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ANNUAL REPORT) Intramural research.

OCTOBER 1, 1977 THROUGH SEPTEMBER 30, 1978

OFFICE OF THE DIRECTOR

EXTRAMURAL ACTIVITIES PROGRAM

NEUROLOGICAL DISORDERS PROGRAM

STROKE AND TRAUMA PROGRAM

COMMUNICATIVE DISORDERS PROGRAM

FUNDAMENTAL NEUROSCIENCES PROGRAM

INTRAMURAL RESEARCH PROGRAM

NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE

ANNUAL REPORT

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INTRAMURAL PROJECTS

OFFICE OF THE DIRECTOR

Office of Program Planning and Evaluation

Office of Biometry and Epidemiology

NEUROLOGICAL DISORDERS PROGRAM

Developmental Neurology Branch

COMMUNICATIVE DISORDERS PROGRAM

INTRAMURAL RESEARCH PROGRAM

Office of Scientific Director

Medical Neurology Branch

Laboratory of Biophysics

NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE

ANNUAL REPORT

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I N T R A M U R A L

PROJECTS LISTED NUMERICALLY

CONTRACT NARRATIVES

OFFICE OF THE DIRECTOR

Office of Program Planning and Evaluation

Office of Biometry and Epidemiology

NEUROLOGICAL DISORDERS PROGRAM

Developmental Neurology Branch

Epilepsy Branch

STROKE AND TRAUMA PROGRAM

COMMUNICATIVE DISORDERS PROGRAM

FUNDAMENTAL NEUROSCIENCES PROGRAM

INTRAMURAL RESEARCH PROGRAM

Infectious Diseases Branch

Annual Report of the Director
of the
National Institute of Neurological and
Communicative Disorders and Stroke
October 1, 1977 through September 30, 1978

BUDGET

Fiscal year 1978 has been a year extraordinarily full of activity and variety for the NINCDS. This was the first year in the last five that there was a significant increase (about $20 million) in our appropriation. At the level of $177 million this still did not totally correct for the inflation over those five years; about $196 million would have been needed to accomplish that. But with the FY 1978 appropriation, the NINCDS has been able to fund well over 40 percent of the extramural research grants recommended by Study Sections for approval, in contrast to about 25 percent funding in the previous two years. Thus, at the end of FY 1978 the NINCDS is funding a total of 1294 research grants at some $122 million, 112 research contracts at some $18 million, 468 trainees (about one-half individual and the rest in 45 institutional awards) at $7.3 million, and Research Career Development Awards (97) and Teacher-Investigator Development Awards (37) at $4.4 million. The remaining $26 million in the FY 1978 budget went for direct operations (intramural research, program directions and NIH management funds). It is of interest that since 1970 the dollars invested in reserach grants have increased from about $49 million to more than $120 million but the numbers of grants have remained relative constant, because of the constantly rising direct and indirect costs per grant. At the same time the number of applications for the final Council session of the fiscal year has nearly doubled. Much of this reflects the growing research activities in the neurological and communicative sciences and disorders.

NATIONAL AND INTERNATIONAL ACTIVITIES

During FY 1978, the final reports of the Epilepsy and the Huntington's Disease commissions were submitted to the Congress and to the President. These were considered by the Congressional appropriations subcommittees (for the FY 1979 appropriation), and planning for implementation of recommendations directed to NINCDS has been initiated by our Neurological Disorders Program, which is responsible for both research areas.

Also during FY 1978 the NINCDS organized or supported a number of important workshops or conferences. These included such topics as: neuroepidemiology, criteria for Guillain-Barré syndrome, mechanisms of action of anticonvulsant drugs, autosomal dominant genetic disorders of the nervous system, language disorders in children, evaluation of the feasibility study of tonsillectomy and adenoidectomy, atypical slow viruses, Alzheimer's disease-senile dementia and related disorders, and Reye's syndrome. Some of these were international, and several were

supported in collaboration with other NIH institutes or other organizations. One was characterized as a consensus exercise (the T and A conference) but in fact most of the meetings could have been so considered. The slow virus workshop marked the occasion of opening the new laboratory and animal holding facilities at the Frederick Cancer Research Center.

The NINCDS Office of Biometry and Epidemiology (OBE) reported good progress in evaluating its probability sampling surveys on incidence, prevalence and costs of brain tumors, stroke, head and spinal cord injuries, multiple sclerosis and epilepsy (the last two still to be completed). In addition the epidemiological survey of neurological disorders in Copiah County, Mississippi, and nationally in conjunction with the National Center for Health Statistics both continue. A new initiative involves feasibility studies to establish stroke and trauma data banks for development of prognostic patient profiles. These OBE activities are especially important in providing us with more accurate dimensions of various disease problems as a basis for more intelligent planning and allocations of research resources.

It was an active year for the NINCDS in its role as one of the eight WHO Collaborating Centers for Research and Training in the Neurosciences. Several of the staff concerned with this program attended the stroke symposium and the following fifth consultation in Firenze, Italy; a special consultation in Montreal, Canada, at the occasion of the Third Foundation of the Montreal Neurological Institute; and special courses given at Marseille, France and at Lima, Peru. In addition the NINCDS Director attended by invitation the annual meeting of the WHO Advisory Committee on Medical Research (ACMR) at Geneva in June, in order to summarize the Collaborating Centers program for the ACMR. As a result of recommendations made to WHO by the ACMR it is likely that the name of the program will be changed from neurosciences to neurological disorders, that it will be shifted administratively from the WHO Division of Mental Health to the office of the WHO Director-General, and that more core support and staffing for the program may be forthcoming from WHO. The NINCDS-FIC sponsored fellowships for this WHO program have finally been implemented. The first two fellows (one from Nigeria and one from the Philippines) were selected at the Firenze consultation and two more (one from Mexico and one from Sri Lanka) were selected at the Montreal consultation. Once these choices have been processed by the Fogarty International Center the chosen fellows will come to NINCDS, Bethesda, to start their fellowships.

PERSONNEL

Fiscal year 1978 saw some very major changes in key staff positions at NINCDS. Four out of the five directors of extramural programs were lost during FY 1978 and there are indications that the fifth will retire in FY 1979. Dr. Wesley Bradley, director of the Communicative Disorders Program (CDP), resigned to return to academic clinical practice after very ably launching this program three years ago and leaving it a well staffed, mature and effective operation. Dr. Karl Frank, director of the Fundamental Neurosciences Program (FNP) retired after a long and distinguished NIH career as researcher, intramural laboratory chief and

scientific director, and finally founder and architect of the FNP extramurally. Search committees for replacement for the CDP and FNP director are already at work. Dr. Murray Goldstein, director of the Stroke and Trauma Program (STP), has been transferred to the post of Deputy Director of NINCDS for Neurological Sciences and Disorders. Until a search committee can identify a new director for STP, Dr. Goldstein continues as acting director of that program. Simultaneously Dr. Eldon Eagles was assigned to be Deputy Director of NINCDS for Communicative Sciences and Disorders. These changes at the deputy director level reflect in part Dr. Eagles' long illness and convalescence from major surgery but more significantly the formidable escalation in work load and demands on the time of the Director, NINCDS, and his immediate staff.

Finally, the tragic death from recurrent cancer of the director of the Extramural Activities Program (EAP), Dr. Kenneth Hisaoka, after less than a year in that position, necessitated search for a replacement for this key post. The NINCDS is fortunate in having recruited Dr. John Dalton, a seasoned top-level scientist administrator in NIGMS, as the new director of EAP. Coincidentally with this and the other replacements, a number of key staff changes have taken place. The increasing work loads in the various program areas have necessitated the appointments of deputy directors (Drs. Ranney-CDP; Streicher-FNP; Brewer-STP; and Bick-NDP), and in EAP a new grants management officer (Mr. Ed Donohue), a new acting chief of the Scientific Evaluation Branch (Dr. John Diggs), and new analysis for classification and budget (Mrs. Charlotte Karel and Mrs. Mary Layman) were installed.

In the OD-NINCDS the retirements of Mr. Robert Sithens (budget) and Mrs. Ruth Dudley (scientific and health reports [OSHR]) necessitated recruitments for these two vital positions. Both retirees left after long and devoted service to the NINCDS. Mr. William Matthews is serving as budget officer, and Miss Sylvia Shaffer has been recruited from the USN Bureau of Medicine and Surgery as the new chief of OSHR. In addition two of the senior NINCDS administrative officers (Glenn Hammond from intramural and George Durall from extramural) retired during the year.

One major recruitment to the Intramural Program (IRP) was achieved in FY 1978. Dr. Paul Kornblith from the neurosurgical department at the Massachusetts General Hospital has accepted the post of chief of the Surgical Neurology Branch, IRP, and brings with him two neurosurgical colleagues, Drs. Barry Smith and Eugene Quindlen, to initiate an exciting program of brain tumor immunochemistry and chemotherapy utilizing novel tissue culture techniques. These appointments culminate a search by a distinguished external committee that has taken more than three years. The search continues for a new chief of the Laboratory of Neurophysiolgy, and a search is being initiated for a new chief of the Medical Neurology Branch to replace Dr. W. King Engel who was relieved of his administrative responsibilities to devote full time to research as chief of the Section on Neuromuscular Diseases.

The number and importance of these personnel actions and shifts warrant two general considerations. First, it is worth noting that despite so

many key turnovers or vacancies, program stability and progress have been maintained--a distinct tribute to the dedication of continuing staff and a good test of organizational soundness. The second consideration is the alarming increase in the inertia of the personnel recruitment and processing system coupled with the disturbing trends in classification downgrading. Recruitment is difficult at best, in view of the salary differentials and other such inherent problems. But when ground rules for searches are changed by the Civil Service Commission (CSC) in mid-course, and when the processing of appointments take 4 to 6 months to get through the system (because of more rule changes and preoccupation with grammatical and semantic minutiae), the ability of NIH and its components to recruit and retain the requisite scientific and managerial expertise is in deep jeopardy. Now we are confronted with civil service reform and the senior executive service provisions thereof, which are likely to further compound these problems. I view the area of personnel management and services as one of the most serious problems with which all of us at the NIH must cope.

PLANNING ACTIVITIES

Three other programmatic activities deserve mention here. In the area of equal employment opportunity (EEO), the NINCDS is fortunate to have dedicated and effective coordinator, counsellor and advisory committee personnel. During this year the NINCDS joined the Minority Biomedical Support program administered by DRR. A major continuing concern is the percentage of minority and women members of Institute staff and advisory groups. Progress is being made but it is obvious that we are already exceeding the overall minority and women distributions at the NIH and in the pools of scientists from which we must recruit. Thus, the competition for any available minority or women scientists tends to be self-defeating. We can document a number of cases in which eligible women scientists have refused to serve because they are already overloaded. If one adds the problems of recruitment and processing by personnel or by the DHEW Special Projects Section, it is not surprising that some of our principal EEO objectives are not being met.

A second area is the activities of the NINCDS Office of Scientific and Health Reports (OSHR). Amongst its traditional activities the OSHR handled 1064 public inquiries, 268 White House and Congressional written inquiries, and 130 freedom-of-information requests, and distributed more than 350,000 publications, primarily to lay requests. With the advent of a new OSHR chief and some new staff, new informational activities have been added. NINCDS "Notes" in such journals as the Annals of Neurology are now published monthly to keep our scientific research professionals abreast of current programmatic developments. A variety of media interfaces (newspapers, news magazines, radio and television) have been successfully developed. Our Scientific Staff have collaborated to facilitate these approaches to informing the public about our research activities and progress.

Finally, FY 1978 saw the NINCDS initiate the process of developing long-range strategic planning for the next 5 to 10 years. Under the supervision of a four-man subcommittee of the NANCDS Council and staff

resources from our Office of Program Planning and Evaluation (OPPE), 7 panels and 2 task forces of external experts have spent the last six months of FY 1978 in assessing the current status of research and research problems across the spectrum of the NINCDS areas of mission responsibilities; evaluating the incidence, prevalence and socioeconomic impacts of the neurological and communicative disorders; and recommending future directions, priorities and necessary resources. Special input was obtained from 44 voluntary health agencies and 35 professional societies at a public forum held here in June. By the end of FY 1978 draft reports from all panels and task forces had been submitted to the NANCDS Council for review. It is anticipated that final editing and preparation of a consolidated summary volume and an overview digest will be completed or in final draft by February 1979. Both staff and advisory Council regard this undertaking as timely and essential for effective investment of future resources.

EXTRAMURAL PROGRAMS

Turning now to research accomplishments during FY 1978, each of the extramural programs deserve some specific comments. At least 60 percent of all NINCDS dollars and 70 percent or more of NINCDS grants are in basic research. Thus, each of the four extramural grant and contract programs contains a large measure of basic research projects. Those that are very fundamental and of wide relevance are the responsibility of the Fundamental Neurosciences Program (FNP), which administers 350 regular research grants, 9 program project grants and 22 RCDA's, representing together 26 percent of the NINCDS total or in dollars 19 percent of the total at $21 million. One characteristic of this research portfolio is the generally high quality (in terms of study section priority scores) of the grant applications--a characteristic which accounts for the need for more monies to meet any given pay-line relative to the other extramural programs. The FNP has two special program areas: one, the neural prosthesis program which in 14 research contracts accounts for 10 percent of the FNP budget--an example is the currently very promising development of a urinary bladder prosthesis; the other special area consists of two information activities, the Neurosciences Research Program (a Seminar-workshop type of informational activity) and the Brain Information Service (primarily an alerting bulletin service). At Senate request, the latter is currently under critical review by the National Library of Medicine and the NINCDS.

The Stroke and Trauma Program (STP) is in contrast a highly clinically-oriented program, funding 14 clinical research centers and 50 research grants in stroke, 5 clinical research centers and 20 research grants in acute spinal cord trauma, and 6 clinical research centers and 14 research grants in acute head tramua. Yet each of the center grants includes a significant percentage of basic research projects, and in the special area of central (especially spinal) regeneration the STP now supports about 60 research grants--a very active field indeed. The STP is engaged in several important new initiatives: One is a multi-institutional prospective randomized controlled clinical trial of the efficacy of the extracranial-to-intracranial arterial anastomosis for preventing transient ischemic attacks (TIA's) and subsequent stroke--more than 30 centers in

North America, Europe and Japan are enrolled in this study. Similarly, the 5 spinal cord injury clinical research centers have embarked on a cooperative randomized prospective clinical trial of the efficacy of high-dose steroid therapy in acute spinal cord injury. Comprehensive center programs for stroke (6 feasibility studies completed; 3 centers awarded) and for CNS trauma (still at the feasibility stage) have been initiated to deal with the interface between research centers and community health care, including the epidemiological and rehabilitation parameters. The development of stroke and tramua computerized data banks to provide prognostic and therapeutic profiles for patients may be expected to complement these comprehensive center projects. Finally, through the STP the NINCDS in consultation with its national advisory Council has begun the preliminary planning for undertaking a special initiative in the new research technique of positron emission transverse tomography (PETT). This technique represents the metabolic and functional analogy of the CAT scanning techniques for visualizing brain and cord morphology and pathology in the living animal or human subject in situ. PETT utilizes the short-lived positron-emitting isotopes ^{15}O, ^{13}N, ^{11}C, ^{18}F, etc. produced on location by cyclotron and incorporated into such metabolites as O_2, CO_2, NH_3, CO, glucose and 2-deoxyglucose or drugs, receptor ligands, immunopeptides, and the like for metabolic mapping of the living brain in situ. The prospects for this research initiative are exciting indeed.

Despite some changes in key personnel, the Communicative Disorders Program (CDP) has continued its excellent progress. Their annual report provides a very comprehensive picture of the types of grant and contract projects being supported. Over one-half of the CDP funds support research on hearing (44%) and diseases of the ear, nose and throat (12%), with much smaller allocations for research on the vestibular system (7%), speech and voice (13%), language (4%), etc. Thus, there is still much to be done in stimulating research and research training in these areas. The situation demands special attention because, in terms of afflicted patients, there are greater numbers with disorders of hearing, speech and language than all the neurological disorders combined. Three special items deserve notice: The multiple electrode arrays developed under contract for auditory or cochlear prostheses are now ready for experimental trials. A literature retrieval system for investigators in the communicative sciences and disorders has been under development by the CDP staff, a special group of consultants, and the NLM-MEDLARS staff. This system is now ready for field testing, and we will be watching the testing with particular interest, since it seems to hold much greater potential for research information needs than the more traditional approaches which NINCDS has supported over the last decade. Finally, the CDP staff have continued to be active in on-campus projects at the NIH Clinical Center, involving audiological services, clinical research studies in speech pathology (e.g. speech correlates of neuropharmacological studies on patients with Parkinson's and Huntington's diseases, Gilles de la Tourette's syndrome, etc.), and otological studies in collaboration with NCI concerned with ototoxicity of a variety of cancer chemotherapy agents. These Clinical Center projects and services clearly suggest the need for a communicative disorders branch within the NINCDS Intramural Research Program. Planning

for such a branch has been initiated by the CDP staff for inauguration when the Ambulatory Care Facility of the NIH Clinical Center opens several years hence.

