibition to the nigra. If so, it might be that tonic administration of an agonist could be as harmful as chronic loss of the GABA innervation.

We are currently examining the effects of THIP, 4,5,6,7-tetrahydroisoxazolo-[4,5-c]-pyridin-3-ol, a new GABA agonist, in hopes that it will have some properties which would give it a therapeutic advantage over muscimol. In the substantia nigra, effects of systemic administration of this drug are similar to those of muscimol, although THIP is less potent. We have also compared the *in vitro* receptor binding properties of this agent and to those of muscimol and GABA and are currently comparing the iontophoretic effects of these agents to obtain a broad picture of THIP's relative potency and actions. We expect to be able to compare these results to those which will be obtained from clinical trials with THIP in this laboratory in the future.

II. Regulation of GABA Synthesis

Methods:

These include analysis of enzyme activity in synaptosomes, homogenates or precipitates of brain tissue obtained from decapitated rats or rats sacrificed with a "brain blowing" device.

Major Findings and Significance to Biomedical Research and the Program of the Institute:

Previous studies in this Unit have suggested that the activity of glutamic acid decarboxylase (GAD), the enzyme which synthesizes GABA, may be related to the amount of enzyme with bound cofactor. Cofactor binding seems in turn affected by levels of endogenous substances such as ATP and inorganic phosphate. From other studies we have had evidence that several anesthetics decrease GABA synthesis rates in vivo. To test our hypotheses that the synthesis of GABA is regulated by changes in the degree of saturation of GAD by cofactor, we examined cofactor saturation of GAD in brain obtained from animals pretreated with the anesthetics most apparently effective at inhibiting GABA synthesis in vivo. For these experiments, it was necessary to use a brain blower device which provides very rapid freezing of brain tissue to obtain artifact-free data. We have had a difficult time getting the brain blower to work properly, but results to date indicate that, contrary to our original hypothesis, the anesthetics which cause an apparent in vivo inhibition of GAD activity do not alter the degree of saturation of GAD in homogenates prepared from rapidly frozen brain tissue. If further results substantiate these observations, we will have cast substantial doubt on the significance of short term alterations in cofactor saturation of GAD as a significant mechanism for regulation of GABA synthesis in vivo.

Publications:

Walters, J.R., Lakoski, J.M., Eng, N. and Waszczak, B.L.: Effect of muscimol, AOAA and Na Valproate on the activity of dopamine neurons and dopamine synthesis. In Krogsgaard-Larsen, P., Scheel-Kruger, J. and Kofod, H. (Eds.): GABA-Neurotransmitters: Pharmacological, Biochemical and Pharmacological Agents. Copenhagen, Munksgaard, 1978, pp. 118-134.

Silbergeld, E.K. and Walters, J.R.: Synaptosomal uptake and release of dopamine in substantia nigra: Effects of gamma-aminobutyric acid and substance P. Neurosci. Letters 12: 119-126, 1979.

Walters, J.R., Baring, M.B. and Lakoski, J.M.: Effects of ergolines on dopaminergic and serotonergic single unit activity. In Calne, D.B. and Fuxe, K. (Eds.): Dopaminergic Ergot Derivatives and Motor Function. New York, Pergamon Press, in press.

Walters, J.R., Baring, M.D. and Lakoski, J.M.: Effects of dopamine agonists on dopaminergic unit activity. In Usdin, E. (Ed.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, p. 631-639.

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Miller, L.P. and Walters, J.R.: Effects of depolarization on cofactor regulation of glutamic acid decarboxylase in substantia nigra synaptosomes. J. Neurochem., in press.

Waszczak, B.L. and Walters, J.R.: Effects of GABA-mimetics upon substantia nigra neurons. In Chase, T.N., Barbeau, A. and Wexler, N. (Eds.): Advances in Neurology, vol. 23. New York, Raven Press, 1979, pp. 727-740.

Baring, M.D., Walters, J.R. and Eng, N.: Action of systemic apomorphine on dopamine cell firing after neostriatal kainic acid lesion. Brain Res., in press.

Waszczak, B.L., Eng, N. and Walters, J.R.: Effects of muscimol and picrotoxin on single unit activity of substantia nigra neurons. Brain Res., in press.

Project Description:

Objectives:

(1) Develop methods for correlating behavioral and functional effects of basal ganglia lesions with extent of neurochemical and anatomic damage; (2) study interactions of putative neurotransmitters in the basal ganglia; (3) study the effects of ergot drugs on neurochemistry and behavior; (4) study effects of neurotoxic compounds for their basic mechanisms of action.

(1) Basal ganglia lesions and neurobehavioral function

Several neurotoxins have been identified as possessing relatively specific mechanisms of action which confer selectivity as to the anatomic or biochemical nature of their target cells when injected locally into brain regions. Kainic acid and ibotenic acid are excitotoxins which appear, under appropriate conditions, to destroy selectively cell bodies of neurons in the injection area, while sparing axons of passage and nerve terminals. 6-Hydroxydopamine (6-OHDA) is a relatively specific toxin for dopaminergic and noradrenergic nerve terminals. The neurochemical and anatomic changes consequent to injections of these toxins have been well characterized; however, little work has been done to investigate their effects on neurobehavioral function as a means of studying the role of specific pathways in basal ganglia control of movement. In the present studies, toxins were injected into the striatum or globus pallidus; aspects of cholinergic, dopaminergic, serotonergic, GABAergic (gamma-aminobutyric acid, GABA) and glutamatergic neurotransmission were then studied. Routine histology was also done on selected animals. Methods for the quantitative study of rat locomotion were developed, utilizing spatial and temporal analyses of movement, and applied to specifically lesioned rats. In addition, rotation and drug-induced behaviors (stereotypy, tremor) were also investigated in lesioned animals.

Major Findings and Significance to Biomedical Research and the Program of the Institute:

We have developed sensitive and quantitative methods for characterizing aspects of normal locomotion in rats. These methods, which describe both temporal and spatial aspects of movement in spontaneously walking rats, were applied to rats with specific brain lesions. The results indicate that destruction by kainic acid of striatal cholinergic interneurons and striatonigral GABAergic efferent neurons can significantly affect locomotion in rats. These effects are distinct from the effects of destroying nigrostriatal dopamine neurons by 6-OHDA.

Rats with kainic acid or ibotenic acid lesions of the striatum also show altered responses to cholinergic agents. Unilaterally lesioned rats rotate ipsilaterally to scopolamine and atropine; bilaterally lesioned rats show potentiated tremor response to arecoline and tremorine. The results may be of significance for the therapeutics of Huntington's disease (since intrastriatal

kainic acid has been suggested to provide an animal model of this disorder) as well as informative on the neuronal bases of tremor and other cholinergically mediated behaviors.

(2) Interactions of putative neurotransmitters in basal ganglia

Methods Employed:

Utilizing synaptosomal preparations of dissected brain regions, the interactions of exogenously added GABA and substance P on dopamine uptake and release were studied *in vitro*. Fluorescent dye monitoring of synaptosomal membrane potential was explored as a means of investigating actions of neurotransmitters at the synaptic level on *in vitro* electrochemical events. The nonhypothalamic interactions of dopamine and sex steroids have also been studied by neurochemical and behavioral methods.

Major Findings and Significance to Biomedical Research and the Program of the Institute:

Tissue from substantia nigra prepared as synaptosomes demonstrates systems for Na-dependent high and low affinity uptake of dopamine, which is readily releasable by 35 mM K⁺ (and calcium dependent). Substance P increased both uptake and release of dopamine by nigral synaptosomes; GABA inhibited release with no effect on uptake. In striatal synaptosomes, substance P inhibited both uptake and release of dopamine. The differences in response to substance P and GABA between nigral and striatal synaptosomes may reflect structural differences in dopamine-containing and releasing processes in these two areas.

Studies of synaptosomal depolarization, utilizing the binding of carbo-cyanine dyes and consequent changes in fluorescence, revealed that GABA added *in vitro* could produce an apparently hyperpolarizing effect on synaptosomal membrane potential, while glutamate and kainic acid both depolarized synaptosomes. The effects of glutamate were K⁻ and Na-dependent but reversible over time, when ATP was present, while the depolarizing effects of kainic acid were not reversible, suggesting toxic damage to the nerve terminal. The results support an excitotoxic role for kainic acid, mediated through prolonged depolarization, and further support the utility of these techniques for *in vitro* membrane potential experiments.

Recent research has suggested that the interactions of central monoaminergic neurotransmitters and the sex steroids may be mutual, in that dopamine may influence release of pituitary factors, and circulating levels of estrogen and prolactin may affect central dopaminergic function. In addition, several halogenated hydrocarbons, such as the polybrominated biphenyls (PBBs), act to increase circulating levels of estrogen. The Unit has investigated the effects of estrogenization of male rats on dopaminergic function outside the hypothalamus. Stereotypy and rotation were studied in unilaterally 6-OHDA lesioned rats. In collaboration with Dr. Tamminga, rats were injected once

with estrogen and 8 days later, their responses of amphetamine and apomorphine measured. Estrogenization was associated with significant potentiation of behavioral activity of both amphetamine and apomorphine. This behavioral supersensitivity is paralleled by an increase in ^3H -spiroperidol receptors in the striatum. The results support an important interaction between dopamine and sex steroids, which may be of relevance to neuropsychiatric disorders and to the symptoms described in humans exposed to estrogenic compounds.

(3) Effects of ergot drugs

Methods Employed:

In order to study serotonergic and dopaminergic mechanisms for the effects of ergot drugs, a combined behavioral and neurochemical approach has been used. Behaviorally, rats were tested for responses using monitoring systems sensitive to dopaminergically mediated effects (stereotypy and rotation) and serotonergically mediated effects (the 5-HTP syndrome, or serotonin syndrome). Neurochemically, the effects of ergots on receptor binding and neurotransmitter uptake and release in vitro were studied.

Major Findings and the Significance to Biomedical Research and the Program of the Institute:

A systemic investigation of the effects of ergots in vitro on specific receptor binding revealed a spectrum of potencies on the specific receptor binding of spiroperidol, LSD, 5-HT, and WB-4101. Of the ergots, bromocriptine and lisuride were the most potent at displacing binding of spiroperidol; methergoline the most potent at displacing binding of 5-HT and LSD; and lergotrile the most potent at displacing WB-4101, the ligand for α -adrenergic receptors. These results are difficult to correlate with the results of behavioral studies, which have concentrated on the interactions of ergot drugs with stereotypy, postdecapitation convulsions and the so-called serotonin syndrome. Of the ergots, lergotrile, bromocriptine, 29-712, 29-717 all produce stereotypy similar to that induced by apomorphine; however, ergot-induced stereotypy shows some reliance upon presynaptic as well as postsynaptic function, in that inhibition of catecholamine synthesis can reduce or block the stereotypy produced by bromocriptine. Bromocriptine and lergotrile also potentiate the behavioral effects of 5-HTP, quantified as the serotonin syndrome. Lisuride, in very low doses, can produce signs of the serotonin syndrome, in a manner indistinguishable from LSD. However, there are differences between lisuride- and LSD-induced behaviors, in that haloperidol can block lisuride but not LSD. As an index of noradrenergic activity, the interaction of ergots with postdecapitation convulsions was studied in rats being used for neurochemical studies. Only lergotrile and methergoline were able to reduce duration of PDC. The actions of the two drugs appeared to be through similar mechanisms, since lower doses of each compound were additive when administered together. These studies suggest that the ergots possess a spectrum of potent neurochemical effects on mono-

aminergic receptors in the CNS. The extrapolation of these data to clinical conditions is difficult, and it is of great importance to know the effects of chronic exposure to ergot drugs in estimating their clinical potential. Studies of semichronic effects of ergots have been initiated using 5-day treatment of rats with bromocriptine, lergotrile, or lisuride. The preliminary results suggest that this treatment is associated with significant decreases in receptor binding, consistent with findings for apomorphine, which has been used as a reference dopamine agonist in these studies.

(4) Neurotoxic compounds

Methods Employed:

Chronic, acute, and in vitro exposure paradigms have been used to study the effects of lead, porphyrinopathic compounds and red dye No. 3 (erythrosin B) on neurochemistry and behavior. Behaviorally, studies have concentrated on the mechanisms of action of lead and porphyrinopathic substances which appear to alter sensitivity to seizures.

Major Findings and the Significance to Biomedical Research and the Program of the Institute:

Seizures have been described as the endpoint of lead intoxication in both humans and animals, and as a severe complication of porphyria. For this reason, the possible neurochemical mechanisms of action of lead, particularly through its actions on heme synthesis were studied in relation to seizure sensitivity and GABAergic function. The results indicate that acute exposure to lead, or acute inhibition of heme synthesis by succinyl acetone, can significantly reduce the threshold dose for convulsant agents such as 3-mercaptopropionic acid and strychnine. The biochemical correlate appears to be an inhibition of GABA release, and an accompanying increase in GABA receptor binding. The time course of these effects is very rapid, but not directly involved with the result of lead itself, rather apparently mediated through altered heme synthesis since the same effects are seen when succinyl acetone is administered. Succinyl acetone acts to inhibit heme synthesis at the same step as inorganic lead.

Because of the hypothesized relationship of artificial food dyes with altered behavior and learning disorders in children, we have investigated possible neuroactive properties of food dyes. Initial experiments concentrated on the interactions in vitro of Red No. 3 with dopamine uptake and release. It was found that this dye can act in an uncompetitive fashion to inhibit the uptake of dopamine by caudate synaptosomes. Further studies have investigated the effects of the dye on sodium and amino acid (glutamate) uptake by synaptosomes. The results suggest that the dye at very low concentrations (nmolar) can act to shift the stoichiometric relationship of an amino acid to sodium in the active, energy-dependent transport of amino acids into neurons.

Proposed Course:

This project will be transferred from the Experimental Therapeutics Branch (ETB) to the Section on Neurotoxicology on September 1, 1979 when the Section is established. At that time, it is anticipated that studies on ergot drugs will be discontinued, since this is of special interest to ETB, but that other studies on animal models of neurological disease will be continued.

Publications:

Silbergeld, E.K., Hruska, R.E., Kennedy, S. and Weir, R.: Dopaminergic and serotonergic effects of ergot drugs. In Fuxe, K., and Calne, D.B. (Eds.): Dopaminergic Ergot Derivatives. London, Pergamon Press, in press.

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Silbergeld, E.K. and Hruska, R.E.: Effects of ergot drugs on serotonergic function: Behavior and biochemistry. Europ. J. Pharmacol., in press.

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Hruska, R.E. and Silbergeld, E.K.: Abnormal locomotion in rats after bilateral intrastriatal injection of kainic acid. Life Sci. 25: 181-193, 1979.

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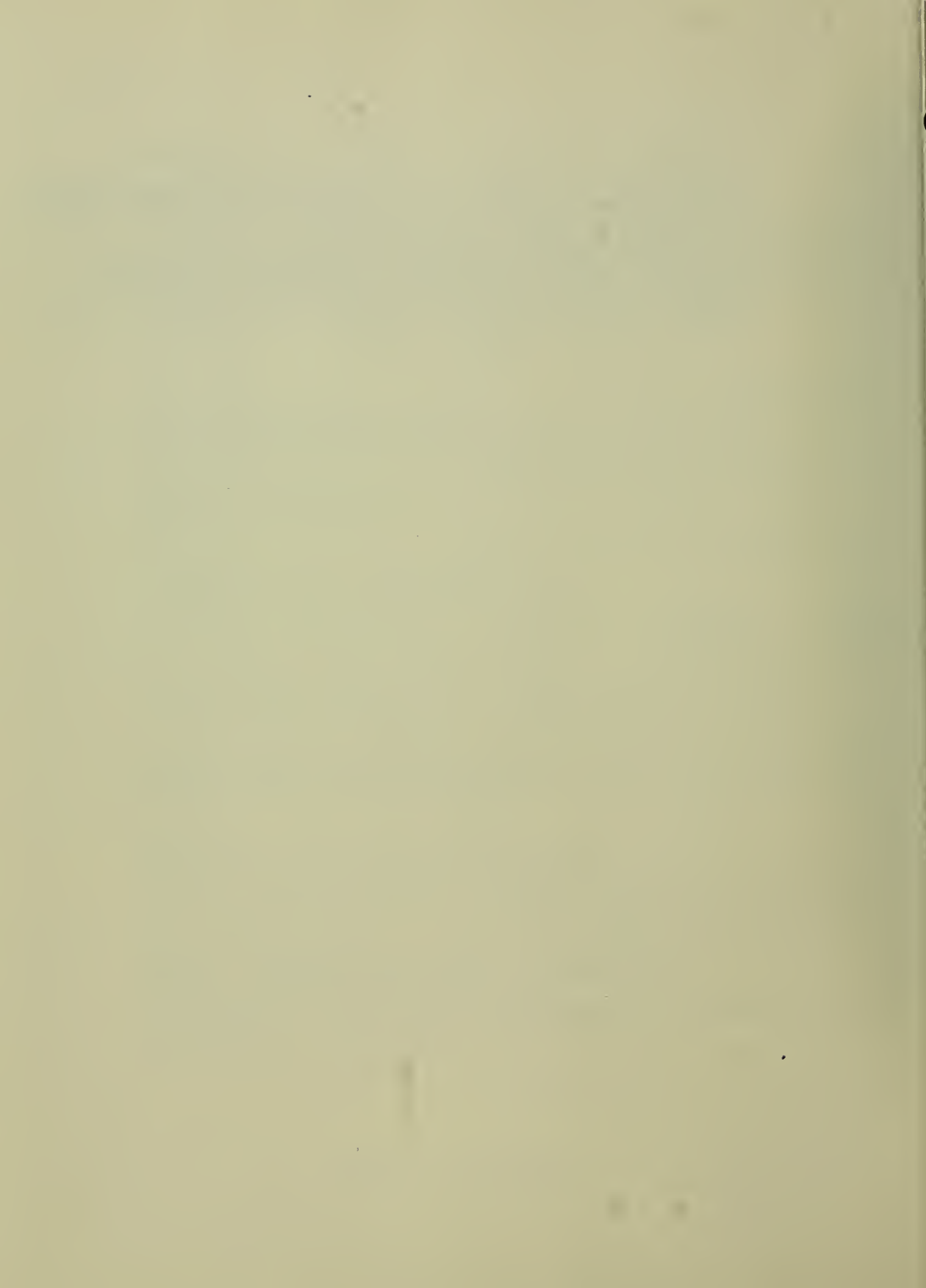
Silbergeld, E.K.: Advances in the neuropharmacology of parkinsonism. Ann. Intern. Med. 90: 219-229, 1979.