Of the four extramural programs, the Neurological Disorders Program (NDP) is the largest accounting for 570 research grants, program project and center grants at some $44 million, plus a research contract program of nearly $8 million. Responsibility for implementation of the recommendations of the Epilepsy and the Huntington's Disease Commissions rests with the NDP. These two commissions completed and submitted their reports during FY 1978, so that NDP staff has devoted considerble time to analysis of the reports and recommendations in anticipation of implementing a number of the recommendations in FY 1979. After a long search the NDP has recruited a neurologist-neuropathologist to head the NDP branch on multiple sclerosis and other demyelinating and sclerosing disorders. The new branch chief is expected to report in FY 1979. Similar recruitment for staffing the branch on degenerative disorders and dementias of the aging nervous system is well under way. The comprehensive epilepsy centers continue to show good progress, with three of the five reviewed this year and extended for another two years. The epilepsy drug screening program is active and received a special boost by the FDA approval for marketing of an effective new anticonvulsant, sodium valproate. In other areas attention is being given to special initiatives in peripheral neuropathies (notably funding of a clinical research center to study diabetic neuropathy), in planning for greater emphasis on neuroendocrine research (including the very active area of peptide neurohormones, neurotransmitters and neuromodulators), and in problems of pain (including headache) and aging (notably the dementias). It is now 20 years since the Collaborative Perinatal Project began, and now it is almost terminated as the analyses and monograph reports near completion (mostly in FY 1979). Thus, the group was reorganized during this year into the NDP Developmental Neurology Branch, with retention of only selected staff members and redirection of program emphases into four principal areas: mental retardation, cerebral palsy, autism, and CNS birth defects.

INTRAMURAL PROGRAMS

The NINCDS Intramural Research Program (IRP) represents a major research activity in its own right. It presently comprises 16 research laboratories and branches on the NIH campus, at the Frederick Cancer Research Center, at the Marine Biological Laboratories at Woods Hole, Mass., and on the island of Guam in the western Pacific. The IRP has 143 current research projects, generating in FY 1978 some 250 research reports in 131 different scientific journals and books. The current mix of research comprises 51% basic research projects, 42% clinical research projects, and 7% applied studies (clinical trials). The IRP commands 295 of the total 538 permanent full-time personnel slots allocated to the NINCDS, plus most of the 143 personnel not occupying slots (guest workers, visiting fellows, etc.). Slots and the tenure problem (relatively little turnover) represent one of the major constraints to taking full advantage of or initiating new initiatives in such areas as molecular virology, neuroendocrinology, neurotoxicology and communicative disorders. The need for personnel flexibility is acute. The other major constraint continues to be the inadequate number of

inpatient beds, which have remained at the original total of 52 beds assigned in 1954, despite the many research and programmatic advances that have occurred in the intervening 24 years. This is clearly unrealistic and grossly unfair.

Despite the many constraints in terms of resources, the NINCDS Intramural Research Program is, in my opinion, an outstanding research endeavor. In addition to our Nobel laureate, there is a sizable group of investigators whom I consider remarkably gifted and working at the very frontiers of some of the best and most promising areas of research on the nervous system. The NINCDS can be very proud of its intramural scientists and their research, much of it uniquely innovative, as it should be in an NIH intramural research setting.

It is not possible to comment comprehensively on the IRP research progress during FY 1978. The accompanying summaries by the laboratory and branch chiefs and the individual project reports must be consulted for the details. Here I can only select some which particularly appealed to me or seemed to me of unusual significance. The selections carry no priority implications.

RECEPTORS TRANSMITTERS AND MOTOR SYSTEMS

Receptors are very much in the research news these days. Two studies from the IRP are pertinent here. Neuropharmacological data from the Experimental Therapeutics Branch establish the presence in brain of multiple dopamine receptors. Biochemically these receptors are of two types: alpha, whose reactivity is unrelated to adenyl cyclase, and beta, coupled to the adenyl cyclase-cyclic AMP transduction system. By neuropharmacological criteria there are three classes of alpha and two classes of beta dopamine receptors, or a total of 5 distinct receptors. For example the presynaptic autoreceptors regulate the activity of tyrosine hydroxylase (a key enzyme in dopamine synthesis) in the nerve terminals, and these autoreceptors, which are of the alpha type differ from the alpha receptors in the anterior pituitary: ergot derivatives act on the latter but not on the former, which are inhibited by apomorphine. Studies in the Laboratory of Molecular Biology have demonstrated that beta-adrenergic receptors can be induced in Hela cells in culture by exposure to butyrate. Detailed examination of this phenomenon has identified four components in the receptor system: the receptor protein, a catalytic subunit of adenylyl cyclase, a GTP binding subunit, and a putative coupling factor. In parallel work the Experimental Therapeutic Branch has identified GTP as the endogenous co-factor required for linkage or coupling of beta-dopaminergic or beta-adrenergic receptors to adenylyl cyclase. The effect of GTP is on the coupling and not on the receptor itself, and thus this seems to provide a mechanism for controlling receptor sensitivity.

The roles of neuropeptides (e.g. enkephalins) and the peptide (opiate) receptors in brain and spinal cord continue to be studied in the Laboratory of Neurophysiology. Three types of neuropeptide actions have been distinguished: neurohormonal and neurotransmitter actions, plus neuromodulation, so that a variety of actions ranging from abrupt depolarization to

depression of excitability and elevation of spike threshold may be encountered. These findings have prompted the investigators to draw the following analogies. Neurohormones can be likened to radio waves broadcast widely and received by those neurons with receptors tuned specifically to the structural characteristics of the neurohormone. Neurotransmitters subserve direct neuron-to-neuron communications like a private telephone conversation. Neuromodulators can be thought of as altering the gain (amplitude) or the sensitivity of such conversations. What needs to be ascertained is whether the same peptide molecule may exhibit all three properties depending upon local circumstances. In any case, these observations greatly expand the mechanisms for the modulation of central nervous system activity and hence the complexity and sophistication of its functions.

The use of special freeze-fracture techniques with a time resolution of 2 milliseconds has allowed electron microscopic study of the details of the synaptic release of transmitters, as reported by scientists in the Laboratory of Neuroanatomical and Neuropathological Sciences. Thus, each quantal event in presynaptic transmitter release results from the fusion of a single synaptic vesicle with the presynaptic plasmalemma. In less than 100 milliseconds the fusion is complete and the vesicle particles aggregate for reincorporation into new synaptic vesicles. By using amoebocytes from Limulus, in which bacterial endotoxin can be used to induce vesicle exocytosis, the very initial event in vesicle exocytosis could be visualized as a puckering of the plasmalemma and the development of a tiny hole through it into the subjacent vesicle. Thus the initial fusion is a very local disruption. This extraordinary technological tour-de-force makes it possible to correlate the most intimate details of molecular morphology with unitary physiological events, and hence to provide means for us to understand precisely the roles of calcium ions, which lies at the heart of neuron-to-neuron communications in the brain and spinal cord.

Additional transmitters are being added to the existing roster. A notable example comes from the studies on the cochlear nucleus and auditory nerve in the Laboratory of Neuro-otolaryngology. The receptors for cochlear nucleus efferent fibers are clearly cholinergic, as ascertained by studies with alpha-bungarotoxin, but the auditory nerve synapses--the input to the cochlear nucleus--are presumably served by glutamic (or aspartic) acid as transmitter. This conclusion is based on direct observation of changes in levels on denervation and of release on stimulation, as well as the effects of kainic acid, a putative neurotoxin for neurons with glutamatergic input. These auditory nerve fibers originate from the cochlear hair cells, which accomplish the mechanotransduction process from sound-generated fluid waves in the cochlear endolymph to coded nerve signals in the auditory nerve. Studies on the statocyst in the primitive nervous system of the marine worm, Hermissenda, are contributing to our understanding of how this transduction process takes place. Scientists in the Laboratory of Biophysics have been able to visualize the moving hairs on the statocyst hair cells in sufficient resolution to permit them to quantify the frequency of movement with a stroboscope. They find a strict correlation between the frequency of

hair movement with hair cell voltage noise and generator potentials, data which they believe will permit them to develop a conceptual model of mechanotransduction. The importance of such studies can be appreciated from the fact that such knowledge is the key to our understanding of sensori-neural deafness.

Other innovative recording techniques come from the Laboratory of Neural Control. There investigators have pioneered in recording from awake, free-ranging cats or monkeys such activities as dorsal root ganglion potentials (the sensory input to the spinal cord), skin stimulation, muscle spindle afferents, joint activity, and the like. Such kinesiological data on limb position, joint angles, muscle lengths and forces produced by individual muscles are providing insights into normal mechanisms and control of motor activity and organization that have not only been impossible heretofore but are generally unanticipated. Where the nerve supply to skeletal muscles has been interrupted, problems of regeneration of connections occupy the attention of many neuroscientists. Studies with nerve allografts being conducted by Laboratory of Neurochemistry scientists are particularly provocative in this context. These investigators have found that nerve allografts containing only minor tissue antigens will survive until the host cells can regenerate through the graft, whereas skin or ganglion allografts from the same donor animal are promptly rejected. Thus, it appears that nerve allografts bearing only minor antigens can rapidly and functionally repair large injuries to peripheral nerve in animals with no immunosuppression measures. The applicability of this approach to human situations and to central (spinal cord) regeneration is under evaluation.

NEUROVIROLOGY AND NEUROIMMUNOLOGY

A great deal of the research in the NINCDS-IRP is concerned with virological and immunological aspects of the nervous system and its disorders. Special interest continues to be focussed on the atypical slow virus agents responsible for the transmissable spongioform encephalopathies of kuru, Creutzfeldt-Jakob disease, scrapie and transmissable mink encephalopathy. At the Institute-sponsored workshop on these disorders,there was a consensus that the atypical slow virus agents are indeed a new kind of microorganism, and there is very preliminary evidence to suggest that they may contain a low molecular weight DNA intimately associated with the host cell plasma membrane. In the Institute Laboratory of Central Nervous System Studies these agents have been shown to cause cell fusion in tissue culture, and although the effect is not specific enough to be of diagnostic use, it does provide a rapid assay procedure--3 weeks for the cell growth test or 2-3 days for histological examination versus 6 to 8 months for mouse titration in vivo. The corneal mode of transmission--from an infected donor to recipient--has been confirmed (and incidentally, the Infectious Disease Branch has verified a comparable corneal transplant transmission of rabies). Moreover, the transmission of Creutzfeldt-Jakob disease by EEG depth electrodes ("sterilized" in formaldehyde) from a known infected patient to several subsequent human subjects and experimentally to primates has now been fully verified. These observations reinforce concern for adequate

precautions in the handling of patients and tissues. During FY 1978 the NINCDS laboratory conducted epidemiological studies of the prevalence of and mortality from Creutzfeldt-Jakob disease. In the United States the annual mortality is 0.26/1 million population. A very thorough study of prevalence was carried out in France, where every neurologist and many more physicians in the entire country were personally visited. The data collected provide very accurate statistics, with a mortality of 0.32/1 million which is approximately the annual incidence. For a dense urban area like Paris, the annual mortality is 1.33/1 million. About 15% of the cases were familial.

Studies of obvious relevance are being conducted in the Laboratory of Molecular Biology on defective infectious (DI) particles, using the experimental-animal vesicular stomatitis virus (VSV). This virus selectively infects neurons and not glial or fibroblast cells. NINCDS scientists have established the RNA sequence and genic localizations for this virus. They find that all types of DI particles share the same nucleotide sequence at the 3'-end of the viral genome, and this sequence competes favorably with the viral genome for the replicase binding site. It is this competition that accounts for the autointerference by the DI particles. Such studies are vitally important to our understanding of the general problems of slow, latent and defective viruses in the mammalian central nervous system. One case in point is the recent demonstration of the development of glioblastomas in the brains of 2 out of 4 owl monkeys inoculated with the JC-strain of papova virus isolated from a patient with progressive multifocal leukoencephalopathy. The experimentally-induced tumors contained "T" antigens but no demonstrable infectious virus or virion antigens in tumor or cultured tumor cells. These studies were done by scientists in the Infectious Diseases Branch in collaboration with colleagues at the University of Wisconsin.

Multiple sclerosis is probably one of the most central problems in neurovirology and neuroimmunology. With the great impetus given to MS research by the MS Commission and the research initiatives fostered by the NINCDS, a number of purported diagnostic tests or transmissable agents are being reported. Each of these require confirmation, and the Infectious Disease Branch has played a major role in studies seeking to confirm such claims. Unfortunately, to date, none of the claims could be substantiated by the careful repetitions of the original studies. Despite the negative nature of these evaluations, it is an especially important and responsible contribution in an area of research where false hopes can be so easily fostered. On the other hand research in the Neuro-immunology Branch has demonstrated that blood serum from selected multiparous wives of MS patients can be used in a cytotoxic assay for the detection of B-lymphocyte antigens. These special sera detect an antigen with considerable specificity for MS and this antigen may be related to the HLA-DW2 haplotype present in the lymphocytes of 68% of MS patients in the United States. We are still a long way from understanding the significance or even the clinical utility of such observations, but progress is indeed being made in this most complex and difficult interface between genetic and autoimmune phenomena.

Progress is also being made in the related areas of specific antigens

to nerve tissue components which may be of diagnostic significance and/or responsible for the autoimmune phenomena in MS. Thus, for example Neuroimmunology Branch scientists in collaboration with colleagues at Emory Medical School have shown conclusively that the encephalitogenic activity of myelin basic protein is localized to the 19 amino-acid fragment at residues 68 to 88 in the protein and that substitution of a serine for a threonine residue at residue 79 markedly enhances the activity. Thus, substitution of a single amino acid in this peptide results in a stimulation of a greater number of T-lymphocytes involved in the induction of experimental allergic encephalomyelitis, in the in vitro proliferative response and in helper function in antibody formation.

In parallel collaborative studies by four IRP laboratories (Developmental and Metabolic Neurology Branch, Laboratory of Neuroanatomical and Neuropathological Sciences Infectious Diseases Branch, and Neuroimmunology Branch), both the myelin basic protein and the myelin associated glycoprotein have been studied in the MS context. With antibodies to myelin basic protein and new histological fixation techniques, the presence of this protein has been demonstrated in oligodendroglia before the myelin sheath is detectable histologically or biochemically, and the changes in distribution of the protein can be followed during maturation of the myelin sheath. Similar studies with antibodies to the myelin-associated glycoprotein show a particular localization to the myelin at the axolemmal interface. In demyelination these techniques demonstrate abnormalities well beyond the definable limits of the MS plaque. The new techniques permit study of paraffin-embedded tissue sections and are applicable to human material. Since the MS patients exhibit in cerebrospinal fluid oligoclonal bands of IgG (in 80% of patients), elevated IgM and detectable antibodies to myelin basic protein (and to myelin associated glycoprotein in experimental animal models), the foregoing studies are highly relevant to the etiology and detection of MS.

Associated studies by these same groups of measles antigens and measles virus-host interactions are also relevant. By electron microscopy, the measles specific antigen in infected neurons has been localized to neuronal cytoplasm, dendrites and post-synaptic receptor areas. In the acute measles encephalopathy there is no inflammatory response, so that the underlying pathophysiology may be a virus-induced dysfunction of synaptic transmission. After treatment with measles antibody there is redistribution and clearing of the antigen-antibody complex, a remodelling of the cell surface and synthesis of new measles antigen. In chronic infections the animals usually develop a subacute disease after 2 to 6 months, with paravascular inflammatory response, detectable virus and a high antibody titer (in contrast to no detectable antibodies in the acute disease). A technique has now been developed permitting isolation of 3 to 5 mg of pure measles virus from 3 liters of supernatant fluid from infected cells, with retention of 70% of the original infectivity. Such preparations permit isolation and immunological studies of the 6 structural proteins, the matrix protein and glycoprotein of the virus. The latter two are both antigenic and react with all 6 structural proteins. These specific measles viral proteins are now being used to search for antibodies in patients with MS or with subacute sclerosing

panencephalitis (due to a defective measles virus).