Project No.: Z01 NS 02264-03 ET

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Silbergeld, E.K. and Hruska, R.E.: Neurochemistry of lead poisoning. In Needleman, H. (Ed.): Lead Poisoning. New York, Raven Press, in press.

Lafferman, J. and Silbergeld, E.: Erythrosin B inhibits dopamine uptake by striatal synaptosomes. Science, in press.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02319-02 ET
PERIOD COVERED October 1, 1978 through September 30, 1979		
TITLE OF PROJECT (80 characters or less) Analytic Electron Microscopy in Neurochemistry		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: E.K. Silbergeld, Head, Behavioral Neuropharmacology Unit, ETB, NINCDS Other: J.L. Costa Medical Officer CNB NIMH		
COOPERATING UNITS (if any) UCSF, Medical School, San Francisco, CA; Bell Telephone Laboratories, Summit, NJ; Johns Hopkins University, Baltimore, MD; University of Maryland, College Park, MD; Clinical Neuropharmacology Branch, NIMH		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Behavioral Neuropharmacology Unit		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20205		
TOTAL MANYEARS: .5	PROFESSIONAL: .5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This project was undertaken to develop methods for application to neuroscience of <u>x-ray microprobe</u> and <u>electron energy loss spectroscopy</u> coupled to high resolution <u>scanning-transmission electron microscopy</u> . Current topics under investigation include: <u>localization of elemental neurotoxins</u> in brain tissue; <u>identification and localization of receptor ligands</u> in synaptosomes; development of quantitative methods for studying <u>element fluxes</u> in neurons.		

Project Description:

Objectives:

(1) To determine applicability of analytic electron microscopy (EM) to qualitative problems in neuroscience; (2) to investigate methods for quantitative analysis of elements within subcellular compartments; (3) to compare resolution of receptor ligand binding sites measured by analytic EM with sensitive autoradiographic techniques.

Methods Employed:

Both conventional fixation and rapid air-drying techniques have been applied to these studies. Conventional methods yield specimens with great morphologic information, at the cost of loss of readily diffusible material. Rapidly air-dried material appears to preserve intracellular elements in compartments, but morphologic information is minimal.

X-ray microprobe analysis was done on a Hitachi H-500 electron microscopy.

Major Findings and the Significance to Biomedical Research and the Program of the Institute:

In qualitative studies, it appears feasible to use x-ray techniques for localizing heavy metals, such as lead and manganese, within neurons and capillary endothelial cells exposed to metals *in vitro*. The results indicate that lead is taken up by cells and deposited, at calcium binding sites, within mitochondria. Manganese appears to bind on the external side of neuronal membranes.

In quantitative studies of air-dried synaptosomes and mitochondria, elemental x-ray analysis confirmed that neuronal cytoplasm is relatively enriched in Na and Cl, while mitochondria are enriched in Ca and P, contained in noncrystalline electron-dense accumulations. Synaptosomes and mitochondria exposed *in vitro* to treatments which affect Ca uptake and retention, showed changes in Ca:P ratios consistent with the predicted effects of these treatments on Ca bindings, which was also studied with ⁴⁵Ca as a tracer for Ca metabolism.

Current studies on receptor ligand bindings have concentrated on localization of iodinated α -bungarotoxin (α -BGT) binding to synaptosomes. This system was chosen because preliminary experiments indicated that other receptor ligands -- GABA, haloperidol, apomorphine -- are rapidly dissociated from synaptic membranes during processes of fixation and substitution. α -BGT is known to bind rapidly and irreversibly to nicotinic cholinergic receptors, and we have studied this binding in CNS tissue using ³H- α -BGT.

Project No.: Z01 NS 02319-02 ET

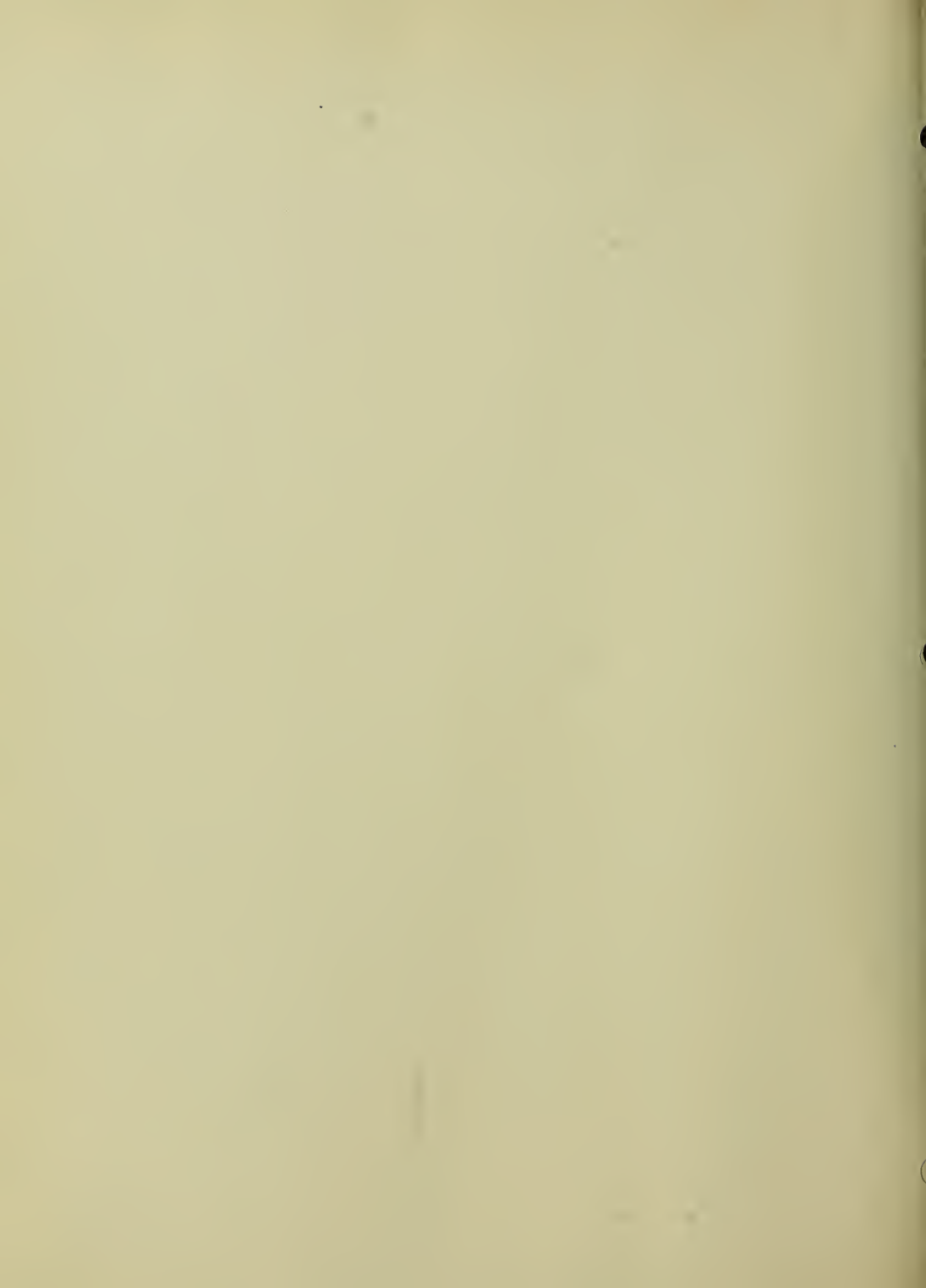
Proposed Course:

This project will be transferred from the Experimental Therapeutics Branch to the Section on Neurotoxicology on September 1, 1979 when the Section is established. At that time, it is anticipated that these studies will be continued and expanded.

Publications:

Silbergeld, E.K. and Costa, J.L.: Synaptosomal Ca metabolism studied by electron microprobe analysis. Exper. Neurol. 63: 277-292, 1979.

Silbergeld, E.K., Goldstein, G. and Wolinsky, J.: Localization of lead in capillary endothelial cells by x-ray microprobe analysis. Exper. Brain Res., in press.



ANNUAL REPORT

October 1, 1978 through September 30, 1979

Neuroimmunology Branch

National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report
October 1, 1978 to September 30, 1979
Neuroimmunology Branch
National Institute of Neurological and
Communicative Disorders and Stroke

Dale E. McFarlin, M.D., Chief

Research in the Neuroimmunology Branch (NIB) is directed at assessment of immunity in both patients with neurological diseases and experimental animals with either infectious or autoimmune diseases. Experimental models of neurological diseases provide a mechanism for controlling multiple biological variables and for developing new experimental approaches which can subsequently be applied to clinical investigation. Progress over the past year in the NIB has been related to several technical advances which are being applied to the research in the Branch. These include: 1) Development and standardization of a routine procedure yielding mgm quantities of pure measles virus which retains infectivity and antigenicity. This technical advance is facilitating detailed investigation of the biology and immunology of this important infectious agent. 2) The application of methodology for detecting antibody directed at minute amounts of antigen after separation by gel electrophoresis. This approach, although qualitative, circumvents the tedious task of purification of antigen present in small quantities. Of particular interest are molecules present in the membranes of myelin and viruses which are believed to have important biological functions. 3) Initial studies indicate that the cell fusion technique (Hybridoma) for producing monospecific antibody can be applied to the investigation of the molecular components of viruses and the myelin membrane.

Investigations designed to identify virus-host interaction in various models of experimental virus infections of the CNS are being conducted in mice, because this species is well defined genetically and immunologically. With measles virus several types of infection have been produced. Inoculation of weanling Balb/c mice with mouse-adapted HNT strain of measles virus produces an acute encephalopathy leading to death. There is a characteristic distribution of virus within the brain, where the major involvement is neurons of the limbic system. Electronmicroscopic studies in collaboration with Electron Microscopy Section, IDB, have demonstrated virus antigen in nerve cell bodies, but more importantly, in the presynaptic regions of the dendrite. No inflammatory response is seen in the acute disease. From these findings it has been postulated that the underlying pathophysiology may result from viral-induced dysfunction of synaptic transmission.

Inoculation of mice with different genetic background or passive transfer of anti-measles antibody into Balb/c mice after infection produces a much different clinical and pathological process. In both types of experiments, only a small number of the animals become acutely ill,

while a significant number develop a subacute disease. In contrast to the acute disease, the subacute disease characteristically is associated with perivascular inflammatory response in the CNS. Elucidation of the mechanisms responsible for the subacute disease will influence our investigation of chronic and subacute human diseases.

Considerable progress has been made in characterizing the antibody response to the measles virus both in animals with experimental disease and patients. In animals hyperimmunized with measles, antibody directed at each of the six polypeptide components of the virus has been identified using the techniques mentioned above. To date antibody against the major glycoprotein has not been detected by these methods in any human serum. This suggests that the antigenicity of the glycoprotein, which is believed to be associated with hemagglutination, requires a specific conformation which is present on the intact virus but is altered during our extraction procedure. In patients recovering from acute measles infection and patients with SSPE, antibody against the other five viral polypeptides has been identified. These data indicate that the immune response to measles and SSPE strains of measles virus is not qualitatively different in individuals with SSPE as compared to normals.

Another area under investigation is the immune response to the components of myelin. A number of factors including antigen source, the type of adjuvant, the amount of adjuvant, as well as the genetic background influence the immune response to myelin basic protein (BP). Investigation of antibody formation to BP in mice has demonstrated that differences exist among the capacities of various strains, to produce a humoral response and that this is related to the histocompatibility background. Results obtained using recombinant and congenic strains of mice indicate that the genes which regulate this immune response map to the K region of the chromosome encoding for the H-2 complex. The capacity to develop the experimental autoimmune disease, Experimental Allergic Encephalitis (EAE), is dissociated from formation of antibody to basic protein. This disease is believed to be cell-mediated and may be under control of genes other than the ones which regulate antibody response.

In mice it has been consistently observed that EAE is easier to induce with whole tissue than the basic protein. This suggests that BP in whole tissue may exist in a more encephalitogenic form or that other components of myelin may contribute to the disease. An important component of the outer surface of myelin is a glycoprotein. This substance has been demonstrated to be antigenic in rabbits. Furthermore, rabbits injected with myelin subsequently develop EAE and, in addition, develop antibody against the major glycoprotein. It seems likely that an immune reaction to glycoprotein may contribute to the immunopathology.

Clinical investigations are directed at extensive assessment of immune response in patients with multiple sclerosis. Factors responsible for immune regulation including the genetic background and the role of subpopulations of immune cells are being studied. Our initial results

confirmed increased representation of HLA-A3, HLA-B7 and HLA-DW2 in patients with multiple sclerosis. The best correlation occurs with B-cell antigens which are believed to be analogous to murine Ia antigens. In an attempt to find antigens relatively specific for the disease, sera from multiparous wives of male patients are being characterized; two have given interesting results; one detects antigens in approximately 56% of our patients and 30% of the control individuals. The second serum detects antigens in 65% of the patients with multiple sclerosis and only 5% of the controls. The patients which are positive with the second serum are also DW2 positive. Of particular interest is that four DW2 individuals in the control group were also negative. These observations indicate that this serum is detecting an antigen with considerable specificity for MS.

An initial investigation of the cellular immune response to measles virus and other human pathogens using a lymphocyte stimulation assay has been completed. The findings indicate that the cellular response to measles virus is significantly less than the response to other viral agents in both patients and controls. The study has been extended to examine the capacity of T-cells and B-cells to respond. T-cells respond to measles, mumps and vaccinia, while B-cells respond to mumps but not to the other viruses. Investigation of all of these parameters has not revealed a significant difference between MS patients and controls.

A major aspect of the clinical research activities has been devoted to an extensive study of monozygotic and dizygotic twins who are either concordant or discordant for multiple sclerosis. To date 32 sets have been evaluated and are now well defined clinically and immunologically. The findings to date indicate that there is a high degree of concordance in both monozygotic and dizygotic twin pairs. Secondly in a high percentage of the monozygotic, clinically-discordant twin sets, the clinically uninfected member has abnormalities of spinal fluid immunoglobulins; these include either an increase in one of the immunoglobulins or oligoclonal banding pattern. This suggests that a subclinical immune reaction may be occurring in such individuals. Furthermore in a group of twins in whom one individual clearly lacks a history of neurological symptoms, careful examination has revealed subtle but definite abnormalities, which is consistent with the concept of mild subclinical disease.

The next phase of the evaluation of twins has been initiated and involves a careful analysis of cellular immune function. Within recent months reports have suggested that abnormalities of immune regulation exist in multiple sclerosis. Twins provide ideal research subjects for investigating this possibility. Such abnormalities when present can be analyzed by mixing immune cells from individuals having the identical genetic background. This approach should provide relevant information about immune regulation in multiple sclerosis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02202-04 NI																																				
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TITLE OF PROJECT (80 characters or less) Immunological Studies in Patients with Multiple Sclerosis and Other CNS Diseases																																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">D.E. McFarlin</td> <td style="width: 25%;">Chief</td> <td style="width: 20%;">NI NINCDS</td> </tr> <tr> <td></td> <td>H.F. McFarland</td> <td>Asst. Chief</td> <td>NI NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>P.M. Moore</td> <td>Clinical Assoc.</td> <td>NI NINCDS</td> </tr> <tr> <td></td> <td>K.W. Rammohan</td> <td>Clinical Assoc.</td> <td>NI NINCDS</td> </tr> <tr> <td></td> <td>A.C. Williams</td> <td>Visiting Assoc.</td> <td>NI NINCDS</td> </tr> <tr> <td></td> <td>J.I. Greenstein</td> <td>Clinical Assoc.</td> <td>NI NINCDS</td> </tr> <tr> <td></td> <td>J.L. Sever</td> <td>Chief</td> <td>ID NINCDS</td> </tr> <tr> <td></td> <td>R. Eldridge</td> <td>Section Chief</td> <td>ID NINCDS</td> </tr> <tr> <td></td> <td>S.A. Houff</td> <td>Clinical Assoc.</td> <td>ID NINCDS</td> </tr> </table>			PI:	D.E. McFarlin	Chief	NI NINCDS		H.F. McFarland	Asst. Chief	NI NINCDS	OTHER:	P.M. Moore	Clinical Assoc.	NI NINCDS		K.W. Rammohan	Clinical Assoc.	NI NINCDS		A.C. Williams	Visiting Assoc.	NI NINCDS		J.I. Greenstein	Clinical Assoc.	NI NINCDS		J.L. Sever	Chief	ID NINCDS		R. Eldridge	Section Chief	ID NINCDS		S.A. Houff	Clinical Assoc.	ID NINCDS
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COOPERATING UNITS (if any) Infectious Diseases Branch, NINCDS																																						
LAB/BRANCH Neuroimmunology																																						
SECTION Office of the Chief																																						
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205																																						
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SUMMARY OF WORK (200 words or less - underline keywords) <p>The general aim of this project is to obtain a more precise understanding of multiple immunological and genetic factors possibly related singly or in combination to the pathogenesis of <u>multiple sclerosis</u>. These include (1) Determination of <u>histocompatibility</u> types in a carefully selected population of MS patients and appropriate controls. (2) Correlation of histocompatibility data with the humoral and cell-mediated immune response to <u>viruses</u>. (3) Identification of new lymphocyte antigens which may show greater correlation with multiple sclerosis than presently identified lymphocyte antigens. (4) Evaluation of <u>cerebrospinal fluid immunoglobulin</u> content and specificity. (5) Evaluation of families with a <u>multiple incidence</u> of multiple sclerosis and examination of affected and nonaffected members of these families with respect to the above. To minimize some of the variables in the disease identical and nonidentical <u>twins</u> who are either discordant or concordant for MS are being studied. (6) Similar studies are being conducted in patients with <u>SSPE</u>.</p>																																						

Project Description:Objective:

A. Studies of Multiple Sclerosis (MS)

The goal of this project is to establish a more precise understanding of genetic and immunological factors in multiple sclerosis. These factors are being studied in clinically well-defined cases of MS. Numerous interrelated questions are being investigated and include:

1. Examination of the histocompatibility make-up in a population of sporadic cases of MS and appropriate control individuals.
2. Identification of lymphocyte antigens occurring with increased frequency in MS and correlation of these antigens with established genetic and immunological parameters.
3. Evaluation of the cellular immune response to a number of antigens including viruses, components of the nervous system and histocompatibility antigens. This also includes investigation of lymphocyte populations and subpopulations responding to these various antigens.
4. Correlation of histocompatibility make-up with the cellular and humoral immune response to various viral antigens.
5. Evaluation of cerebrospinal fluid (CSF) immunoglobulin content. This includes measurement of IgG, IgM and IgA. Antibody specificity in each Ig class is being examined.
6. The above investigations are being carried out in families with a multiple incidence of MS and in monozygotic and dizygotic twins concordant or discordant for MS. The segregation of histocompatibility antigens and differences in immune function between affected and nonaffected individuals are being studied in both families and twins.