CONCLUSION

From all the foregoing examples of research in the IRP of NINCDS during FY 1978 it is obvious that our IRP is very much a microcosm of the country-wide macrocosm of research on the neurological and communicative disorders. I would particularly stress the neurovirological and neuroimmunological studies, utilizing tissue culture, electron microscopy, immunochemical and related techniques of the most sophisticated and advanced nature. These areas of research promise to inform us richly about the reactions of the central nervous system to viral infections and the perturbations of its immune competence. Finally, it is obvious that almost every example IRP research depends on novel, innovative and advanced technologies. Yet the techniques have been resorted to to solve problems and to test hypotheses rather than the obverse. The NINCDS is particularly fortunate in its intramural research staff and the array of major research contributions for which they are responsible.

In concluding this report, I say again that FY 1978 has been a year extraordinarily full of activity and variety and accomplishments for the NINCDS. With the prospect of a Congressional appropriation of $212 million for 1979, a $35 million increase over FY 1978, the NINCDS looks forward to being able to fund more research and to encourage new initiatives that will bring us all closer to a full understanding of the nervous system and its disorders. Clearly the health and quality of life of this country and of the world depend on a healthy nervous system. And such a healthy nervous system implies the integrity of those uniquely human attributes that comprise communication in its broadest sense: the continuous and complex varieties of sensory input; their transduction to encoded nerve signals; in turn their registration, central processing, storage and retrieval; and finally appropriate motor, speech or other language responses--all contributing to human behavior. These are the ultimate research challenges for the NINCDS--indeed for all of us.

ANNUAL REPORT
October 1, 1977 through September 30, 1978
Office of Administrative Management
National Institute of Neurological
and Communicative Disorders and Stroke

The Office of Administrative Management provides general business and administrative management advice and services to the Office of the Director and to the various programs of the Institute. Services provided by this office include budget formulation, administration, monitoring and reporting, personnel management services, space and property management and accounting, central filing services, management analysis services, and advice and consultation on travel issues. The office provides technical supervision over operations of the Program Administrative Offices. In addition, the office coordinates general business and administrative activities with the grants and contracts administrative management activities in the Extramural Activities Program.

Among the goals and achievements of the Office of Administrative Management are the following:

1. Personnel
   To conduct the second year of the three year position classification review. By the end of Fiscal Year 1978, 51% of NINCDS General Schedule positions had been reviewed. Although no positions have been formally identified as being overgraded, the review has resulted in the identification of a number of management and organizational problems which have been or are in the process of being resolved.

2. Budget
   To improve the functional relationship between the Budget Officer, Extramural Activities Program and the Budget Officer, NINCDS. During the past year the supervisory relationships over the Budget Officer, EAP have been clarified with the NINCDS Budget Officer assuming technical supervision of this function. The result has been an improvement in the accuracy and timeliness of extramural grant and contract financial data.

3. Administration
   To consolidate Administrative Offices supporting the NINCDS extramural programs. Formerly, three separate administrative offices supported NINCDS extramural programs. During the past year two were consolidated with a resultant improvement in efficiency and a saving of staff.

   Key administrative staff changes during the past year include:

   1. Mr. Robert Sithens retired as Financial Management Officer and was replaced by Mr. William Matthews as Budget Officer.

   2. Mr. Glenn Hammond retired as Administrative Officer for the Intramural Research Program and was replaced by Mr. Robert Knickerbocker.

3. Mr. Joseph Barnard assumed the position of Administrative Officer, Office of the Director.

4. Mr. George Durall retired as Administrative Officer for the Neurological Disorders Program.

5. Mr. John Jones assumed the position of Administrative Officer for the combined Neurological Disorders Program, Communicative Disorders Program, Fundamental Neurosciences Program and Office of Biometry and Epidemiology pursuant to the consolidaton described above.

ANNUAL REPORT
For Period October 1, 1977 through September 30, 1978
Office of Program Planning and Evaluation
Office of the Director
National Institute of Neurological and
Communicative Disorders and Stroke

The Office of Program Planning and Evaluation, as principal advisory staff to the Director and the Institute on program development and analysis, assists the Director and other Institute program managers in the analysis and evaluation of their programs, and in the development of strategic and operational program plans to meet the long-range goals and immediate objectives of the Institute. The Office provides staff support to facilitate the integration of the program planning, analysis, and evaluation efforts in the categorical program areas and provides the Director and the Executive staff with assistance in setting and articulating the goals of the Institute programs and strategies for meeting those goals. The Office has developed with the staffs of program areas of the Institute, a process to prepare annual implementation plans which form the basis for resource allocation decisions and a development of Institute Forward Plan and budget requests for future years.

Late in FY '77 the Institute judged it timely and appropriate to reassess its long-range goals and develop long-range research strategies as a consciously articulated framework within which the annual operational program planning can take place. With the council, the Institute established seven categorical panels of experts to review and assess the health problems and research needs in those specific areas assigned to each panel. The panels were further asked to review the current state of science in those areas and to recommend research efforts the Institute should seek to foster in the decade ahead. In conjunction with these panels, a public forum was held to which were invited all voluntary health organizations concerned with and associated with the Institute's research concerns. In workshops of this public forum, the voluntary associations and other public interest groups met with panel members and Institute staff and discussed the problems and patient needs from their point of view. In addition, they discussed the research which they hoped the Institute or other federal organizations could support. Based on these considerations and advice from hundreds of ad hoc advisers and consultants, the seven panels have prepared draft reports outlining their findings and proposing recommendations to the Institute. These draft reports will be reviewed by the Institute's Council, at the October 1978 meeting, and will be published in the spring of 1979. In support of these panels, two additional task groups were formed. The first task group is to provide epidemiologic and biostatistics data as well as estimates of the economic impact of the disorders of concern. The second task group is to summarize the basic neurosciences research needs in the future growing out of the deliberations of these panels and to articulate the role of basic research and its potential contributions in the future. Based on the reports of the panels, task groups and the public forum, the Institute will develop, early in FY '79,

an Institute document describing the long-range research goals and the strategic plans for addressing those goals. This report will summarize the panel findings and their recommendations, articulate Institute goals which encompass and respond to panel findings and recommendations, and set the goals for the Institute for the next decade. It will then describe how the Institute's programs intend to address these goals in forseeable years ahead. It is expected that this document will also be published in the spring of 1979.

The Office has, during the past year, continued the development of an integrated Program Information System for the Institute. This will combine scientific program data with fiscal and management data into an integrated Institute-wide data base which will provide necessary information required to effectively and efficiently plan and manage program activities at all levels of the Institute. A review and analysis of Institute program and management information needs has been carried out. Further, an analysis of available information data systems both within and outside of the Institute was completed. A comprehensive system design was developed to provide the identified needs and implementation specifications written. Programming, testing and installation will begin in the coming year.

The Office has been a focus within the Office of the Director, NINCDS, and of assistance to all parts of the Institute, in gathering and coordinating input for, and in developing special reports (oral or written) and issuing papers concerning the Institute's program efforts in specific areas of special interest to NIH.

The Office has developed, within the Institute, an implementation planning process which offers an opportunity to all programs to provide comparable input about program needs, opportunities, priorities, etc., in a uniform fashion for use in resource allocation decisions by the Director. On the basis of these submissions, discussions and negotiations, OPPE develops an Institute operational plan which documents program plan agreements and resource allocation agreements to carry out the Institute programs for the coming year. It is intended that this multiyear implementation plan can form the basis for development of the annual Forward Plan.

The Office provides a central management focus for the Institute's science information programs, which provide summarized literature review and bibliographic references to the research and clinical community in fields related to the Institute's mission. A plan is being developed for the evaluation of these programs including the analysis of the options, and possible alternative methods of providing for the objectives of these services. It is hoped that this evaluation can provide to the Director necessary information for the decisions concerning the supplementation and/or restructuring of these information services.

As a legislative focus for the Institute the Office worked with appropriate staff of the Office of the Director, NIH and a special task group in developing a revised statute for the Institute as part of the legislative package of NIH last year. If and when adopted, this revised statute would become a new part under Title IV of the Public Health Service Act and would

define the Institute's mission and that of its council and also provide the Institute with special authorities in training, in clinical demonstration, technology transfer and public education.

The Office continued to work on the following research projects:

1. Study of Mental Retardation -- (See Annual Report Project Number ZO1 NS 01144-16 OPPE). More careful comparison is underway to assess the distinctive features, if any, of retrospective vs prospective data collection and the degree of bias in the Georgetown sample due to incomplete data.

2. Public Health Implication Study -- (See Annual Report Project Number ZO1 NS 01146-16 OPPE). The manuscript prepared by Dr. Bartlett in collaboration with OPPE on COLR populations helped meet the thesis requirements for a graduate degree in Western Reserve University. The thesis is being prepared to be submitted for publication. Data on cities were found incomplete and thus dropped from further analysis.

3. Study of Labor -- The pediatric outcome of vaginal deliveries in normal pregnancy compared along selected parameters with the outcome of elective Caesarean deliveries, with intent to discover, if possible, the effect of uterine contraction, on time and prematurely, on normal growth and development of the offspring. This study was reactivated during FY'77 in collaboration with NICHHD; analysis is actively under way. An obstetrician in another Institute (since moved to Howard University and later to Hershey Medical Center), is interested in collaborating in the analysis and writing of the manuscript because C/S is more and more used in obstetrical practice and, as such, needs careful evaluation. Computer retrieval of data on normal deliveries has been delayed on account of difficulty in coding the "normal pregnancies" in COLR data. Further delay in availability of computer time has occurred.

4. A Study of Comparative Health (.01 man years) -- In selected ethnic U.S. groups, a survey of incidence and prevalence of MS, ALS, Parkinson's disease, etc., and other bio-psycho parameters to compare with adequate controls from samples of population of the same ethnic origin in other lands. The comparison will be made in a selected period of time and the analysis will hopefully reveal some insight into the mechanism (genetic or environmental) of production of these selected parameters. Contacts with interested investigators in Lebanon and Israel have been made and data collection shall proceed as soon as proper population samples have been selected here and abroad. Political Strain in the Middle East is delaying further progress in pursuit of this work.

5. Comparative Study of Schools of Thought in Medical Science and Practice (A study in medical care) (.01 man years) -- In the U.S., allopathic medicine is thought and practiced side by side with homeopathic medicine, etc. Many systems of medicine are prevalent in India. In Lebanon, the American University has a faculty of medicine and the French University has another faculty, thereby offering a chance to compare French and American medicine. This has implications not only to the systems of delivery of medical care but also to the growing concern in training physician aides even in the developed

countries. Contact with interested collaborators in Lebanon and the Mid East Center in the University of Pennsylvania has been made. Data collection is imminent. Political unrest in the Mid East has delayed design and data collection. Attention is shifting to an exploration of a comparative study of Holistic Medicine in the U.S.

6. Administration Research in Collaboration with NICHHD (.02 man years) -- This is a test of the hypothesis that authority and responsibility in any bureaucratic hierarchy are equal only in middle levels and that authority disproportionately increases the higher the level, and conversely, responsibility disproportionately increases the lower the level. A draft manuscript has been prepared and will be submitted for publication jointly with a political scientist from another Institute. Work on the manuscript has been delayed because it was not given enough priority.

Members of the Office staff continue to be active in a number of associated activities such as the Committee on Science and Human Values (an informal NIH effort established several years ago to help expose the NIH community to intellectual and ethical issues relevant to a human commitment to science and biomedical research), and the NINCDS EEO Advisory Committee.

CONTRACT NARRATIVE
Office of Program Planning and Evaluation, NINCDS
Fiscal Year 1978

NINCDS PROGRAM INFORMATION SYSTEM (PINS) (NO1-NS-6-2345), JRB Associates, Inc., 8400 Westpark Drive, McLean, Virginia 22101.

Title: Design and Specifications for a NINCDS Program Information System (PINS)

Contractor's Project Director: Laurence C. Novotney

Allocation: $125,000

Objectives: The Program Information System (PINS) is an evaluation project to develop the methodology for, and system design to support the efficient input, storage and retrieval of the scientific and programmatic data required to effectively plan, analyze, evaluate, and manage program activities at all levels of the NINCDS. In addition to assessing the present and future informational needs of the Institute, available information data systems both within and outside of the Institute will be reviewed and analyzed. The PINS will be designed to enable Program Managers to have more effective command and control of their program's information in terms of the scientific substance, as well as the budgetary and administrative data.

Major Findings: During the first year of the contract the contractor completed interviews with the NINCDS staff to ascertain the Institute's information requirements. After analysis, these requirements were documented in the Functional Description and Requirements Report. This report then constituted the basis for the Conceptual Design for the Program Information System. Five components of the PINS were identified. For the Budget/Program Analysis component of the PINS, NCI's Fiscal Projection Model (FPM) was evaluated to determine its feasibility to satisfy the fiscal reporting and projection techniques portion for the NINCDS. FPM demonstrated its ability to satisfactorily provide these functions. An integral part of the Project/Program Status and Reports component of the PINS is a scientific classification system. Recognizing the inadequacies of the present classification system, Institute staff and the contractor are designing the structure of a new and more descriptive and retrievable Institute classification system which will consolidate information from both Extramural and Intramural Research and provide accurate and timely responses to questions from the Administration, the Congress, or the public. In order to determine the most appropriate data base management system for use with the PINS, the contractor with the support of an independent consultant completed an analysis of commercial data base management systems and submitted his recommendation to the Institute.

During the second year of the contract, the contractor developed detailed program specifications, based upon the approved Conceptual Design, which incorporate data processing capabilities compatible with the recommended data base management system. The contractor provided specifications as well as analytical and technical support to the staff of OPPE who developed a pilot

element of the PINS - the new Intramural Research Program's information system. In addition the contractor provided the support to analyze, develop and evaluate additional information requirements of the NINCDS.

Significance to the Program of the Institute: The scientific and programmatic information concerning grant, contract and project management, financial (e.g. budget and operating expenses), program projection requirements, and personnel continue to increase in size and complexity. Therefore, rapid access to pertinent facts on a timely basis is essential for effective management. By utilizing a database management system (DBMS) which provides controlled access to the Institute-wide database, PINS will meet this need and make data accessible when and where it's needed, and in a form that can readily be used. The DBMS greatly simplifies programming, maintenance and operation of such applications and data thereby providing an improved level of data access and management. In addition, the PINS will demonstrate a practical means of maintaining data currency, validity and compatibility between it and existing NIH systems such as IMPAC, CAS, CRISP, etc.

Proposed Course: The work on the JRB contract has been favorably received; therefore, the Institute is initiating the implementation phase of this project.

a. A competitive award for the program development and implementation of the PINS will be made first quarter of FY'79.

b. A sole source award for the technical support and validation of the PINS was made on September 30, 1978 to the present contractor.

CONTRACT NARRATIVE
Office of Program Planning and Evaluation, NINCDS
Fiscal Year 1978

SUPPORT SERVICES FOR DEVELOPMENT OF A NATIONAL RESEARCH STRATEGY FOR NEUROLOGICAL AND COMMUNICATIVE DISORDERS (NO1NS82382), CDP Associates, Inc., 6000 Executive Blvd., Suite 510, Rockville, Md. 20852

Title: Support Services for Development of a National Research Strategy for Neurological and Communicative Disorders

Contractor's Project Director: Gregory W. Lewis

Allocation: $348,438 + (225,000) = $573,438

Objectives: The contract provides support to NINCDS, and particularly to the Office of Program Planning and Evaluation, to develop a long-term national research strategy or plan for neurological and communicative disorders. The Institute and its National Advisory Council is evaluating the current research and training programs and overall priorities of NINCDS in allocating scarce resources in the light of changing public health needs and new research opportunities. The evaluation is a joint effort of the Institute, the National Advisory Council, panels of biomedical investigators and clinicians, and the health voluntary and professional bodies related to the subject areas of concern. Reports from seven panels, covering the Institute's programs, and from two cross cutting task groups together with proceedings of a Public Forum which captured the views of voluntary health organizations, will be used by the Institute to formulate its long-range goals strategic plans.