B. Studies of Subacute Sclerosing Panencephalitis (SSPE)

Because of the established relationship of SSPE to measles, humoral and CMI in this disease are being assessed in a few patients. A therapeutic trial of Tilorone, an agent reported to be efficacious was being undertaken. The possible effect of this agent on humoral and CMI was determined.

Methods Employed:

Patient Populations. All patients included in these studies have been evaluated as either inpatients or outpatients on the NIB service at NIH. Each individual receives a complete medical and neurological evaluation

with appropriate diagnostic studies. Patients classified as possible or definite MS are included in the studies.

Studies of familial MS involve families with two or more clinically confirmed cases of MS. Twins either concordant or discordant for MS are being admitted to the Clinical Center in order to document the clinical aspects of each case, to perform extensive laboratory evaluations, and to clinically classify each pair of twins. These studies are performed in collaboration with Neurogenetics Section, IDB. SSPE patients are studied which have the characteristic clinical EEG, CSF and serological findings.

Histocompatibility. Histocompatibility testing for HLA-A and HLA-B antigens are being performed under contract by Dr. Paul Terasaki. HLA-D typing for DW2 antigen is done by mixed lymphocyte culture in our laboratory. In addition, sera from multiparous wives or mothers of patients are being employed in cytotoxicity assays to identify antigens on T or B lymphocytes from MS patients. Since the HLA types of the patients are known, appropriate absorptions can be done to establish the specificity of the sera and to identify differences from the HLA A, B and D antigens.

Humoral Immunity. Conventional assays (CF, HI and neutralization) for antibody to various viruses including measles, rubella, mumps, and vaccinia are performed on serum and CSF. In addition, antibody levels to these viruses are evaluated in serum and CSF using radioimmunoassay. This assay permits the identification of antibody in either the IgG or IgM, and because of its sensitivity allows highly accurate assessment of the CSF/serum ratios of antibody.

Cell-Mediated Immunity. Cell-mediated immunity to viral antigens are studied in patients and controls using a lymphocyte stimulation assay. The CMI response to measles virus is also studied using purified viral antigens and macrophage inhibition assays. The responses of T lymphocytes and B lymphocytes are studied in these assays. These populations are prepared from peripheral blood using immunoabsorbant columns and rosetting methods. Studies have been initiated to investigate the function of T-cell subpopulations. These are obtained by fractionating T-cells on the basis of their capacity to react with Fc receptors of IgG and IgM.

Cerebrospinal Fluid. IgG, IgM and IgA levels are quantitated in each CSF by radioimmunoassay. CSF is also being examined for the presence of oligoclonal IgG in collaboration with the Infectious Diseases Branch. Further, preparative isoelectric focusing is being used to obtain immunoglobulins with restricted charge heterogeneity. The immunoglobulin in each fraction will be quantitated by RIA and antibody specificity studied.

Major Findings:

Results of Clinical Studies

1. A population of patients and controls who have been well-characterized clinically have been identified. Further, these individuals have been characterized with respect to their make-up at the A, B, and D loci of the major histocompatibility region. These individuals have been subsequently used for investigations of cellular immune function and of B-cell antigens. To date 32 pairs of twins who are either concordant or discordant for MS have also been investigated.

Studies of CMI function using a proliferative assay have shown a significantly lower response to measles virus as compared to mumps, rubella or vaccinia viruses. This has been found in both patients and controls and suggests that the normal immune response to measles differs from that to other common viruses. No significant difference in CMI has been seen between individuals with MS and controls. The lower cellular response to measles virus is in contrast to the antibody levels; both patients and controls have generally had higher antibody levels to measles virus than to mumps vaccinia. These suggest an inverse relationship may exist between the cellular and humoral immune status to these viruses.

Studies of purified T and B-cells show that T cells are the major responding cell to each of the viruses tested. There was no difference between controls and MS patients. Differences in the magnitude of the cellular response to these viruses did not correlate with histocompatibility background.

Two sera obtained from multiparous wives of patients with MS have proven effective for identification of antigens restricted to B-cells. One serum detects antigen in approximately 60% of MS patients and 30% of the controls. A second serum detects an antigen in 68% of MS patients and 7% of the controls. The majority of the MS patients who gave a typing response with this serum were DW2 positive while 3 of 4 DW2 controls did not react with this serum. Enriched fractions of T-cells and B-cells, prepared by anti-immunoglobulin absorbant columns, were examined by indirect immunofluorescence. The antigens detected by the absorbed maternal antisera were found to reside on B-cells, but not T-cells. These antigens are most likely related to or identical to the DRW antigens found on B-cells and coded for by genes in the major histocompatibility region. The correlation with disease provides further evidence of a genetic influence in MS.

Studies of serum and CSF immunoglobulins have focused on IgM, because of the important biological function of IgM. However, to date we have been unable to identify antibody associated with this immunoglobulin in the CSF of patients with MS. Isoelectric focusing studies continue

to show restricted heterogeneity of charge in both CSF and sera from not only SSPE, but also MS patients. Some, but not all, of the isoelectrically prepared immunoglobulin peaks co-migrated with oligoclonal bands demonstrable by agarose electrophoresis.

A major emphasis of the clinical research program has been the extensive study of monozygotic and dizygotic twins who are either concordant or discordant for Multiple Sclerosis. This work represents an extension of the investigations of familial MS and was undertaken to more precisely identify the importance of genetic and environmental factors in the etiology of MS. To date, three major findings have been noted. First, the number of concordant sets was greater among the monozygotic twins as compared to the dizygotic sets. Second, in a large number of the monozygotic clinically discordant twin sets, the clinically uninfected twin had abnormalities of CSF immunoglobulins. These abnormalities included increased content of one of the immunoglobulins, or oligoclonal banding patterns. Third, a significant number of twins lacking a history of neurological symptoms were found on neurological examination to have subtle but definite abnormalities. Together, these findings indicate a relative but important role for genetic make-up in susceptibility to MS. In addition, the incidence of clinical and CSF abnormalities in presumptive nonaffected twins is consistent with the concept of subclinical disease.

2. Findings in SSPE. Using the gel overlay technique described in Project #Z01 NS 02203-04 NI, 6 patients have been studied. Serum and CSF from these patients contain antibody against 5 of the 6 polypeptides of measles virus. Reactivity against the major glycoprotein was not detected, even though these sera contained high levels of HAI antibody. However, sera from rabbits and mice hyperimmunized with inactivated measles virus did bind this protein which is believed to be responsible for hemagglutination. These observations are consistent with the idea that multiple antigenic determinants exist on the glycoprotein and that the one related to hemagglutination is altered by treatment of the virus with sodium dodecyl sulfate (SDS). Other antigenic determinants which are not altered by SDS treatment stimulate antibody formation during hyperimmunization.

The antibody specificities of the SSPE patients did not differ from that seen in serum from normal individuals after acute measles infection. Of particular interest was that reactivity against the matrix protein was found. One contemporary theory concerning the pathogenesis of SSPE is that a mutation of virus genome coding for the matrix protein occurs and that this protein is not produced. The demonstration of antibody against this protein indicate that it is present and provides the antigenic stimulus.

In all cases of SSPE the CSF IgG is increased, but the levels of IgM and IgA varied. Oligoclonal IgG has been documented in the CSF.

Preparative isoelectric focusing has provided a means for obtaining populations of pure CSF immunoglobulins. The specificity of these is being examined.

Significance to Biomedical Research and the Program of the Institute:

The total effort in this project is directed toward the investigation of human diseases of the nervous system. Contributions from other basic projects within the Branch are applied to the study of clinical problems. The majority of the effort is aimed at understanding mechanisms involved in multiple sclerosis. This is a major health problem which affects young individuals at the prime of life. Over the past years a number of fragmentary bits of evidence, suggesting possible etiologies and factors contributing to pathogenesis has been suggested. The present investigation is aimed at intensive study of a small group of well-characterized patients. In addition, the use of families in which there is more than one case of multiple sclerosis and twins eliminates some of the variables encountered in studying sporadic cases. Although SSPE is a rare disorder, it is important because of the documented association between this disease and measles virus. Defining the parameters of the immune response to measles may not only provide insight of the pathogenesis of SSPE, but in addition this may provide important information about other disorders of unknown etiology.

Proposed Course:

The course of continued work on this project will be directed at establishing a more precise understanding of the findings described above. More specifically this work will focus on more clearly defining genetic make-up as it relates to susceptibility to MS and to undertake a more detailed examination of immune regulation in MS. These studies will continue to employ sporadic cases of MS but in addition will involve a more detailed examination of families and twins with MS. Further, a continuing effort will be made to integrate methods and approaches resulting from the nonclinical projects of this Branch into the clinical research.

Publications:

McFarland, H.F., and McFarlin, D.E.: Cellular immune response to measles, mumps and vaccinia viruses in multiple sclerosis. Ann. Neurol. (in press).

McFarlin, D.E., Mingioli, E.S., Behar, T.N., and McFarland, H.F.: Detection of B-cell antigens in multiple sclerosis: Use of sera from multiparous wives. Arch. Neurol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02203-04 NI
PERIOD COVERED October 1, 1978 to September 30, 1979		
TITLE OF PROJECT (80 characters or less) The Immune Response Against Membrane Antigens		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	J.F. Poduslo W.J. Bellini	Staff Fellow Staff Fellow
OTHER:	D.E. McFarlin H.F. McFarland A.G. Trudgett C.L. Koski	Chief Asst. Chief Visiting Assoc. Guest Worker
		NI NINCDS NI NINCDS NI NINCDS NI NINCDS NI NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Neuroimmunology		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.5	OTHER: 0.5
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<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>The goal of this project is to characterize the immune response to components of <u>myelin</u> and of <u>measles</u> virus. This study has focused on the composition of the outer membrane surface. One of the myelin <u>glycoproteins</u> has been partially purified and demonstrated to be antigenic in rabbits. An immune response against this surface component of myelin may contribute to immunopathology involving the nervous system. The measles membrane antigens are being characterized; immuno-reactivity against these components is being analyzed. This research uses pure virus which is being assessed chemically and immunologically.</p>		

Project Description:Objectives:

The surface components of the myelin sheath and the oligodendroglial plasma membrane should provide important clues in defining the alterations which occur in certain human nervous system disorders involving demyelination, such as multiple sclerosis. Such components may not only play important recognition roles in the process of myelination or myelin maintenance, but they may also be readily susceptible to immunological damage or even act as specific viral receptors. Consequently, the identification, isolation, and immunological characterization of these surface membrane components should permit elucidation of their role in the initial mechanism of both the myelinating and demyelinating process.

Several of the external surface components of this myelin sheath complex have been identified using the enzymatic membrane probe, galactose oxidase, on an intact spinal cord preparation. A major glycoprotein associated with central nervous system myelin was shown to have an external surface localization. This myelin-associated glycoprotein (MyGPI) has been isolated in a water-soluble form using a similar approach as developed by Marchesi and Andrews (*Science*, 174, 1247-1248 (1971)) for isolating the major glycoprotein of the human erythrocyte membrane. The immediate objectives of this project, therefore, are to demonstrate the immunogenicity of this glycoprotein in rabbits and define a membrane surface antigen associated with central nervous system myelin or the closely associated oligodendroglial plasma membrane.

Insertion of measles virus envelope proteins into the host cell plasma membrane occurs during the replication of the virus. Thus, the immune response to these surface components may constitute an especially important event with respect to susceptibility or resistance to disease. Furthermore, the immune response to individual viral proteins may vary such that one component may elicit an antibody response while another may stimulate T cells to produce a cell-mediated response. A major objective of this study is to assess the immune response to measles virus components. Animals with experimental measles infection and hyperimmunized with inactivated virus are being used to develop methods applicable to the study of normal individuals as well as patients with chronic diseases such as subacute sclerosing panencephalitis (SSPE) and multiple sclerosis (MS).

Methods Employed:

A. Membrane myelin

Lewis rat myelin was isolated according to the procedure of Norton and Poduslo from 18 day old rats after 48 h incorporation of 8.3 nmole (100 μ Ci per rat) of L-6-³H fucose (New England Nuclear) (intracranial

injection; 50 μ Ci per hemisphere). The radioactive fucose profile after separation of the myelin proteins by sodium dodecyl-sulfate polyacrylamide gel electrophoresis revealed at least 3 radioactive peaks. These quantitatively minor components of myelin have been partially characterized.

At least 94% of the fucose label was solubilized by using 0.1 M lithium diiodosalicylate as a membrane perturbant of the isolated myelin. Treatment of this extract with 50% phenol resulted in the selective partitioning into the aqueous phase of only MyGP1. The other myelin glycoproteins remain in the phenol phase along with the rest of the myelin proteins. The reasons for the selective partitioning of this glycoprotein into the aqueous phase are not clear although high levels of carbohydrate associated with the protein remains as a distinct possibility. This preparation of MyGP1 represented a 22-fold purification comparing the specific activities found in isolated myelin (58 cpm/ μ g total protein) with the isolated protein (1273 cpm/ μ g glycoprotein). The radioactive fucose profile indicated that a highly enriched preparation of MyGP1 has been obtained. This [3 H]fucose-labeled glycoprotein was used in a radioimmunoassay to quantitate antibody in rabbits immunized with this protein and with isolated Lewis rat myelin.

New Zealand white rabbits were injected with 100 μ g of MyGP1 in Freund's complete adjuvant (FCA) (2.5 mg Mycobacterium butyricum/ml). Animals were boosted 21 days later with 280 μ g of glycoprotein in incomplete Freund's adjuvant and bled at day 30. These animals showed no evidence of EAE at day 30. The capacity of the antisera to bind [3 H]fucose-MyGP1 was tested using a double antibody procedure with sheep anti-rabbit IgG as the second antibody. The antiserum to rabbit IgG, which was purified from rabbit γ -globulin protein by DEAE-cellulose, was produced in sheep by primary immunization in CFA followed by successive secondary immunizations in incomplete Freund's adjuvant. Equivalence was determined in this double-antibody system at each dilution of both the immune and normal sera. After incubation of normal and immune rabbit serum with the labeled MyGP1 for 1 h at 37 and 1 h at 4°C, an equivalent amount of the sheep anti-rabbit IgG was added. After an overnight incubation, the precipitate formed was washed 3 times with borate buffered saline (pH 8.0) and hydrolyzed for 30 min with 200 μ l 1 N NaOH. The radioactivity was then determined by liquid scintillation spectrometry to a 2-sigma counting error of $\pm 4\%$.

B. Measles virus membrane antigens

Plaque-purified Edmonston measles virus is grown in Vero cells to titers exceeding 1×10^7 PFU/ml. Supernatant virus is first concentrated by ultrafiltration and purified by two successive velocity sedimentations in sucrose. During virus replication in Vero cells, the various components of the virus may be specifically radiolabeled with nucleotides, sugars and/or amino acids depending upon the intent of the study.

The ability of the purified virus to evoke an antibody response in animals directed against the surface components of measles is assessed by conventional serology, such as hemagglutination inhibition, hemolysin inhibition and neutralization. In addition, a solid phase radioimmunoassay performed in polyacrylamide gels is used to assess the antibody response to the individual polypeptides of the virus, i.e., both surface and internal components. The latter method is also used to examine the antigenicity of the purified measles components.

Non-ionic detergent extraction methods have been successful in removing the major envelope antigens from the measles virion. The internal antigens or core proteins are then separated from the solubilized surface antigens by differential centrifugation. Envelope antigens are further purified by lectin-affinity chromatography which permits the purification of the major glycoprotein and fusion protein. Purity of the envelope and core constituents of the virus is determined by two dimensional electrophoresis consisting of isoelectric focusing in the first dimension followed by polyacrylamide gel electrophoresis in SDS in the second dimension.

Major Findings:

A. Myelin Membrane

A series of titration curves for the antiglycoprotein sera were made at several antigen concentrations. The ratio between two concentrations of antigen was 0.24 while the respective titration curves had a titer ratio at the 50% maximal binding level of 0.29 which indicated that the immune precipitation was proportional to the antigen in this concentration range. At a dilution of 1:213, 33% of the antigen was bound which represented an antigen binding capacity of 2.54 μ M. Absorption of this antiglycoprotein serum with brain or myelin significantly reduced the antigen binding capacity, although absorption with heart, liver, or thymocyte homogenates did not affect its capacity to bind the labeled antigen.

Rabbits were also injected with isolated Lewis rat myelin (440 μ g myelin protein) in CFA followed by successive secondary immunizations and bleedings. These rabbits developed EAE by day 12 and produced substantial levels of antibody to the glycoprotein. An experimental binding of 32 and 22% was obtained at a dilution of 1/10 for two different rabbits immunized with whole myelin, while an experimental binding of 66 and 59% was obtained at a similar dilution with rabbits immunized with the isolated glycoprotein preparation.

The present finding of antibody to MyGP1 in animals with EAE that were challenged with isolated myelin supports the possibility that the immune response to other antigenic components in nervous tissue may contribute to the pathogenesis of this experimental disease. It is well known that, on a dry weight basis, the potency of CNS tissue to induce

EAE in certain experimental situations is greater than basic protein itself or its encephalitogenic determinant. The presence of additional myelin-associated antigens operating in conjunction with the major encephalitogenic determinant of basic protein could account for the greater encephalitogenicity seen with whole tissue in this disease process.