Major Findings: Nine Reports from Panels of outside consultants from the biomedical community have reached draft form for presentation and review by the National Advisory Council of Neurological and Communicative Disorders and Stroke. These reports constitute: 1) a comprehensive review of the current health problems and status of knowledge in the areas of neurological disorders, stroke, central nervous system trauma, communicative disorders, neoplasms of the central nervous system, all aspects of pain, neuroprosthesis, behavior, neuroendocrinology, regeneration, basic neuroscience, and epidemiology; 2) an overview of recent progress in the neurological sciences; 3) an identification and enumeration of research opportunities, approaches and needs for future research and application; 4) recommendations for new initiatives, strengthening of existing programs, redirection of current resources and realignment of priorities. In addition, the study will furnish systematic and relatively reliable estimates of the impact of neurological and communicative disorders and stroke on our society in terms of morbidity, mortality and social and economic costs. Finally, the study provides the basis for assessing Institute programs, for future evaluation and reporting of program progress and effectiveness, and a base line for reevaluation in the near future (5 years).

Significance to the Program of the Institute: The strategic plan or "National Research Strategy for Neurological and Communicative Disorders" will form the framework and overall guidance within which operational planning and resource allocation decisions can be developed. It will further supply an overall guidance to the research community associated with the Institute by identifying the important health problems of the future, research opportunities to be addressed and the role of individual research projects in the overall effort. At the present time, no such overall plan exists. Therefore, this evaluation and planning effort is of the highest importance.

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 ZO1 NS 01144-16 OPPE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
An Instrument for the Conduct of a Retrospective Study of Seizures, Cerebral Palsy, Mental Retardation (M.R.) and Other Neurological and Sensory Disorders of Infancy and Childhood.

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

PI:	Z. A. Shakhashiri, M.D.	Special Assistant	OPPE, NINCDS
OTHER:	Mr. Ernest Harley	Systems Analyst	EBRP, NICHHD

COOPERATING UNITS (if any)

LAB/BRANCH
OD-NINCDS

SECTION
Office of Program Planning and Evaluation

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.04	.04	

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 retrospective instrument has been designed (index cases, with sib and non-sib controls) for record-anchored Study of Mental Retardation (cases selected from Georgetown (G.T.) University Hospital Specialty Clinic) as a pregnancy outcome. Ten hypotheses were being tested, related to the biological and psycho-social mechanisms underlying the occurrence of damage in the growing white child by age 5 years: anoxia, toxic influences on brain, metabolic influences, trauma to the head, infection of brain, dehydration of child, genetic or familial factors, socio-economic status, prematurity, and nutrition. Heart disease, prenatal hypertension, breech presentation, bleeding, Caesarean Section (C.S.) and Cephalopelvic Disproportion (C.P.D.), were noted more frequently in mothers of M.R.; and meconium B.W.<2500, and neonatal head injury more frequently in M.R. children themselves, than in the corresponding sib and non-sib controls. Findings from G.T. data base are being compared to findings from prospective Collaborative Perinatal Research (COLR) data base, using the same retrospective instrument. In view of less incomplete data in the latter, this comparison would confirm or invalidate the initial findings from the former.

Project Description:

Objectives: Design an instrument for the conduct of a retrospective study of seizures, cerebral palsy, mental retardation and other neurological and sensory disorders of infancy and childhood in order to test certain basic and important hypotheses concerning the occurrence of neurological damage.

Methods Employed: Recognized damaged outcomes of pregnancy, especially mental retardation, have been studied and related to defined perinatal or postnatal events which are involved in the biological or psycho-sociological mechanism underlying the following hypotheses: (1) anoxia, (2) toxic influences on the brain, (3) metabolic influences, (4) trauma to the head, (5) infection of the brain, (6) dehydration of the child, (7) genetic or familial patterns, (8) socio-economic status, (9) prematurity, and (10) nutrition.

Current Status: Upon review, the manuscript was criticized on the basis of possible bias inherent in incomplete data as presented. Since the data base is available on tape, another extended analysis is under way, comparing as well, the emerging findings from this retrospective study with those from the prospective COLR study when subjected to the same instrument. The validation of findings from the retrospective study would thus be possible.

The retrospective instrument designed for the collection and analysis of the Georgetown Study data has retrieved data from the COLR data base. Preliminary comparison of data retrieved from G.T.S. sample and from COLR sample reveals some common and some different antecedant factors or events associated with mental retardation as defined. This discrepancy is being scrutinized to assess whether it is valid (due to real differences in the two populations compared) or artificial (due to differences in availability of specific data based on definitions and codes used in the two studies). A manuscript will emerge during FY'79 after consultation with a psychologist at NINCDS, on the issue of this discrepancy.

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 ZO1 NS 01146-16 OPPE

PERIOD COVERED
October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)
Public Health Implications Study of Perinatal Mortality in the Collaborative Study (COLR) and in the Collaborative Study Cities

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

PI:	Z. A. Shakhashiri, M.D.	Special Assistant	OPPE, NINCDS
OTHER:	Mr. Ernest Harley	Systems Analyst	EBRP, NICHHD

COOPERATING UNITS (if any)

LAB/BRANCH
OD-NINCDS

SECTION
Office of Program Planning and Evaluation

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.02	.02	

CHECK APPROPRIATE BOX(ES)

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

X ☐ (a1) MINORS X ☐ (a2) INTERVIEWS

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

Perinatal mortality of COLR children (single births 1959-1969) was compared by institution, race, sex, birth weight (b.w.), gestation length (g.l.). Regional trends appeared in a steady decline, except for 1962. Females, for both races, have lower b.w.; non-whites, for both sexes, have lower b.w., and shorter g.l. Perinatal mortality, as well as fetal mortality, is lower for non-whites at short g.l. and low b.w., and higher at long g.l. and high b.w. These data formed the basis of a term paper by Dr. G. Bartlett, in a Sociology course at Western Reserve. This paper will be published.

Similar data were being analyzed from those COLR cities which supplied data upon request. After an unsolicited consultation with NICHHD, it became apparent that the incomplete data gathered from the cities were not adequate for any further serious work and it was decided that any further comparative analysis is not possible.

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 02355-01 OPPE

PERIOD COVERED
October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effect on Pregnancy Outcome, of Vaginal vs C/S Delivery, in Normal Pregnancy

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

P.I.: Z. A. Shakhashiri, M.Sc., M.D., M.P.H.
Special Assistant, OPPE, OD, NINCDS

OTHER: ErnestHarley, Computer Specialist, Epidemiology & Biometry Branch, NICHHD
David Smith, Computer Specialist, OPPE, OD, NINCDS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH
OD-NINCDS

SECTION
Office of Program Planning and Evaluation

INSTITUTE AND LOCATION
NINCDS/NIH - Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.08	.08	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)

From the overall sample of the Collaborative Perinatal Study, infants of normal pregnancies delivered vaginally are compared with those delivered by elective C/S, as to mortality, Apgar scores, mental and motor scores at eight months and I.Q. at four years, controlling on race; b.w., g.l., and sex. The comparison would throw light on the merits/demerits of spontaneous natural delivery vs delivery by elective caesarean. Search for adequate size samples for comparison continues. There have been unavoidable delays in computer data retrieval, which when overcome, would expedite progress unto writing of a manuscript, hopefully by end of FY'79. Previously, # NDS-(CF)-68-PR/OC 1618; in FY'71 was referred to Ad Hoc Task Force on Labor and Delivery, which, it was discovered this year, did not address the main comparative thrust of this study.

ANNUAL REPORT
October 1, 1977 through September 30, 1978

Office of Scientific and Health Reports (OSHR)
Office of the Director
National Institute of Neurological and Communicative Disorders and Stroke

National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) information, publications, press, and public affairs activities are centered in the Office of Scientific and Health Reports (OSHR). The Office is divided into two sections: Scientific Publications and Public Inquiries.

The Office advises the Director and executive staff on ways to enhance the Institute's public and professional image, and on the effective interpretation and reporting of Institute-conducted and supported research findings. These findings are of interest and concern to many audiences, including Congress, the Department and other agencies of government, scientists, physicians, voluntary health agencies, and the general public. The Office initiates many projects designed to convey public and scientific information, and responds to many Congressional, Departmental, NIH, internal, and public requests for information.

This year and for the past 22 years the OSHR has prepared Special Reports on disease problems designated by the House Subcommittee on Appropriations. These state-of-the-art reviews describe the disorder and the Institute's research program to counter it, report research advances of the past year, including any new developments in therapy, and briefly project the outlook for the future. Nine Special Reports were requested this year: Cerebral Palsy, Epilepsy, Hearing, Speech and Language, Multiple Sclerosis, Neuromuscular Disorders, Parkinson's Disease, Spinal Cord Injury, Stroke, and Amyotrophic Lateral Sclerosis. Two of these reports--Stroke and Spinal Cord Injury--were written by OSHR staff members; of the remaining seven, all drafted by program area staff members, four received extensive editing in the OSHR. Additionally, the OSHR staff wrote approximately half of an inter-Institute report on genetic disorders.

Coordinating the Institute's program with the programs of some 44 private voluntary agencies and 35 professional societies is a major function of the OSHR. This work continued in Fiscal Year 1978 with the performance of many information services, including publication of an annual directory of voluntary agencies; preparation of speeches and information materials as requested on specific disorders; meetings with agency representatives to provide advice and to obtain information about their programs; assisting with annual, scientific, and press meetings of voluntary agencies; and various other types of assistance. At an Institute-sponsored Public Forum on May 11-12, at which some 50 institutions, professional societies, and voluntary agencies were represented, OSHR staff distributed publications and discussed publication needs. The discussions resulted in plans for new pamphlets and fact sheets on tuberous sclerosis, narcolepsy, stroke, and Reye's syndrome. Toward the end of the year, OSHR

undertook development of an information kit for voluntary agencies. This kit will be sent to agency directors and editors of agency newsletters, and will contain brief reports of current research, lists of NINCDS publications, results of recent meetings, news from the agencies themselves, and other information useful to the agencies and their members. A highlight of the kit will be an accompanying letter from the Institute director in which he will discuss topics of mutual interest to the Institute and the agencies.

A growing concern of the Institute has been better disemination of information to physicians and paramedical personnel. Last year the OSHR inaugurated NINCDS Notes, a monthly service for journals in the fields of neurology, neurosurgery, otolaryngology, speech, and the neurosciences. Notes consists of approximately four pages of copy and covers program and administrative developments in the Institute. The audience is primarily heads of departments, chairmen of training programs, grantees, potential grantees, and clinicians.

OSHR also contributes to an NIH service to the Journal of the American Medical Association, providing items of interest to clinicians for a special section appearing monthly. In Fiscal 1978, NINCDS contributed two items to this service: a report on new tests for multiple sclerosis diagnosis, and a discussion of the prognosis of children with febrile seizures.

With the loss of its Health Reports Section and the transfer of audiovisual functions to a central NIH source, OSHR audiovisual productions decreased sharply. This year, however, OSHR helped plan and coordinate a "Project Reach" program on pain, produced in cooperation with the NIH Task Force on Communications and televised through use of the communications technology satelite. The program was introduced by the Institute director; following his remarks, other participants--many of whom were NINCDS grantees or former grantees--discussed advances in pain research and answered questions from an audience at the University of South Carolina Medical School. Also this year, OSHR began work with the NIH Audiovisual Branch on a public service announcement to alert parents to the problem of hearing loss in young children.

The OSHR staff grew by two positions this fiscal year. First to arrive was a new public information officer whose duties include coordinating and responding to increasing numbers of requests from the media for information about Institute activities and interviews with Institute officials. Among the media interest evidenced this year was an interview between the Institute director and reporters from the NEW YORK TIMES and U.S. MEDICINE; an interview between an Institute speech pathologist and U.S. NEWS & WORLD REPORT; and numerous requests for Institute staff members to participate in various radio and television interviews. Also joining the staff was a trainee writer-editor added to the Public Inquiries Section.

OSHR set up press rooms and provided information and assistance to media representatives for several meetings, including the ninth annual meeting of the American Society for Neurochemistry, the NINCDS Public Forum, a workshop on the subacute spongiform encephalopathies and the unconventional viruses that cause them, and a workshop held in Guam to review research on amyotrophic lateral sclerosis and Parkinson's disease.

Scientific Publications Section

The Scientific Publications Section produces and distributes publications for the general public and for a variety of scientific and professional audiences. When a sufficient need is demonstrated, publications production services are provided to various administrative units of the Institute, ad hoc committees preparing reports, and outside organizations in the neurological and communicative disorders fields. The services include planning, writing, and editing; design and layout; clearance, distribution and storage of publications; and subsequent revision and reprinting according to demand. The Section cooperates with the NIH Printing Unit, the Medical Arts and Photography Branch, and the Government Printing Office in producing publications, and also serves as the Institute's supply center for these materials.

The Institute's Monograph Series continues, with 19 publications in this series to date, 11 of which are still distributed by the Section and GPO. The four new monographs published in Fiscal Year 1978 were manpower surveys in neurology, speech pathology and audiology, otorhinolaryngology, and basic and communicative sciences. As of July 1978 one new monograph, Workshop on Antiepileptic Drug Development, was in press.

The Institute's highly popular Hope Through Research pamphlets and companion fact sheets on neurological and communicative disorders continue to be revised and reprinted as necessary. This year a new pamphlet on amyotrophic lateral sclerosis was published, and a new pamphlet on multiple sclerosis was in press. Fact sheets on Friedreich's Ataxia and Alzheimer's Disease were published, and four others, on Narcolepsy, Autism, Tuberous Sclerosis, and Essential Tremor, are in various stages of preparation. Two booklets on Diabetic Neuropathy--one for laymen, the other for physicians--were written by OSHR staff and sent forward for printing.

Annual special reports to Congress, used as background material for appropriation hearings, are printed as pamphlets following the hearings and used as public information materials.

The Section has assumed responsibility for storing, distributing, and reprinting reports of two recent commissions: the Commission for the Control of Huntington's Disease and Its Consequences, and the Commission for the Control of Epilepsy and Its Consequences. Some 18,274 copies of the HD Commission Reports were distributed this year, and approximately 11,000 copies of the Epilepsy Commission Reports. Reprintings were ordered for 1,500 copies of the Epilepsy Commission Reports and 1,000 copies of the HD Commission Reports.

The Section also now stores and distributes two reports of the National Committee for Stroke Resources: Guidelines for Stroke Care and Fundamentals of Stroke Care.

Public Inquiries Section

This section is responsible for responding to written inquiries and telephone calls concerning research findings on some 600 neurological and sensory

disorders. Many of the inquiries involve difficult subject matter and staff members of this section must coordinate closely with intramural scientists and grantees before fully factual responses can be written. Likewise, policy inquiries require close interaction with the NINCDS Office of the Director, Institute program directors, and the NIH and HEW Secretariat.

Inquiries addressed to the NINCDS are not confined to research; they also cover patient care, rehabilitation, health care services, and the economics of neurological and sensory disorders. For complete answers to these inquiries, staff members must obtain pertinent information from other components of the Department, other agencies of Government, and from state agencies through which services and financial aid are funneled.

Last year 1,064 individually prepared responses were sent to the Institute's lay and medical audiences. In addition, 268 responses to controlled letters from the Congress and the White House were written and coordinated with the NIH Secretariat. Many other inquiries were answered with printed materials. In all, NINCDS responded to requests for over 350,000 publications.

Responsibility for scheduling and manning the Institute's exhibit rests with the Public Inquiries Section. In Fall 1977 the exhibit was shown at the American Academy of Ophthalmology and Otolaryngology meeting in Dallas; the American Speech and Hearing Association meeting in Chicago; and the Society for Neuroscience meeting in Anaheim, California. In early 1978, the exhibit was shown at meetings of the American Society for Neurochemistry, the American Academy of Neurology, and at a special meeting in Atlanta devoted to minority biomedical support. The Section also helped staff a modular exhibit for the Institute's Communicative Disorders Program at a Chicago meeting of the ASHA. These showings generated hundreds of requests for information about the Institute's programs. The ASHA meeting is especially valuable for getting NINCDS publications for the lay public into the hands of students and paraprofessionals.

The Public Inquiries Section provides materials for the Institute's Advisory Council meetings, updates the Council Directory, and plans the annual Council dinner. The head of the Section also serves as information liaison with the Extramural Activities Program and with the Program Directors in the Federal Building, is responsible for identifying grantee research appropriate for use in Institute reports and publications, and writes annual special reports for Congress on cerebral palsy and spinal cord injury.

The head of the Section also serves as the focal point for the Institute in discharging Institute responsibilities under the Freedom of Information Act. Under this Act, the Public Inquiries Section responded to 95 requests for summary minutes of the meetings and to 35 requests for substantive information about the Institute's programs.