B. Measles Membrane Antigens

A purification procedure for measles virus has been developed which maintains the infectious nature of the virus as well as the antigenicity of the viral polypeptide components. Between 2 and 3 mg of virus is routinely recovered from 3 to 4 liters of supernatant fluids.

The development of a solid phase RIA performed in polyacrylamide gels has made possible a study of the anti-measles polypeptide specificities of animal sera as well as patient sera and CSF. Briefly, measles virus proteins are separated by PAGE-SDS and the gels are fixed and washed to remove SDS. Gels are then overlaid with serum, CSF or lectins and following incubation, washed exhaustively. In the case of sera and CSF, iodinated anti-IgG or Fc is employed as the developing antiserum. Gels are washed exhaustively and autoradiographed.

Flyorographic studies using either ^{35}S -methionine, ^3H -leucine and/or ^3H glucosamine labeled virus indicate that purified virus is composed of 7 major structural polypeptides. To date, only a single glycoprotein has been identified ie., the HA or hemagglutinin. This has been confirmed using specific lectins in the gel overlay method.

Rabbit anti-measles sera react with all polypeptides of measles virus in the gel overlay procedure and give high titers in conventional serologic assays indicating that the purified virus retains immunogenicity.

A comparison of normal adult, convalescent and SSPE sera using the solid phase RIA indicate that all sera react with measles virus polypeptides. Although these sera vary with respect to the polypeptides recognized, no apparent differences in the general antibody response to measles has been observed.

Antibody binding in the gel overlay method is unquestionably specific, since whole cell lysates of persistently infected cells have been employed as antigen and only those polypeptides of measles virus have been found to react with anti-measles sera. SSPE sera do not react with any components of uninfected cell lysates.

One of the polypeptides of measles virus appears to be cellular actin with respect to isoelectric point and molecular weight determinations. However, immunologic reactivity with this polypeptide occurs only with infected cell lysates or with the viral polypeptide. This event remains

unexplained and is currently being studied. It may be that virus infection in some way alters the antigenic properties of actin. Alternatively, this protein may be a viral encoded antigen which co-migrates with actin.

Detergent treatment of intact virions in the presence of high salt (1M KCl) has led to the selective solubilization of the membrane antigens of measles virus. Two proteins have been found to be associated with the envelope: a) the glycosylated HA and b) the F_1 , non-glycosylated component of fusion protein. Variable amounts of the matrix protein and cellular actin have also been observed in this fraction. The majority of the latter components partition with the internal antigens or core proteins. Lectin affinity columns have been useful in the isolation of the HA protein of measles. Studies using iodinated membrane components indicate that F_1 component of fusion protein is also bound to the column. There are no data to indicate that this protein is glycosylated; however, a complex between F_1 and a smaller glycopeptide (F_2) has been suggested. Preliminary results using the gel overlay method of unreduced measles antigen with iodinated lectins indicate binding in the 60,000 dalton region consistent with the proposed F_1 - F_2 complex glycoprotein (F_0 or fusion protein).

Significance to Biomedical Research and the Program of the Institute:

The demonstration that a membrane surface glycoprotein associated with the myelin sheath complex is antigenic should provide a basis for investigating its precise cytochemical localization. In addition, antisera against MyGP1 can be used to study differentiation in the nervous system as well as provide the means for searching for the release of MyGP1 during the demyelinating process of certain human neurological diseases. Such an approach has recently been pursued with a less accessible component of myelin, the basic protein. The accessible, surface membrane, location of MyGP1 as well as its susceptibility to degradation after autolysis compared to other myelin proteins could provide a sensitive indication of disease activity.

The capacity for antibody production against this myelin-associated glycoprotein should facilitate further investigations of both fundamental neurobiological and pathogenic mechanisms operative in the nervous system.

The immune response to measles virus is of considerable importance because this virus is related to SSPE and has been implicated in MS. In addition widespread immunization with live virus is currently practiced nationwide. However, little is known about those components of measles that invoke a humoral and/or cellular immune response in man. Through examination and comparison of the immune response elicited in normal individuals with that of individuals suffering from measles-induced disease states, certain differences may appear which are important in

understanding the mechanisms leading to disease. Emphasis has been placed on membrane antigens due to the accessibility of these components to the immune system and the feasibility for these proteins to alter normal surface topography.

Proposed Course:

Future studies involve using antibody against the myelin glycoprotein to develop a radioimmunoassay for quantitating this component in tissue and spinal fluid. Other components of the myelin membrane will be analyzed using highly sensitive methods now being developed. Evidence of antibody against these components will be sought in human and experimental demyelinating disease. Antibody against the components of purified measles virion will be measured. Studies of cellular immunity will be expanded in experimental infections and human diseases. These efforts are integrated with projects Z01 NS 02202-04 NI and Z01 NS 02205-04 NI.

Publications:

Bellini, W.J., Trudgett, A., and McFarlin, D.E.: Purification of measles virus with preservation of infectivity and antigenicity. J. Gen. Virol. (in press).

Poduslo, J.F., and McFarlin, D.E.: Immunogenicity of a membrane surface glycoprotein associated with central nervous system myelin. Brain Res. 159: 234-238, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02204-04 NI												
PERIOD COVERED October 1, 1978 to September 30, 1979														
TITLE OF PROJECT (80 characters or less) Immunologic Mechanisms Operative in Experimental Allergic Encephalomyelitis (EAE) & Myasthenia Gravis (EAMG)														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">D.E. McFarlin</td> <td style="width: 20%;">Chief</td> <td style="width: 20%;">NI NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>J.F. Poduslo</td> <td>Staff Fellow</td> <td>NI NINCDS</td> </tr> <tr> <td></td> <td>A.M. Brown</td> <td>Guest Worker</td> <td>NI NINCDS</td> </tr> </table>			PI:	D.E. McFarlin	Chief	NI NINCDS	OTHER:	J.F. Poduslo	Staff Fellow	NI NINCDS		A.M. Brown	Guest Worker	NI NINCDS
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SUMMARY OF WORK (200 words or less - underline keywords) The aim of this project is to identify the relative role of various mechanisms operative in the production of <u>experimental allergic encephalomyelitis</u> , a model of autoimmune disease. The immune response to <u>myelin basic protein</u> is being assessed by measuring antibody and in vitro proliferative responses in various strains of mice. The chemical components of <u>myelin basic protein</u> responsible for disease production, <u>T-cell proliferation</u> , and reactivity with antibody are being studied. In all of these studies there is focus on the relationship to the host genetic background.														

Project Description:

Objective: EAE, a model autoimmune disease of the CNS, bears a number of clinical, histological, and immunological features similar to human demyelinating diseases and to chronic encephalitis. The object of this project is to delineate the mechanisms responsible for the pathogenesis of EAE. Susceptibility to EAE and the immune response to myelin basic protein (BP), the established encephalitogenic antigen in a number of species, is being studied in various strains of mice with different genetic backgrounds in order to elucidate the relationship between histocompatibility background and disease mechanisms.

Methods Employed:

a) In the past EAE has been studied in rats, guinea pigs, and mice. Investigations now in progress and future studies will be performed in the mouse because this species is well characterized immunologically and because other research in the Branch is being conducted in this species (Project Z01 NS 02205-04 NI). A number of inbred strains of mice are challenged with either whole central nervous tissue or BP in various adjuvants. BP from several sources is being employed. Clinical disease, CNS pathology, cell-mediated immunity and antibody formation are being evaluated.

b) Lymphocyte stimulation (LS) with mitogens and BP. Optimal culture conditions have been defined for these assays which are performed in conjunction with column separations and transfer experiments described below. LS responses of cells treated with specific antisera and complement are measured. Lymphoid cells from different sources have been studied. These include lymph node cells (LNC), spleen cells (SC), and peritoneal exudate cells (PETELS).

c) Macrophage migration inhibition. This assay is performed with peritoneal exudate macrophages incubated with antigens at various concentrations.

d) Separation of B lymphocytes on nylon wool columns and on an immunoabsorbant column with anti IgG coupled to Sephadex. Lymphoid cells are separated by these methods and characterized by FA, cytotoxicity, and LS as measured by thymidine incorporation.

e) Transfer of EAE with sensitized cells. LNC, SC and column-purified T-cells from these sources are evaluated for their ability to transfer EAE. The encephalitogenetic responses versus dose of transferred cells are determined by clinical and histological grading of the recipients.

f) Anti-BP antibody is measured by solid phase RIA.

Major Findings:

EAE can be induced by the injection of murine spinal cord in complete Freund's adjuvant followed by two boosts of *H. pertussis* given intravenously 1 and 3 days later. SJL, A.SW, DBA/1, B10.S animals develop severe clinical disease with associated CNS pathology. A.TH and A.TL mice show mild disease, primarily manifested by weight loss which is accompanied by low grade histological lesions. Balb/c and P/J are generally resistant but histological lesions are found in an occasional animal. No evidence of disease was seen in AKR and C57BL/10 animals. The disease developed earliest (11-13 days post-inoculation - PI) and was most intense in the SJL strain. It occurred later in DBA/1 and A.SW mice (14-18 days PI) and frequently was non-fatal. Morphologically, lesions were inflammatory and demyelination was not always prominent. There was a marked predilection for lesions to develop at spinal nerve root entry zones. Frequently, a generalized parenchymal response (edema and glial reactivity) was apparent together with some destruction of nerve fibers. EAE lesions in the mouse, in comparison to those in other species, display less demyelination. The explanation for this dissimilarity is not yet understood.