Annual Report
for Period October 1, 1977 through September 30, 1978
Office of Biometry and Epidemiology
Office of the Director
National Institute of Neurological and Communicative Disorders and Stroke

The Office of Biometry and Epidemiology (OBE) is responsible for the development and implementation of research programs in biometry, epidemiology, and systems analysis to advance medical knowledge and reduce the impact on society of neurological and communicative disorders.

The activities of OBE may be divided into three categories of studies: descriptive, analytic and experimental.

Descriptive Studies - Disease Surveys

In FY 1978, NINCDS will publish monographs on the major findings of national sample surveys of brain tumors, head and spinal cord injuries, and stroke. These findings will include estimates of incidence, prevalence and economic costs to the U.S. population and for special population subgroups. Additional material will also be published; for example, from the stroke survey, information on type of stroke, past medical histories of stroke patients, functional status, fatalities and autopsies, signs and symptoms, laboratory findings, diagnostic tests, and surgical procedures will be reported. A stroke questionnaire was developed to augment the NINCDS stroke survey, and is in use as part of the Health Interview Survey of the National Center for Health Statistics (NCHS). A monograph on the survey of multiple sclerosis will appear in early FY 79.

A survey of epilepsy has recently begun. Its recent appearance in the sequence of surveys belies its high priority to NINCDS. Epilepsy is an important disorder for the Institute, but this large-scale survey awaited the resolution of methodological problems. For example, the presumed stigma attached to epilepsy created problems of patient cooperation in past attempts at assessment of the magnitude of that disorder. NINCDS has developed and is testing a new and innovative approach -- a survey of anticonvulsant drug prescriptions in pharmacies, with follow-back to the physician to identify the purposes for which the drugs were prescribed.

The Comprehensive Disease Statistics Survey, a multipurpose survey of several neurological and non-neurological disorders, is under development in collaboration with the NCHS and with the

cooperation of the Bureau of the Census. This survey will "piggy-back" on the NCHS Hospital Discharge Survey, which is an annual survey of about 450 short-stay hospitals. This multipurpose survey should provide the following advantages:

1. Organization responsibilities for the various aspects of the survey are assigned in conformity with organizational mandates. NIH is providing the medical input; it is selecting the disorders of interest, creating disease algorithms which define the diseases, provide for its subclassifications, and establish criteria for the disease to be classified as definite, probable, or undocumented. NIH is also identifying the additional peripheral medical information to be collected on each case. For this survey, NIH will be a health statistics data user, rather than a data collector.

2. Information on many disorders will be collected, rather than on the few that could be obtained from individual surveys. For example, data on 18 disorders of interest will be collected during the feasibility stage of this survey. A several-fold increase in that number will be considered for the main phase. Since the data will be collected according to standardized protocols from the same hospitals, comparisons will be possible across disease lines.

3. The survey should be highly cost effective. Seven other NIH Institutes, as well as the Center for Disease Control, are cooperating in this study to develop algorithms of their diseases of interest for inclusion in the feasibility trial. Since the major costs of the survey are those incurred in travel to the survey hospitals, the addition of disorders to the list for cases to be abstracted adds only marginally to the total cost.

4. Since the Hospital Discharge Survey is accomplished annually, trends in the incidence of these disorders can be determined.

A more specifically epidemiologically oriented study is the Mississippi Survey of Major Neurological Disorders. The Bureau of the Census is carrying out a screening interview of the members of every household in Copiah County, Mississippi, a community of 25,000 persons equally divided in the number of blacks and whites. Those found to be positive in the screen for senile dementia, stroke, mental retardation, epilepsy, Parkinsonism or cerebral palsy are examined by neurologists or pediatric neurologists on the staff of the University of Mississippi Medical School. This survey is the first that will provide precise comparisons of the prevalence of these disorders between blacks and whites in a rural setting. To date, the response rate for compliance with the screening interview exceeds 99 percent.

An epilepsy questionnaire is under test in the Mississippi Survey for possible use in the NCHS Health Interview Survey in

1980. In addition to these surveys, a study of stroke mortality is under way. This study undertakes to explore the potential biases associated with the assessment of age-specific death rate trends with the use of vital statistics data.

OBE is preparing a series of disease definitions and classifications. These disease algorithms will be used in surveys and in other epidemiologic studies. Their eventual publication in a disease algorithm monograph will support the design of studies with standardized disease definitions and classifications.

Analytical Studies

NINCDS is developing a new program to permit neurologists and neurosurgeons to study the natural history of a number of disorders of importance, and to obtain answers to many research issues. This program involves the collection of longitudinal patient data for stroke and for traumatic coma, by means of multicenter, clinical, interactive computer data banks.

A clinical data bank is a clearly defined, easily accessed collection of data from multiple individual patient records which records past experience to aid in clinical decisions. It allows for the association of patient outcome with the clinical processes of symptom recording, tests, and treatment interventions. It provides access to the physician of information for either individual patients or subgroups of patients, and provides research leads that cannot otherwise be obtained without the uniformity of definitions, efficient collection, and timely access provided by the recent data bank technology.

These data banks will provide uniform, high quality data on an appropriate core of variables for a large number of cases with a specific disorder.

The construction of these clinical data banks has become feasible in the recent past with the advances in computer technology and the growing sophistication of software packages.

Pilot studies for the stroke and traumatic coma data banks will each include four clinical research centers and a data center (all by contract mechanism), with the data analysis centered at NINCDS. Their objectives will be twofold: to improve patient care by asssisting the physician (primarily by providing prognostic data from similar patients), and to provide leads in regard to important medical issues, for new clinical research studies.

Uniform vocabularies for stroke and for coma, each consisting of approximately 450 variables, have been constructed and will undergo review, revision and pilot testing by the contract clinical centers.

Several major epidemiological research contract studies will be completed during this fiscal year, the most important of which is the Study of Multiple Sclerosis in the Shetland and Orkney Islands and Caithness. Several manuscripts have been received from the principal investigator, and several more are in preparation. Perhaps the most significant finding that will stem from this research in this very comprehensive study of a region with the highest reported prevalence of multiple sclerosis in the world is that heredity is not found to be an important etiologic factor in multiple sclerosis..

A number of epidemiological investigations in neurology are in various stages of development and implementation. Briefly, they include: a descriptive epidemiologic study of dementia, a study of the association between myasthenia gravis and multiple sclerosis, a study of cardiac disease and/or hypertension as risk factors for subsequent stroke or TIA, a case-control study of Alzheimer's Disease, the development of system and protocols for an ALS Registry and a Registry for Creutzfeldt-Jacob Disease, analysis of U.S. and international mortality data for neurological disease (including Huntington's Disease in particular), a study of life expectancies in neurological diseases, studies of space/time clusters in neurological disease, and the development of protocols and strategies for studying neurological diseases in developing countries.

Brain tumor research includes studies of racial differences in the occurrence of brain tumors; studies of extracranial malignancies and their intracranial metastases; and descriptive epidemiological studies of primary intracranial neoplasms including analysis of discrepancies in their incidence.

Cerebrovascular studies include the measurement of the incidence rate for this disease in children, unusual patterns of the disease, and the incidence during pregnancy and the postpartum period.

Experimental Studies

OBE continued a broad range of investigative activities. The most significant of these is the analysis of the Collaborative Perinatal Project data, with intensive analysis in the areas of Febrile Seizures, Neonatal Seizures, Convulsive Disorders in general, Cerebral Palsy, Minimal Brain Dysfunction, and Maternal Infection During Pregnancy. These studies have a potential for a dramatic impact on obstetrical care and the practice of pediatric medicine. For example, in the febrile seizure study it has been shown that febrile seizures for most children are a benign condition with small attendant risks of later afebrile seizures, no apparent risk of IQ deficit, and no risk of death or cerebral palsy. The child's medical history and characteristics of the febrile seizures can be used to recognize the small remainder of children at relatively high risk for later morbidity. Still

at issue is the continued publication of instructions to treat all children with febrile seizures with phenobarbital for several years to prevent later epilepsy. For most children it has been demonstrated clearly that the risks of treatment and the unknown efficacy of such a program when compared with the small risk of potential afebrile activity make such a program unwarranted. A proposal for a definitive study of the prophylactic efficacy of phenobarbital in the small subgroup of children considered to be at relatively high risk for later afebrile seizures is currently being developed.

Intramural research collaboration in biometry and mathematical statistics includes studies on clinical and genetic aspects of Tourette's Syndrome, Toxemia in Patas monkeys, detection of immune complexes in MS patients, ventilatory lung function studies, a study of immunoglobulin levels in offspring of PD and ALS patients, examination of viral interferences and biological interaction with Scrapie, Kuru and CJD viruses, a study of the half-time measure of survival in evaluation of the effectiveness of treatments of glioblastoma, a study of L-Dopa treatment of PD patients on Guam, a study of neuronal and glial response to axon injury in the immature rat, an investigation of the application of Sensory Decision Theory to the study of pain, and of the association of certain serological parameters with three different types of MS.

Other activities of OBE include training and education in neurological epidemiology, the planning of international cooperative studies and symposia, the preparation of lectures and reviews and the development of appropriate scientific exhibits. For example, a textbook on the principles of neuroepidemiology will be published in Advances In Neurology. Videotapes on this subject have been developed and are being distributed as training films.

Office of Biometry and Epidemiology
Section on Mathematical Statistics

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3. The survey should be highly cost effective. Seven other NIH Institutes, as well as the Center for Disease Control, are cooperating in this study to develop algorithms of their diseases of interest for inclusion in the feasibility trial. Since the major costs of the survey are those incurred in travel to the survey hospitals, the addition of disorders to the list for cases to be abstracted adds only marginally to the total cost.

4. Since the Hospital Discharge Survey is accomplished annually, trends in the incidence of these disorders can be determined.

A more specifically epidemiologically oriented study is the Mississippi Survey of Major Neurological Disorders. The Bureau of the Census is carrying out a screening interview of the members of every household in Copiah County, Mississippi, a community of 25,000 persons equally divided in the number of blacks and whites. Those found to be positive in the screen for senile dementia, stroke, mental retardation, epilepsy, Parkinsonism or cerebral palsy are examined by neurologists or pediatric neurologists on the staff of the University of Mississippi Medical School. This survey is the first that will provide precise comparisons of the prevalence of these disorders between blacks and whites in a rural setting. To date, the response rate for compliance with the screening interview exceeds 99 percent.

An epilepsy questionnaire is under test in the Mississippi Survey for possible use in the NCHS Health Interview Survey in

1980. In addition to these surveys, a study of stroke mortality is under way. This study undertakes to explore the potential biases associated with the assessment of age-specific death rate trends with the use of vital statistics data.

OBE is preparing a series of disease definitions and classifications. These disease algorithms will be used in surveys and in other epidemiologic studies. Their eventual publication in a disease algorithm monograph will support the design of studies with standardized disease definitions and classifications.

Analytical Studies

NINCDS is developing a new program to permit neurologists and neurosurgeons to study the natural history of a number of disorders of importance, and to obtain answers to many research issues. This program involves the collection of longitudinal patient data for stroke and for traumatic coma, by means of multicenter, clinical, interactive computer data banks.

A clinical data bank is a clearly defined, easily accessed collection of data from multiple individual patient records which records past experience to aid in clinical decisions. It allows for the association of patient outcome with the clinical processes of symptom recording, tests, and treatment interventions. It provides access to the physician of information for either individual patients or subgroups of patients, and provides research leads that cannot otherwise be obtained without the uniformity of definitions, efficient collection, and timely access provided by the recent data bank technology.

These data banks will provide uniform, high quality data on an appropriate core of variables for a large number of cases with a specific disorder.

The construction of these clinical data banks has become feasible in the recent past with the advances in computer technology and the growing sophistication of software packages.

Pilot studies for the stroke and traumatic coma data banks will each include four clinical research centers and a data center (all by contract mechanism), with the data analysis centered at NINCDS. Their objectives will be twofold: to improve patient care by asssisting the physician (primarily by providing prognostic data from similar patients), and to provide leads in regard to important medical issues, for new clinical research studies.

Uniform vocabularies for stroke and for coma, each consisting of approximately 450 variables, have been constructed and will undergo review, revision and pilot testing by the contract clinical centers.

Several major epidemiological research contract studies will be completed during this fiscal year, the most important of which is the Study of Multiple Sclerosis in the Shetland and Orkney Islands and Caithness. Several manuscripts have been received from the principal investigator, and several more are in preparation. Perhaps the most significant finding that will stem from this research in this very comprehensive study of a region with the highest reported prevalence of multiple sclerosis in the world is that heredity is not found to be an important etiologic factor in multiple sclerosis..

A number of epidemiological investigations in neurology are in various stages of development and implementation. Briefly, they include: a descriptive epidemiologic study of dementia, a study of the association between myasthenia gravis and multiple sclerosis, a study of cardiac disease and/or hypertension as risk factors for subsequent stroke or TIA, a case-control study of Alzheimer's Disease, the development of system and protocols for an ALS Registry and a Registry for Creutzfeldt-Jacob Disease, analysis of U.S. and international mortality data for neurological disease (including Huntington's Disease in particular), a study of life expectancies in neurological diseases, studies of space/time clusters in neurological disease, and the development of protocols and strategies for studying neurological diseases in developing countries.

Brain tumor research includes studies of racial differences in the occurrence of brain tumors; studies of extracranial malignancies and their intracranial metastases; and descriptive epidemiological studies of primary intracranial neoplasms including analysis of discrepancies in their incidence.

Cerebrovascular studies include the measurement of the incidence rate for this disease in children, unusual patterns of the disease, and the incidence during pregnancy and the postpartum period.

Experimental Studies

OBE continued a broad range of investigative activities. The most significant of these is the analysis of the Collaborative Perinatal Project data, with intensive analysis in the areas of Febrile Seizures, Neonatal Seizures, Convulsive Disorders in general, Cerebral Palsy, Minimal Brain Dysfunction, and Maternal Infection During Pregnancy. These studies have a potential for a dramatic impact on obstetrical care and the practice of pediatric medicine. For example, in the febrile seizure study it has been shown that febrile seizures for most children are a benign condition with small attendant risks of later afebrile seizures, no apparent risk of IQ deficit, and no risk of death or cerebral palsy. The child's medical history and characteristics of the febrile seizures can be used to recognize the small remainder of children at relatively high risk for later morbidity. Still

at issue is the continued publication of instructions to treat all children with febrile seizures with phenobarbital for several years to prevent later epilepsy. For most children it has been demonstrated clearly that the risks of treatment and the unknown efficacy of such a program when compared with the small risk of potential afebrile activity make such a program unwarranted. A proposal for a definitive study of the prophylactic efficacy of phenobarbital in the small subgroup of children considered to be at relatively high risk for later afebrile seizures is currently being developed.

Intramural research collaboration in biometry and mathematical statistics includes studies on clinical and genetic aspects of Tourette's Syndrome, Toxemia in Patas monkeys, detection of immune complexes in MS patients, ventilatory lung function studies, a study of immunoglobulin levels in offspring of PD and ALS patients, examination of viral interferences and biological interaction with Scrapie, Kuru and CJD viruses, a study of the half-time measure of survival in evaluation of the effectiveness of treatments of glioblastoma, a study of L-Dopa treatment of PD patients on Guam, a study of neuronal and glial response to axon injury in the immature rat, an investigation of the application of Sensory Decision Theory to the study of pain, and of the association of certain serological parameters with three different types of MS.

Other activities of OBE include training and education in neurological epidemiology, the planning of international cooperative studies and symposia, the preparation of lectures and reviews and the development of appropriate scientific exhibits. For example, a textbook on the principles of neuroepidemiology will be published in Advances In Neurology. Videotapes on this subject have been developed and are being distributed as training films.

Office of Biometry and Epidemiology
Section on Mathematical Statistics

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CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1978

WESTAT, INC. (NO1-NS-6-2333)

Title: Nationwide Study of Stroke

Contractor's Project Director: Morton Robins

Current Annual Level: $118,078

Objectives: The primary objective of this survey is to produce national estimates and accompanying measures of precision for the incidence, prevalence, and economic costs of stroke. It would be accomplished by devising and testing a method for carrying out the survey, and for presenting the statistical results of the survey, including the sampling errors.

Major Findings: The field work of the survey and the data analyses have been completed. A clinical algorithm was applied to the abstracted hospital records which classified the records according to type of stroke and degree of confirmation of the diagnosis in the medical records. Analyses have been made relating the clinical data with the type of stroke and selected age-sex groupings. The final report is being prepared and the draft copy will be available in July 1978 for initial review.