An important variable in the studies is the source and dose of the *H. pertussis*. It is not known whether the adjuvant exerts action on the immune system or by altering the permeability of the nervous system. Only mild disease has been produced by the administration of BP, and no difference in the encephalitogenicity of BP from various sources has been identified to date. The observation that BP is less encephalitogenic than whole spinal cord suggests that other antigens may be involved. For example, one of the myelin glycoproteins as discussed in Z01 NS 02203-04 NI. Alternatively determinants may exist on the BP molecule in situ which contribute to the immune response but are destroyed during the purification.

Investigation of the immune response to BP has shown that the presence and magnitude of antibody formation is related to a number of variables including the type of Mycobacteria used, the dose of Mycobacteria, the use of pertussis and the strain of mouse. The relationship of this response to the genetic background, and specifically to the H-2 haplotype, was investigated. H-2^K and H-2^D animals were found to be high responders with all types of adjuvants; H-2^S animals were intermediate responders; H-2^P, H-2^Q and H-2^B animals were poor responders, and H-2^D responded poorly with *M. butyricum*, but were intermediate responders with *M. tuberculosis*. These observations coupled with those on susceptibility indicate that production of antibody to BP and susceptibility to EAE may be dissociated and under the control of different genes. EAE is believed to be cell-mediated. Studies of the T-cell response to BP in the mouse have been initiated. Proliferate responses can be obtained with PETELS while only minimal responses can be elicited with LNC and SC. Macrophage migration inhibitory factor is produced when lymphoid cells from responder strains are incubated with BP.

Significance to Biomedical Research and the Program of the Institute:

A major function of the immune response is to provide protection against infectious agents. Similar mechanisms can lead to disease through either autoimmunity or immunopathologic reactions. It is becoming increasingly apparent the control and regulation of the immune response is complex and varies greatly. In the present project, experimental animals which can be controlled in regards to age, sex and genetic background and well-characterized antigens are being used to dissect various parameters of immune regulation which can lead to neurological disease. In addition to producing new information about pathogenesis of experimental disease, this project is providing background for the development of new approaches and techniques to study human diseases as outlined in other projects (Z01 NS 02202-04 NI).

Proposed Course:

Future studies will focus on prevention and modification of EAE and investigation of immuno-regulatory factors which influence the immunopathologic process. This will be analyzed in greater detail with routine histology, immunolabeling techniques and electron microscopy. Our studies to date have surveyed a number of mouse strains; future investigations will focus on selected strains which are high responders, low responders and those which develop EAE.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02205-04 NI																												
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TITLE OF PROJECT (80 characters or less) Interaction Between Viruses and the Host Immune-System																														
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SUMMARY OF WORK (200 words or less - underline keywords) <p>The purpose of this study is to examine the role of the host <u>immune response</u> in both <u>acute</u> and <u>chronic viral infections</u> of the central nervous system. These studies will examine the host immune response with relationship to mechanisms of protection as well as disease production in a viral-infected host. In addition, the effect of virus on the host immune response will be examined. This will include the effect of virus on the functional capacity of both T and B lymphocytes.</p>																														

Project Description:

Objective: This project is designed to examine the virus-host interaction of various models of virus-induced central nervous system disease. These studies are being focused on the role of several variables thought to be important in disease production. These include the biological properties of the virus, the immune response of the host to the virus and the influence of the genetic background of the host on disease susceptibility. In particular, the roles of these variables in establishing chronic viral infections of the CNS, as well as in control or potentiation of disease are being examined. These investigations are being performed in the mouse since the immunogenetic parameters of the host are well defined and easily manipulated.

Methods Employed:

A. Virus-induced CNS disease

Acute virus-induced disease of the CNS is being studied in animals infected with either mouse adapted measles virus or vesicular stomatitis virus (VSV). In addition, these viral infections are being used to study the mechanisms by which the acute disease can be modified to produce a subacute or chronic infection.

1. Measles virus. The hamster neurotrophic strain of measles virus adopted to the mouse is being used in these studies. This virus produces an acute CNS disease when inoculated IC into susceptible mice. The pathogenesis of this infection is being studied using the techniques of histopathology, immunofluorescence, and electron microscopic examination. Further, mechanisms of persistence are being examined in strains of mice which are less susceptible. Both susceptible and nonsusceptible animals are being examined with respect to their ability to support virus replication, and their ability to respond to the virus immunologically. Immunological studies include the determination of antibody using a radioimmunoassay and evaluation of the cellular immune response using a lymphoproliferative assay on virus infected monolayers. In addition, attempts to establish a cytolytic assay are underway.

2. VSV. Inoculation of animals with wild type VSV produces an acute disease of the CNS resulting in death in 2-3 days post inoculation. The mechanisms by which this acute infection can be modified are being examined in studies using the R_1 mutant of VSV. This mutant is not temperature sensitive and replicates readily both in vitro and in vivo. However, R_1 produces considerably less tissue damage than does WT VSV. This has been reported to be due to a mutation which eliminates the virus-induced shutdown of host cell protein, an event associated with virus-induced cell damage.

The neuropathological characteristics of the diseases produced by WT and R₁ viruses are being studied using routine histological technique, immunofluorescence, and electron microscopy. The nature of the immune response to these viruses is being carried out using techniques similar to those employed for measles virus. In addition the growth curves for both viruses is being studied in brain and spinal cord and the R₁ virus is being examined for reversion to WT in vivo.

B. Virus specific immune response

The immune response to measles and VSV is being studied as described above. In addition, the cellular and humoral response to measles virus is being studied using concentrated, purified Edmonston strain of measles virus. Following inoculation of animals with the purified virus, the T-cell reactivity is being examined using a lymphoproliferative assay and macrophage inhibiting factor (MIF) production. The humoral response is also being measured in these experiments using a RIA for measles virus. Further, the specificity of the antibody response with respect to measles virus polypeptides will be examined using methods described in project Z01 NS 02203-04 NI.

Major Findings:

A. Acute viral-induced disease.

1. Measles virus

Measles virus infection in susceptible Balb/c mice is characterized by a consistent pattern of clinical disease consisting of hyperactivity and death in approximately 10 days following inoculation. Viral antigen has been demonstrated in neurons throughout the brain although there is a preferential localization in the structures of the limbic system. Infectious virus cannot, however, be recovered. Further, little tissue damage is noted by light microscopy and no inflammatory reaction is seen. Electron microscopic examination of these animals in collaboration with Electron Microscopy Section (IDB) confirms the nonproductive nature of the infection since no assembled virus is found although abundant viral antigen is present in neurons. Of interest is the frequent localization of measles protein in the postsynaptic terminals. This has suggested that a functional impairment of synaptic transmission may be related to the pattern of clinical disease.

It has been further shown that the acute disease is less frequent in other mouse strains, such as SJL mice following IC inoculation with measles virus. Only a small number of SJL mice die during the acute period. Viral antigen is present although its appearance is delayed by a few days as compared to Balb/c mice. No apparent quantitative difference in the distribution, or nature of the viral antigen can be demonstrated between these two mouse strains. However, SJL mice have a mean onset

in clinical disease significantly later than the Balb/c. Further, a portion of the mice develop disease as late as 30-60 days with viral antigen persisting to that time.

Only a small amount of antibody is produced during the acute phase of the disease and none can be detected before two weeks in either strain. A quantitative difference in AB has not been identified.

2. VSV

The acute disease produced by WT VSV consists of death in 2-3 days post inoculation. Histological examination shows some necrosis and mild cellular infiltrate. In distinction, mice inoculated with R₁ mutant of VSV became ill 4-6 days after inoculation, the onset of disease is manifested by paralysis of the hind limbs in distinction to the general moribund state of animals inoculated with WT VSV. Death follows approximately 7 days post inoculation.

Immunofluorescent staining reveals considerable amounts of viral antigen in the spinal cord of the animals inoculated with R₁. This viral antigen is localized previously to the anterior horn cells associated with the hind limb paralysis. In addition a more substantial cellular infiltrate is seen. However, the disease does not appear to have a significant immunopathological component since it is not substantially modified by immunosuppression.

The immune response to the WT and R₁ viruses has been studied following intraperitoneal inoculation. Although both viruses elicit a substantial Ab response the magnitude of the response is somewhat greater using WT VSV.

Significance to Biomedical Research and the Program of the Institute:

The importance of genetic and immunological factors in susceptibility, potentiation or protection in viral infections is not well established. Further, although the properties of the infectious agent are certainly of major importance in determining the effect of infection on the host, the actual virological characteristics important in producing persistent infection in distinction to cell death are largely unknown. It is hoped that the studies outlined in this project will help to identify the host and virological factors involved in the production of chronic neurological diseases, such as Subacute Sclerosing Panencephalitis (SSPE) and possibly Multiple Sclerosis (MS) and Amyotrophic Lateral Sclerosis (ALS).

Proposed Course:

These studies will focus on the various host mechanisms responsible for persistence of virus and modification of acute viral infections

using the measles virus and VSV models. Major attention will be given to the role of immunological mechanisms in protection and disease production in these examples of persistent viral infections.

Publications:

None



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