Significance to the NINCDS Program and Biomedical Research: This survey is important for two reasons. First, it provides national estimates of the incidence, prevalence, and economic costs of stroke. These estimates are especially useful to NINCDS for purposes of program planning and allocation of funds. Second, the survey will demonstrate to health investigators the value of probability sampling as a tool in sample selection. Probability sampling is the only general method available which can provide a measure of the precision of an estimate. Though this method is widely used in other areas, it is largely neglected in health studies. This survey, and the others of the NINCDS Survey Program, will demonstrate that probability sampling is both desirable and feasible for certain types of health studies.

Proposed Course of the Project: The medical records were abstracted and pertinent information on the patient's demographic characteristics, the diagnostic categorizations of the stroke, date of onset, medical history of previous strokes or TIA's, clinical signs and symptoms, diagnostic findings, treatment, and outcome of hospital care were recorded for each stroke discharge selected in the sample hospitals. In addition, the hospital's business office was asked to furnish data on the direct medical charges to the patient or third party payer on those cases discharged during 1975. A subgroup of surviving patients (or close relatives) was also interviewed to obtain information on direct expenditures related to the stroke incurred during 1975 by the patient or the family. Additional facts on other aspects of economic impact of a stroke on the patient and the family were collected.

Moreover, for those discharged patients in the sample that were presumed to have had an initial stroke attack, their survival status was determined as of December 31, 1975, in order to calculate prevalence. The final report is now being prepared and the study will be completed by the end of fiscal year 1978.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1978

NATIONAL ANALYSTS, INC. (NO1-NS-4-2335)

Title: Survey of the Incidence, Prevalence, and Costs of Multiple Sclerosis

Contractor's Project Director: Lorna B. Sherman

Current Annual Level: $103,591

Objectives: The primary objective of this survey is to produce national estimates and accompanying measures of precision for the incidence, prevalence, and economic costs of multiple sclerosis.

Major Finding: The first stage of the study, the data collection of cases of multiple sclerosis identified by hospitals and physicians during the six year period from January 1970 to December 1975, has been completed and duplicated cases eliminated.

Significance to the NINCDS Program and Biomedical Research: This survey is important for two reasons. First, it provides national estimates of the incidence, prevalence, and economic costs of multiple sclerosis. These estimates are especially useful to NINCDS for purposes of program planning and allocation of funds. Second, the survey will demonstrate to health investigators the value of probability sampling as a tool in sample selection. Probability sampling is the only general method available which can provide a measure of the precision of an estimate. Though this method is widely used in other areas, it is largely neglected in health studies. This survey, and the others of the NINCDS Survey Program, will demonstrate that probability sampling is both desirable and feasible for certain types of health studies.

Proposed Course of the Project: In the second stage of the study a sample of patients will be drawn and interviewed to obtain information relevant to their health status. These patients will then keep a daily log for the next 90 days, recording medical visits, auxiliary services, and direct and indirect costs incurred by the disease. A second interview will inquire into their health status at the end of the 90-day period, so that an attempt can be made to relate fluctuations in costs to changes in activity of the disease. Estimates of incidence and prevalence will be calculated for different groups of patients.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1978

WESTAT, INC. (NO1-NS-4-2336)

Title: Survey of Intracranial Neoplasms

Contractor's Project Director: Thomas G. McKenna

Current Annual Level: $29,734

Objectives: The primary objective of this survey is to produce national estimates and accompanying measures of precision for the incidence, prevalence, and economic costs of intracranial neoplasms.

Major Findings: The objectives of this survey have been realized and the findings have been presented to NINCDS by the contractor in the form of a final report. NINCDS staff will edit the final report and will submit it for publication as a supplement to a major journal.

Significance to the NINCDS Program and Biomedical Research: This survey is important for two reasons. First, it provides national estimates of the incidence, prevalence, and economic costs of intracranial neoplasms. These estimates are especially useful to NINCDS for purposes of program planning and allocation of funds. Second, the survey will demonstrate to health investigators the value of probability sampling as a tool in sample selection. Probability sampling is the only general method available which can provide a measure of the precision of an estimate. Though this method is widely used in other areas, it is largely neglected in health studies. This survey, and the others of the NINCDS Survey Program, will demonstrate that probability sampling is both desirable and feasible for certain types of health studies.

Proposed Course of the Project: The main study has been completed and the final report was submitted by the contractor. After careful examination of the final report it became evident that certain ICDA categories needed to be examined in order to validate the findings before publication. Some of the participating hospitals will be asked to furnish photocopies of selected patient records. The contract has been extended in order to complete the study.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1978

WESTAT, INC. (NO1-NS-7-2379)
NATIONAL CENTER FOR HEALTH STATISTICS (YO1-NS-7-0030)

Title: Comprehensive Disease Statistics Survey

Contractor's Project Director: Westat, Inc. - Dr. Anita Schroeder
NCHS - Dr. Monroe Sirken

Current Annual Level: Contractor - $219,543
NCHS - 25,000

Objectives: The objectives are to test the feasibility of obtaining hospital incidence and prevalence data for cases identified from abstracted hospital records of a number of neurological and other disorders, from a redesigned Hospital Discharge Survey of the NCHS. A key objective of a successful pilot study would be to develop a survey program that would permit the annual collection of data on these disorders in order to develop trends of their incidence and prevalence.

The national sample of short-stay hospitals would provide a stable base for special studies. These studies would include methodological problems such as multiplicity. It would also provide an unbiased sample of patients, for periodic studies of special interest such as costs of illness, degree of disability, duration of illness, etc. Comparability of data collection methods, and protocols from the same sample of short-stay hospitals, would also permit comparison across disease lines.

Major Findings: The study has been designed and the disease algorithms are being prepared. The contractor is now preparing detailed protocols for the feasibility study to be conducted in the fall of 1978 in 20 hospitals. NCHS has been involved in a cooperative effort with NINCDS to plan and design the study and to work on the many methodological and statistical problems involved in the survey.

Significance to the NINCDS Program and Biomedical Research: NINCDS has current contracts for surveys of four neurological disorders, and other surveys are underway and in the planning stage. For several reasons there is a need to consider a more comprehensive approach to the collection of disease statistics. First, there is a considerable degree of redundancy in the present approach, both within NINCDS, and, probably, across Institute boundaries. Redundancy leads to higher than necessary costs associated with the collection of disease statistics data. Second, the present approach leads to delays in obtaining current information, since there are a limited number of surveys which can be conducted at any one time. Third, the methods and protocols used by each contractor differ, and this affects the comparability of data across disease lines. Fourth, and perhaps most important, these data provide planning information based on a limited time period, when in fact

trend data, obtained on an annual, prospective basis, would better serve the program planning and program evaluation functions.

The development of a comprehensive system for the collection of disease statistics on a wide variety of diseases would be of great value to NINCDS and other NIH Institutes for it would eliminate the four above-mentioned major problems.

This proposal would establish a cooperative and joint relationship between NCHS and NINCDS, and would provide for an NCHS collection of national health statistics of considerable interest to NINCDS, and potentially, to other NIH Institutes. It would, to the extent that incidence and prevalence data can be obtained from records at short-stay hospitals, supplant NINCDS data collection efforts. NINCDS would continue to analyze the data collected to meet its own program planning needs.

Proposed Course of the Project: This project is a joint venture. The contractor will be responsible for only part of the study. The contractor will develop the methodology and protocols for the feasibility study and the pilot study, will provide the field staff, and will conduct the study and process the data. The disease algorithms will be prepared by NINCDS staff with the aid of other participating NIH Institutes. The sampling plan, counting rules for non-duplication of cases, estimation of sampling variances, and coordination with participating hospitals will be conducted by the National Center for Health Statistics, HRA, under a separate interagency agreement.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1978

RESEARCH TRIANGLE INSTITUTE (263-78-C-0132)

Title: Case Verification and Supplemental HSCI Analyses

Contractor's Project Director: Dr. William D. Kalsbeek

Current Annual Level: $14,810

Objectives: After careful examination of the final report of the Head and Spinal Cord Injury Survey (Contract NO1-NS-4-2334), it became evident that not all needed analyses had been anticipated and carried out prior to the expiration of the contract. The primary objective of this project is to undertake new analyses which will enhance the value of the monograph to be published on major findings from the survey. In addition, the algorithm which has been used in the main survey to determine medical eligibility for cases of spinal cord injury is to be evaluated in a small-scale specificity investigation.

Major Findings: The new analyses have been completed and NINCDS is awaiting the results. The validity check is nearing completion.

Significance to the NINCDS Program and Biomedical Research: This project is of critical importance to NINCDS because the findings are needed for the major report on the Head and Spinal Cord Survey which will be published in a prestigious medical journal. The report will likely have a significant impact on the health care providers who have an interest in injuries to the head and spinal cord.

Proposed Course of the Project: This contract is of short duration and the objectives should be reached without any serious problems.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1978

RESEARCH TRIANGLE INSTITUTE (NO1-NS-8-2383)

Title: Test of Study Design and Pilot Study for a National Survey of Epilepsy

Contractor's Project Director: Mr. Benjamin S. H. Harris, III

Current Annual Level: $414,570

Objectives: This project was initiated to develop a new casefinding approach for ascertaining the incidence and prevalence of epilepsy. The previously used methods have serious deficiencies, and this proposal seeks to remedy them. The goal is to use pharmacies which fill prescriptions for anti-convulsive drugs, to lead to the physicians providing care and thus to the epileptics. By using techniques of probability sampling, estimates of the scope of the epilepsy problem could be obtained for the U. S. population.

Major Findings: The design test has been conducted by the Contractor and the proposal for the pilot study has been submitted to the Project Officer for approval.

Significance to the NINCDS Program and Biomedical Research: Morbidity surveys of relatively rare disorders are difficult to carry out for the U.S. population. One fundamental problem is that adequate numbers of cases for meaningful analyses may not be included in the sample of individuals selected for study due to stringent requirements for sampling a population. With epilepsy, the problem is compounded because of the perceived social stigma associated with having the disorder. The approach being tested in this contract will, if feasible, yield a cost-effective strategy for the sampling of epileptics who take anti-convulsive drugs. Furthermore, the privacy of the persons included in the study will be safe-guarded to a great extent. If this strategy proves successful, a national survey of epileptics could be mounted which would be invaluable to NINCDS and other organizations responsible for the planning of programs for epilepsy.

Proposed Course of the Project: The project is divided into two parts: a design test and a pilot study. The design test is on a very small-scale and its chief purpose is to lead to the development of methodology for data collection from pharmacies and physicians and to aid in the design of the pilot study. It is scheduled to be completed in September 1978. The pilot study is of a larger scale and its purpose is to resolve methodological issues which are raised by the investigators or are apparent after the design test. In addition, the pilot study will serve as a dry run for the procedures of the main survey. The pilot study will begin in fiscal year 1979.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1978

RESEARCH TRIANGLE INSTITUTE (NO1-NS-4-2334)

Title: Survey of the Incidence, Prevalence and Costs of Head and Spinal Cord Injury

Contractor's Project Director: Dr. Daniel G. Horvitz

Current Annual Level: No cost to the Government

Objectives: The chief aim of the NINCDS National Head and Spinal Cord Injury was to develop estimates of incidence, prevalence, and economic costs for the United States population and selected subpopulations. These estimates were based on a sample of cases which have been selected using the techniques of probability sampling. These techniques are generally more expensive to use than other techniques of sampling but they offer the only general approach which permits the determination of levels of precision for the sample estimates. In this NINCDS survey, levels of precision were obtained for the major estimates of interest.

Major Findings: The objectives of this survey have been realized and the findings have been presented to NINCDS in the form of a final report. NINCDS staff have been carefully reviewing the final report and will be responsible for preparation of a manuscript on substantive findings which will be published as a supplement to a major journal.

Significance to the NINCDS Program and Biomedical Research: The Head and Spinal Cord Injury Survey of NINCDS will have major impact on Institute programs in two distinct ways. First, the survey was designed as a tool for program planning. It provides an indication of the scope of this head and spinal cord injury problem and the economic costs (both direct and indirect costs) of the problem. With this information, and information from surveys of other disorders, program planners are better able to justify their research priorities and needs.

Second, this survey and its companion surveys established that national surveys of relatively rare disorders can be feasible in certain instances using a case finding approach. This realization led to the development of a multipurpose survey which encompasses many disease disorders. The multipurpose survey will be a periodic survey, which means that estimates at various points in time (yearly, every five years, etc.) can be obtained and analyzed for trends. This multipurpose survey will be major in scope and will probably have a significant impact on the programs of several NIH Institutes, the National Center for Health Statistics, and the Center for Disease Control.

Proposed Course of the Project: The study is completed and the final report has been submitted. The contract expired on December 15, 1977.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, OD, NINCDS
Fiscal Year 1978

UNITED STATES BUREAU OF THE CENSUS (Y01-NS-70031)
UNIVERSITY OF MISSISSIPPI (N01-NS-7-2357)

Title: Survey of Major Neurological Disorders in Copiah County Mississippi

Contractor's Project Director: Mr. Robert W. Mangold (Bureau of the Census); Dr. Armin F. Haerer (University of Mississippi)

Current Annual Level: $385,000 (Bureau of the Census);
$32,000 (University of Mississippi)

Objectives: The primary objective of the proposal is to establish the prevalence of six major neurological and developmental disorders (cerebrovascular disease, convulsive disorders, cerebral palsy, psychomotor delay, Parkinson's disease, and dementia) in a well-defined population of southern blacks and whites. A secondary objective is to evaluate the sensitivity and specificity of certain screening questions by means of an item analysis at the close of the study. This analysis is needed because effective screening questions will be used in other morbidity surveys (e.g., the Health Interview Survey of NCHS).

Major Findings: To date, the data collection phase is ongoing and is expected to end next fiscal year. Detailed plans are being developed for the data processing and analysis phase.

Significance to the NINCDS Program and Biomedical Research: At present, there are no adequate data on the prevalance of the six disorders of interest among southern blacks and whites in the United States. A number of studies suggest that stroke is more common among the black population. Mortality data and a few morbidity studies suggest that Parkinson's disease is less common among blacks. A biological explanation of this observation is that both melanin and dopamine are involved in the same metabolic pathway. Dopamine-deficiency in the basal ganglia has been found in patients with Parkinson's disease and is the rationale for the treatment of this condition with L-dopa. Blacks have a higher concentration of dopamine in the basal ganglia than whites which could explain a lower frequency of Parkinson's disease. On the other hand, it may be that blacks with this condition do not seek medical care or receive inadequate care. Mortality tabulations, with all of their biases, suggest that blacks have a predominance of epilepsy and cerebral palsy, but this requires confirmation with morbidity data. The magnitude of the dementia problem has not been studied in any United States population and Copiah County will provide some indication as to whether there is a racial and sex differential in the frequency of this group of conditions.

Proposed course of the project: The field operations for the main study are divided into two types of operations. The first is a household screening operation which is conducted by the Bureau of the Census. Residents of the study area are screened in their homes by means of a questionnaire administered by lay interviewers who are trained and supervised by the Bureau of the Census. The second type of operation is the examination of persons suspected of having one or more of the disorders of interest on the basis of responses given to questions from the screening questionnaire. The University of Mississippi is providing senior, board-certified neurologists to accomplish the neurological examinations and to record the medical findings on forms designed especially for this study. After the close of the field operations, the data will be sent to the Bureau of the Census for processing. When the data tapes and files are available for analysis, the Project Director and Associate Project Director from the University of Mississippi will assist NINCDS in the data analysis and the preparation of scientific papers based on the findings of the survey.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, NINCDS
Fiscal Year 1978

THE UNIVERSITY OF NEWCASTLE UPON TYNE (NO1-NS-6-2337)

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

Contractor's Project Director: Dr. D. F. Roberts

Current Annual Level: None from the 1978 budget. A sum of $39,179 was alloted to this contract out of the 1976 budget. To date, Dr. Roberts received $19,654.82 of this money.

Objective: (See contract NO1-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 eleven specimens of blood obtained during the March 1976 field trip to the Orkney Island were determined. The pedigree as well as all serology data for Orkney were analyzed in a study of the genetic aspects of multiple sclerosis among the inhabitants of Orkney Island. A similar analysis of Shetland data is underway.

Major Findings: Three manuscripts were recently received by OBE:

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 Immunglobulin 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 series of contiguous and discontiguous 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 anticipate publication of these manuscripts in the British Journal of Preventive and Social Medicine in the near future.

Significance to the NINCDS Program and Biomedical Research: (See this item as stated for contract NO1-NS-4-2321.)

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

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".

The following reports required by the contract are still outstanding:

1) Shetland pedigree analysis.

2) The final report on the genetic aspects of multiple sclerosis among the inhabitants of Shetland and Orkney Islands.

The contractor promised completion of these two items by August 1978.

CONTRACT NARRATIVE
Office of Biometry and Epidemiology, NINCDS
Fiscal Year 1978

MASSACHUSETTS GENERAL HOSPITAL (NO1-NS-4-2321)

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

Contractor's Project Director: Dr. Raymond D. Adams

Contractor's Co-director: Dr. David C. Poskanzer

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 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 (to be 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 NO1-NS-6-2337). The principal investigators of contract NO1-NS-4-2321 and contract NO1-NS-6-2337 are cooperating in interpretation and publication of results.

Major Findings: Findings determined to date were reported during The American Academy of Neurology meetings in April 1977 as follows:

(1) "A bimodal age at onset curve was observed for Orkney Islands patients 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: 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."

Significance to the NINCDS Program and Biomedical Research: To date research of the etiology of multiple sclerosis follows a divergent path; the quest for environmental and genetic factors continues at comparable rates. Study of genetic and environmental factors in a defined population with the world's highest prevalence of multiple sclerosis should 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. To date, however, only one manuscript is being considered for submission for publication in the near future. The author deemed one of the manuscripts unsuitable for publication. According to the contractor's co-director, three manuscripts (all dealing with histocompatiblity determinants) are not publication ready due to lack of funds.

Analytic epidemiologic studies will be developed from disease-specific repositories of patient data. A computer data base system, which is now in the planning stage, will permit the collection of medical information from standardized forms on large numbers of patients registered in medical centers. The computer system will be applicable to any disease of interest. For example, it will be possible to measure the relative importance of selected patient and disease factors in determining prognosis for the individual patient.

The system will first be applied to patients with stroke, preferably by establishing a data base system for the Comprehensive Stroke Centers. If the system is successful it will next be considered for application to patients with head trauma.

Experimental epidemiologic studies. Discussions are under way with the staff of the Communicative Disorders and Stroke and Trauma Programs for collaboration between these Programs and OBE in the area of clinical trials. If negotiations are successful, OBE may act as a data coordinating and biostatistical center for a number of future clinical trials, such as tests of the effectiveness of the tonsillectomy procedure, effect of heavy doses of steroids on spinal cord injury cases, etc.

Several major epidemiological research contract studies will be completed during this fiscal year, the most important of which is the Study of Multiple Sclerosis in the Shetland and Orkney Islands and Caithness. Several manuscripts have been received from the principal investigator, and several more are in preparation. Perhaps the most significant finding that will stem from this research in this very comprehensive study of a region with the highest reported prevalence of multiple sclerosis in the world is that heredity is not found to be an important etiologic factor in multiple sclerosis.

A major international conference on neurological epidemiology was held in Washington, D.C. Six of the papers were presented by members of OBE, and the Section on Neuroepidemiology held a one-day training session on that topic in conjunction with the conference. The reports will be published in a book entitled "Neurological Epidemiology".

A number of epidemiological investigations in neurology are in various stages of development and implementation. Briefly, they include: a descriptive epidemiologic study of dementia, a study of the association between myasthenia gravis and multiple sclerosis, a study of cardiac disease and/or hypertension as risk factors for subsequent stroke or TIA, a case-control study of Alzheimer's Disease, the development of system and protocols for an ALS Registry and a Registry for Creutzfeldt-Jakob

Disease, analysis of U.S. and international mortality data for neurological disease (including Huntington's Disease in particular), a study of life expectancies in neurological diseases, studies of space/time clusters in neurological disease, and the development of protocols and strategies for studying neurological diseases in developing countries.

Brain tumor research includes studies of racial differences in the occurrence of brain tumors, extracranial malignancies metastasizing to a primary intracranial neoplasm, and descriptive epidemiological studies of primary intracranial neoplasms and their patterns, and analysis of discrepancies in their incidence.

Cerebrovascular studies include the measurement of the incidence rate for this disease in children, unusual patterns of the disease, and the incidence during pregnancy and the post partum period.

The Section on Epidemiology is also involved in training and education in neurological epidemiology, in the planning of international cooperative studies and symposia, in the preparation of lectures and reviews and in the development of appropriate scientific exhibits. For example, a textbook on the principles of neuroepidemiology will be published in Advances In Neurology. Videotapes on this subject have been developed and are being distributed as training films.

OBE is preparing a series of disease definitions and classifications. These disease algorithms will be used in surveys and in other epidemiologic studies. Their eventual publication in a disease algorithm monograph will support the design of studies with standardized disease definitions and classifications.

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 ZO1 NS 02238-02 OBE

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Pilot Data Bank Project Network in Stroke previously titled Computerized Interactive Data Bank for Comprehensive Stroke Centers

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

PI: Selma C. Kunitz, Head of Section on Systems Design & Data Processing, OBE, OD, NINCDS

Other: James M. Dambrosia, Ph.D., Mathematical Statistician, Section on Mathematical Statistics, OBE, OD, NINCDS

COOPERATING UNITS (if any)
Dr. Murray Goldstein, Director of Stroke & Trauma Program, NINCDS and Stroke Steering & Data Committees

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Systems Design & Data Processing

INSTITUTE AND LOCATION
National Institute of Neurological & Communicative Disorders & Stroke, Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	.3

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 goal of developing a computerized interactive data bank for the Comprehensive Stroke Centers is to provide a development model for neurological diseases which will aid both the clinician and reseacher in the treatment, prognosis, rehabilitation, and possible prevention of such diseases. The objectives of the project are: a. To develop a uniform method of data collection utilizing standard clinical nomenclature and vocabulary, patient histories, diagnosis, and treatment. b. To implement an interactive comprehensive data bank network enabling pooling of clinical data among institutions, collaborative inter-institutional studies, rapid access to large quantities of clinical data, and computer consultation. c. To demonstrate the feasibility of such a network, including the computer aspects, the collaboration among a number of institutions, to serve as a model for neurological diseases and disorders.

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 02340-01 OBE

PERIOD COVERED
November 15, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Pilot Data Bank Network Project in Coma

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

P.I.: Selma C. Kunitz, Head of Section on Systems Design & Data Processing, OBE, OD, NINCDS

Other: James M. Dambrosia, Ph.D., Mathematical Statistician, Section on Mathematical Statistics, OBE, OD, NINCDS

COOPERATING UNITS (if any)

Dr. Murray Goldstein, Director of Stroke & Trauma Program, NINDCS & Coma Steering & Data Committees

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Systems Design & Data Processing

INSTITUTE AND LOCATION
National Institute of Neurological & Communicative Disorders & Stroke, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.6	.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)

A pilot multi-centered interactive data bank project restricted to traumatic coma is proposed. The objectives of the study are:

1. To utilize a uniform clinical vocabulary and method of data input
2. To evaluate the feasibility of a multi-centered traumatic coma data bank system to examine:
   a. The ability of diverse institutions to participate and collaborate effectively
   b. The ability to collect data of high quality
   c. The ability to insure the quality and quantity of data from diverse institutions
   d. The preliminary utility of the data for patient management and in indicating research leads
   e. The ability to provide guidelines and protocols for expansion to additional centers and other neurologic disorders

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

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 NS 02354-01 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Clinical Trial of Efficacy of Phenobarbital as Prophylaxis for Children with Febrile Seizures

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

P.I.: Jonas H. Ellenberg, Head, Section on Mathematical Statistics, OBE, NINCDS

P.I.: Karin B. Nelson, Neurologist, DNB, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Mathematical Statistics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.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)

Six percent of all children with febrile seizures are at relatively high risk for later afebrile activity. A proposal is being developed for a clinical trial which will examine the efficacy and toxicity of chronic and short-term prophylaxis with phenobarbital for this group of children. At present chronic prophylactic medication is recommended by several authors without available information as to the potential success or side effects of such medication.

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 02240-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Descriptive Epidemiology of Dementia

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., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

Dr. Wayne Massey, National Naval Medical Center

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	

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 the prevalence of dementia in well-defined populations are planned. Protocols have been developed and are currently being tested.

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 02241-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Study of Pre-existing Cardiac Disease and/or Hypertension as Risk Factors for Subsequent Completed Stroke and Transient Ischemic Attacks

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., Head, Section on Epidemiology, OBE, NINCDS

OTHER: Devera G. Schoenberg

COOPERATING UNITS (if any)

Dr. Jack P. Whisnant, Department of Neurology, Mayo Clinic

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This investigation is aimed at evaluating the effect of heart disease and hypertension as potentially treatable precursors of stroke and transient ischemic attacks. The objectives of the study are 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. Data collection for this study has been ongoing since 1973, and it is estimated that an additional year will be required to complete the investigation.

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 02243-02 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cerebrovascular Disease in Children

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., Head, Section on Epidemiology, OBE, NINCDS

OTHER: Devera G. Schoenberg

COOPERATING UNITS (if any)

Dr. James F. Mellinger, Department of Neurology, Mayo Clinic

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The Mayo Clinic experience with cerebrovascular disease in children was examined for the years 1965 through 1974. The medical record linkage system available for all medical facilities caring for the Rochester population makes it possible, for the first time, to establish an incidence rate for cerebrovascular disease in children. These data have been analyzed. Five papers on this subject have been prepared; three papers have been published and two have been accepted for publication.

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 02244-02 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Unusual Patterns of Cerebrovascular 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., Head, Section on Epidemiology, OBE, NINCDS –

COOPERATING UNITS (if any)

Dr. Jack P. Whisnant, Department of Neurology, Mayo Clinic

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	

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)

Unusual clinical patterns of cerebrovascular disease in the experience of the Mayo Clinic are being examined (e.g., the occurrence of more than 15-20 transient ischemic attacks/day). Such material is being critically evaluated in an attempt to link unusual clinical presentations with specific pathological lesions.

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 02245-02 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Cerebrovascular Disease During Pregnancy and the Postpartum Period

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., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

Dr. Burton Sandok, Department of Neurology, Mayo Clinic

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	

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 study will attempt to define the incidence and nature of cerebrovascular disease in the young female as well as the incidence of such events in the pregnant and non-pregnant population. This may provide us with information to better understand the increased risk of cerebrovascular events presumed to occur in association with the use of oral contraceptives. This investigation is just getting underway.

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 02305-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Descriptive Epidemiology of Primary Intracranial Neoplasms

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., Head, Section on Epidemiology, OBE, NINCDS

OTHER: Dr. Barbara W. Christine, Connecticut State Department of Health
Dr. Jack P. Whisnant, Department of Neurology, Mayo Clinic

COOPERATING UNITS (if any)

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The descriptive epidemiology of primary intracranial neoplasms (including tumors of the pituitary gland) has been studied with regard to incidence, survival, and the association of these neoplasms with primary malignancies of other sites. A paper describing incidence patterns by age, over time, and by histologic type is being prepared for publication. Data on primary intracranial neoplasms from the Third National Cancer Survey have been obtained from the National Cancer Institute and will be similarly analyzed. These data, however, include only those neoplasms specified as "malignant".

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 02306-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Resolution of Reported Differences in the Incidence of Primary Intracranial Neoplasms

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., Head, Section on Epidemiology, OBE, NINCDS

OTHER: Dr. Barbara W. Christine, Connecticut State Department of Health
Dr. Jack P. Whisnant, Department of Neurology, Mayo Clinic

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Age-specific incidence curves from the state of Connecticut reveal a small peak in childhood followed by a much taller, sharper peak with a maximum between ages 55 and 65. This also holds true for data collected from most large population-based registries. In contrast to this are the age-specific incidence curves for Rochester, Minnesota, which show a steady increase in incidence with increase in age. In addition, the reported incidence rate at any particular age for Rochester, Minnesota, is higher than comparable rates from other registries. The reasons for these discrepancies are currently being analyzed. A manuscript concerning the findings has been prepared and has been accepted for publication.

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 02304-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Extracranial Malignancies Metastatic to Intracranial Neoplasms

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., Head, Section on Epidemiology, OBE, NINCDS

OTHER: Dr. R. Jean Campbell, Department of Pathological Anatomy, Mayo Clinic
Dr. Albert Heck, Department of Neurology, University of Maryland
Dr. R. Simon, Berkeley, California

COOPERATING UNITS (if any)

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	

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 study represents analysis of a series of cases in which an extracranial malignancy metastasized to a primary intracranial neoplasm. The pathological specimens are being carefully examined in hopes of providing clues to the etiology of this phenomenon. A paper is being prepared for publication.

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 02303-02 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Racial Differentials in the Occurrence of Brain Tumors

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., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)
Dr. Lawrence W. Mahalak, Jr., Division of Neurology, University of Mississippi

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Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	

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)

African studies suggest that the brain tumor experience of blacks is both quantitatively and qualitatively different from that of whites. No data are yet available for brain tumors on a well-defined population of blacks in the United States. However, the experience of a southern medical center caring for a large black population will be examined.

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

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

PROJECT NUMBER
Z01 NS 02344-01 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

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 Epidemiology, OBE, NINCDS

OTHER: Bruce S. Schoenberg, M.D., M.P.H., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)
Dr. David Hanson, Otolaryngology, Communicative Disorders Program, NINCDS

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Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.25	

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 research was presented at a NINCDS sponsored Workshop on Otitis Media and Child Development 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 otitis media with developmental disability, and a proposal for a retrospective study of this association in a defined population.

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 02299-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Analysis of National and International Mortality Data for Huntington's Disease

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

PI: Judith E. Hogg, M.D., Neurologist, Section on Epidemiology, OBE, NINCDS

OTHER: Bruce S. Schoenberg, M.D., M.P.H., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	

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)

At the request of the Huntington's Disease Commission, the Section on Epidemiology is preparing a set of standard mortality indices for Huntington's disease in the United States and for other countries where suitable data are available. The results of these studies will be published as part of the Commission's final report. A presentation at the conference on Huntington's Disease is planned.

PHS-6040
(Rev. 10-76)

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 02246-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 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., Head, Section on Epidemiology, OBE, NINCDS

OTHER: Judith E. Hogg, M.D., Neurologist, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

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

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Development of a suitable epidemiologic protocol and questionnaire for a case-control study of Alzheimer's disease.

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 02345-01 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

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 Epidemiology, OBE, NINCDS

OTHER: Bruce S. Schoenberg, M.D., M.P.H., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

Mayo Clinic, Rochester, Minnesota

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.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)

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 neurologic manifestations of Graves' disease in a defined population.

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 02346-01 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Investigation of Guillain-Barre Syndrome in San Joaquin County, CA.

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

PI: Judith E. Hogg, M.D., Neurologist, Section on Epidemiology, OBE, NINCDS

OTHER: Bruce S. Schoenberg, M.D., M.P.H., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (If any)

Dr. Donald E. Kobrin, Neurologist, Stockton, CA.

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Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

An epidemiologic study of Guillain-Barre syndrome in the well-defined population of San Joaquin County, California was done. The results of this study have been submitted for publication.

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 02347-01 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Observational Study of a Clinic Population of Cerebral Palsy Patients

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

P.I.: Leon Root, Director of CP Clinic, Hospital for Special Surgery, NYC, NY

P.I.: Robert Richter, Mathematician, OBE, NINCDS

COOPERATING UNITS (if any)

Hospital for Special Surgery, N.Y.C.
Cerebral Palsy Clinic, Leon Root, Director

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Mathematical Statistics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.25	

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 is an observational study of a large urban clinic population of cerebral palsy patients. The population to be examined consists of approximately 1000 Cerebral Palsy patients of the Hospital for Special Surgery in New York City.

Staff of OBE collaborated on the definition of the standardized vocabulary designed the data collection protocols and established a quality control mechanism for data collection. The pilot phase for data collection is nearing completion.

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 02242-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Association Between Myasthenia Gravis and Multiple Sclerosis

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., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

Dr. Drake Duane, Department of Neurology, Mayo Clinic

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Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	

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)

There have been numerous reports of the association between myasthenia gravis and multiple sclerosis. The Mayo Clinic experience with these two diseases will be reviewed for cases of association, and this experience will be analyzed in an attempt to determine whether this association occurs more often than might be expected simply on the basis of chance occurrence.

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 02296-02 OBE

PERIOD COVERED
October 1, 1977 through September 30. 1978

TITLE OF PROJECT (80 characters or less)

Registry for Creutzfeldt-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., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

Charles Poser, Department of Neurology, University of Vermont

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Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	

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 protocol for the implementation of a registry for Creutzfeldt-Jakob disease to include epidemiologic, pathologic, and clinical information.

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 02300-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Life Expectancies in Neurological Diseases

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., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

Mayo Clinic, Rochester, Minnesota

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The study uses a review and abstraction of data from records for a selected group of neurological disorders. It obtains the items of data which will develop onset of the disorder, duration, date and cause of death or current status. These data will be used to construct modified life tables to estimate the expectation of life after diagnosis, the survival curve and morbidity and severity estimates.

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 02297-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Analyses of U.S. Mortality Data for Neurological Disease

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

PI: Judith E. Hogg, M.D., Neurologist, Section on Epidemiology, OBE, NINCDS

OTHER: Bruce S. Schoenberg, M.D., M.P.H., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

Dr. Wayne Massey, National Naval Medical Center
Dr. Thomas Mason, National Cancer Institute, NIH

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Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.03	0.03	

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)

Analysis of recent U.S. mortality data with regard to neurological data in order to examine the geographic distribution and identify areas with particularly high or particularly low death rates. These preliminary analyses may lead to etiologic clues.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02301-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Space/Time Clusters of Neurological Diseases

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., Head, Section on Epidemiology, OBE, NINCDS

OTHER: Judith E. Hogg, M.D., Neurologist, Section on Epidemiology, OBE, NINCDS

Tatiana Kudrjavcev, M.D., Neurologist, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

Dr. Richard Kaslow, Center for Disease Control, Atlanta, Georgia

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Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Development of protocols and strategies for the investigation of space/time clusters of neurological disease. This also involves studies of neurological diseases resulting from environmental toxins.

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 02307-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Conference on 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, M.D., M.P.H., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

D. Schoenberg, Research Epidemiologist, Bethesda, MD.

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SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

An international two-day conference on neurological epidemiology was held in Washington, D.C., on May 15-17, 1977. Approximately 50 representatives from Asia, Africa, Europe, Latin America, and the U.S. attended. The various speakers and discussants discussed current knowledge in neuroepidemiology and stressed the applicability of this information to the practice of neurology and neurosurgery. A national one-day course on the principles of neurological epidemiology was offered. The papers and discussions for this conference have been edited and will be published 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 02298-02 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Mortality in 33 Countries from Diseases of the Nervous System

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., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

Dr. Wayne Massey, National Naval Medical Center

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Epidemiology

INSTITUTE AND LOCATION
NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Analysis of international mortality data on neurological disease collected through the auspices of the World Health Organization. Portions of this work were presented at the 11th World Congress of Neurology. Preparations for publications are being carried out.

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 02302-02 OBE

PERIOD COVERED October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

International Programs in Neuroepidemiology

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., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

Neurosciences Program, World Health Organization, Geneva, Switzerland

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Development of protocols and strategies to study the epidemiology of neurological disease in developing countries. Emphasis will be placed on uniformity and standardization so that results of investigations can be compared.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02348-01 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Simulated CAT Display

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

P.I.: Alan J. Talbert, Mathematical Statistician, OBE, NINCDS

P.I.: Rodney Brooks, Physicist, SN, NINCDS

COOPERATING UNITS (if any)

Neuroradiology Section, NINCDS

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Mathematical Statistics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.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)

A method was devised to simulate CAT scans of phantom subjects on a local printer. This eliminated a lengthy procedure involving writing and transferring tapes to and from an EMI scanner in a remote building. The new procedure reduces the turnaround time for CAT experiments from hours to minutes, greatly speeding up research in interpolation methods and aliasing errors in image reconstruction.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02350-01 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Statistical Methodology for Medical Data Bases

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

P.I. : J.M. Dambrosia, Mathematical Statistician, Section on Mathematical Statistics, OBE, NINCDS

Other: S. Kunitz, Head, Section on Systems Design and Data Processing, OBE, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Mathematical Statistics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.25	

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 proposed data bases for stroke and traumatic coma will provide data that require both critical application of existing statistical methodology and development of new statistical techniques in order to extract meaningful information and to draw valid conclusions from the accumulated data. A continuing review of the statistical activities by existing medical data bases and a search of the relevant literature was initiated. Some of the major problems and associated methodologies that have been formulated are: validation and quality control of the data, analysis of variation due to observers, centers, and patient cohort characteristics; association of indicators and treatment with patient outcome, and analysis of trends in time oriented data. A set of descriptive and associative statistics are available for the analysis of symptoms, indicators, and outcomes. Problem areas that have been identified and that require additional study are: recognition of and adjustment for biases, derivations of life tables with competing risks and utilizing concomitant information, and the use of stratification and how it affects analysis.

PROJECT NUMBER (DO NOT USE THIS SPACE)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02351-01 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Improving Current Methods of Analysing Molecular Hybridization Experiments

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

P.I.: Rosalind B. Marimont, Research Mathematican, OBE, NINCDS

P.I.: Lawrence D. Grouse, Medical Officer, Laboratory of Developmental Neurobiology, NICHHD,

COOPERATING UNITS (if any)

Laboratory of Developmental Neurobiology, NICHHD

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Mathematical Statistics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.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)

Many hybridization experiments are analyzed by fitting the time curve of per cent of DNA or RNA hybridized to various forms of the second order rate equation. Empirical correction factors must be applied for proper interpretation of results. Since the precise assumptions on which this method of analysis are based have not always been made clear, there is some uncertainty about applicability of particular equations. Also some laboratories lack sufficiently general curve fitting programs.

The computer system MLAB, available at NIH and some other installations has been recommended for its power and generality in curve fitting and model building. One simple but important question under what ranges of initial tracer to driver concentration are two of the commonly used approximations to the second prior rate equation valid has been answered by computing and displaying the appropriate curves over a large range of initial concentrations.

Improving the model by finding a theoretical basis for the empirical correction factors is the next goal of this project.

PROJECT NUMBER (DO NOT USE THIS SPACE)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02352-01 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Nearest Neighbor Algorithms for High Dimensional Spaces

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

P.I.: Rosalind B. Marimont, Research Mathematician, OBE, NINCDS

P.I.: Marvin B. Shapiro, Mathematician, LSMM, DCRT

COOPERATING UNITS (if any)

LSMM (Laboratory of Statistical and Mathematical Methodology), DCRT

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Mathematical Statistics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.4	.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 geometric nearest neighbor problem is to choose from a fixed collection of points (the library) in a d dimensional space that point nearest to a given query point. Many classification and pattern recognition problems are exact analogues of the geometric problem if coordinates and distance measure are suitably defined.

Most cutoff algorithms effective in low dimensional spaces ($2 \leq d \leq 10$) deteriorate for $d > 10$. Analysis of these algorithms in terms of mapping from a high to low dimensional space shows that if the mapping function and image space are suitably chosen, cutoff algorithms can be effective at higher dimensionality. It is shown that in most cases, the image space must be of dimension greater than one, and that principal components analysis can be used to optimize the choice of the image space.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02353-01 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Synthesis of Graph and Matrix Theory

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

P.I.: Rosalind B. Marimont, Research Mathematician, OBE, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Mathematical Statistics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.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)

This is a long-term effort to combine the concepts and methodology of graph theory and matrix theory to facilitate understanding of and solution of a large class of problems for example, linear compartmental systems.

A previous matrix-graph theorem was extended. A series important in statistics was summed and its general behavior described by deriving its recursive form through graph-matrix methods.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02342-01 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Association of Certain Serological Parameters with Types of Multiple Sclerosis

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

PI: T.C. Chen, Ph.D., Mathematical Statistician, OBE, NINCDS

Other: Bruce Schoenberg, M.D., Head, Section on Epidemiology, OBE, NINCDS

COOPERATING UNITS (if any)

George W. Ellison, M.D., Associate Professor, Reed Neurological Center, UCLA

Lawrence W. Myers, M.D., Associate Professor, Reed Neurological Center, UCLA

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.10	.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)

In this study of Multiple Sclerosis, the serological measurement of IgG, IgM, Measle and EBV titers of three types of multiple sclerosis patients were compared with non-multiple sclerosis patients which serve as controls. The purpose is to see whether different types of multiple sclerosis (as defined by Reed Neurological Center, UCLA) can be differentiated on the basis of these serological parameters. Statistical investigation with both univariate and multivariate analyses indicated that each type of multiple sclerosis differs significantly in some way from the control.

Further work is considered to collect and analyse more clinical data of multiple sclerosis to confirm this finding.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02310-02 OBE

PERIOD COVERED
October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)
A Sensory-Decision Theory Approach to the Measurement of Pain Components.

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

PI: T.C. Chen, Mathematical Statistician OBE, NINCDS

COOPERATING UNITS (if any)

Brenda Vanlunen, Computer Aid, Section on Mathematical Statistics, OBE, NINCDS

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.25	.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)

This project is aimed at investigating the use of Sensory-Decision Theory in measuring sensory discriminability and response bias in experimental pain responses. The current work revealed that the indices of these two pain components, d' and B, as proposed by Clark (1971), are not completely independent; and that in an experimental condition with treatment (or analgesic drug) the estimation of change in response bias through using index B is not independent of the change in sensory discriminability before and after treatment. The sampling behavior of these two components and their changes due to treatment has been extensively investigated by simulation method. A new valid estimate of the change in response bias has been developed.

The report of this study has been accepted to be presented at the Second World Congress on Pain in August 1978.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02309-02 OBE

PERIOD COVERED
October 1, 1978 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Statistical Methods in Neuronal Spike Train Analysis

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

PI: G.L. Yang, Research Fellow OBE, NINCDS
T.C. Chen, Mathematical Statistician OBE, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	

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 the currently available statistical methods of neuronal spike-train analysis has been extensively reviewed and evaluated from the point of view of stochastic processes. Several optimal statistical tests for validating the use of these methods have been developed.

The report of this project has been published in Mathematical Biosciences, March 1978.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02341-01 OBE

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Stroke Mortality Study

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

PI: Frederic D. Weinfeld, Head, Section on Disease Statistics Surveys, OBE, NINCDS

Other: Prof. Felix Moore (University of Michigan)
Robert Richter, Mathematician, Section on Mathematical Statistics, OBE, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Disease Statistics Surveys

INSTITUTE AND LOCATION
NINCDS, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This is a demographic study of stroke mortality. It undertakes to explore the potential biases associated with the assessment of age-specific death rate trends based on stroke deaths during the period 1950 to the present. Trends by sex, race, and specific ICDA categories of stroke for these age-specific groupings will be produced and assessed. The limitations of the data, the underlying assumptions of the analysis, and the implications of the findings will be considered available on multiple causes of death, an analysis will be undertaken to compare these more complete data with the primary cause of death data which have been used heretofore.

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 02311-02 OBE

PERIOD COVERED

October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

Recovery of Corrupted EMI Data

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

PI: A.J. Talbert, Statistician OBE NINCDS

Other: R.A. Brooks, Physicist SN NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Mathematical Statistics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.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 nature of a process corrupting EMI scanner data was discovered by analysis of representative listings. The process involved spurious cyclic application of logical OR and NOT operations to the underlying bits of the binary data. The errors occurred in a contiguous 6-bit sequence in every consecutive 40-bit string in the files. Relying on an understanding of the corruption process and limiting values of the data, a method was devised by which the data could be restored sufficiently for clinical use. A lengthy and critical reconstruction was made of existing corrupted data. This project has been completed and an article submitted for publication.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02312-02 OBE

PERIOD COVERED
October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)
Methodology for systematic statistical analysis of multiple antibody readings on matched controlled studies.

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

PI:	A.J. Talbert, Statistician	OBE	NINCDS
	J.H. Ellenberg, Head, Section on Math. Stat.	OBE	NINCDS
Other:	J.L. Sever, Chief, Infectious Diseases Branch	OBE	NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry and Epidemiology

SECTION
Mathematical Statistics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.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)

A system was developed for analysis of multiple antibody response data in paired sera from matched control studies. The system includes an exacting data quality control step and comprehensive statistical testing to compare abnormals with controls, and to detect and flag seroconversions. For chi-square tests an interval function was developed which automatically lumps cells to prevent low cell frequencies, in a logical manner. It was necessary to extend existing Kolmogorov-Smirnov tables of critical values to accommodate the frequencies which could be encountered in studies of this type. This project is ongoing.

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 02237-02 OBE

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Development and Design of a Pilot Study for a National Survey of Epilepsy

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

PI: William Weiss, Cheif, OBE, NINCDS

PI: Bernard H. Kroll, Associate Chief, OBE, NINCDS

Other: Frederic Weinfeld, Head, Section on Disease Statistics Surveys, OBE, NINCDS

COOPERATING UNITS (if any)

W. Allen Hauser, Dept. of Neurology, St. Paul-Ramsey Hospital, St. Paul, Minn. 55101
Joseph Steinberg, Survey Design, Inc.

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.70	.50	.20

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 pilot was initiated to develop a new and untried method of ascertaining the incidence and prevalence of epilepsy. The previously used methods have serious deficiencies and this proposal seeks to remedy them. The goal is to use pharmacies filling anti-convulsive prescriptions to lead to the physicians providing care and thus to the epileptics. By using nationwide probability sampling techniques valid estimates of the U.S. epileptic population should be made. The contract was let and the pilot study is about to get underway.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02239-02 OBE

PERIOD COVERED

October 1,1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Design of Stroke and Convulsive Disorder Questionnaires

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

PI: Bernard H. Kroll, Associate Chief, OBE, NINCDS

Other: Bruce Schoenberg, Head, Section on Epidemiology, OBE, NINCDS

William Weiss, Chief, OBE

COOPERATING UNITS (if any)

Clint Burnham, Health Interview Survey, NCHS
W. Allen Hauser, Dept. of Neurology, St. Paul-Ramsey Hospital
St. Paul, Minn. 55101

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.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 design and field testing of questionnaires to be used as a supplement to the NCHS Health Interview Survey (HIS). The first was designed to determine the persons with some degree of stroke, diagnosed or undiagnosed; and hospitalization. This questionnaire was included in the 1977 HIS. A second has been designed to measure those persons with convulsive disorder and is being proposed for the 1978-79 HIS. The stroke questionnaire has been submitted to NCHS and is included in their current household interview survey.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02308-02 OBE

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

An Algorithm for a Morbidity Index

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

PI: T.C. Chen, Mathematical Statistician, OBE, NINCDS

Other: B.H. Kroll, Associate Chief, OBE, NINCDS
William Weiss, Chief, OBE, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Biometry and Epidemiology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.75	

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)

To develop a mathematically acceptable algorithm for the design of a morbidity index to permit comparisons of various diseases as to their impact on the persons affected, their families and society. Have reviewed extensively the existing literatures about various kinds of health indicators. The model derived by Chiang (1976) has been selected for preliminary study. Due to some difficulties in obtaining needed data to (1) determine statistically the weight coefficients in the model and (2) to validate the model, this project is temporarily postponed. Consideration has been given to acquire such data from a comprehensive survey of neurological diseases and disorders.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02295-02 OBE

PERIOD COVERED

October 1, 1977 through September 30, 1978

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., Head, Section on Epidemiology, OBE, OD, NINCDS

OTHER: Judith E. Hogg, M.D., Neurologist, Section on Epidemiology, OBE, OD, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Office of Biometry and Epidemiology

SECTION

Epidemiology

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Development of operational systems and a series of questionnaires to be used in an ALS registry being implemented by the ALS Society of America.

ANNUAL REPORT
October 1, 1977 through September 30, 1978
Director's Report
Extramural Activities Program
National Institute of Neurological
and Communicative Disorders and Stroke

The Extramural Activities Program (EAP), NINCDS, was organized in July 1975 to serve as the Institute center for science administration and fiscal management of the grant, fellowship, and research contract programs. The structure of EAP includes components responsible for manpower, scientific merit review, contract management, grants management, committee management, data reporting and analysis, and program support services including processing. Fiscal Year 1978 was characterized by the stabilization of these organizational components, the definition of responsibilities, and the development of operational patterns for the conduct of Institute extramural activities.

The Extramural Activities Program coordinates grant and contract programs for the NANCDS Council, the Program Directors, the Contract Review Board, the Training Board, the Program Staff, and the Extramural Staff. The EAP undertakes periodic re-examinations of program processes, e.g., distribution of awards during the four quarters of the fiscal year; the preparation of summary data, e.g., the Research Grant and Fellowship Data Books; and the provision of fiscal information, e.g., Fiscal Status Reports, Percentage Funding Rates, and the development of alternative strategies for various budget levels.

Major changes in personnel occurred during the past year. The tragic illness and death of Dr. K. Kenneth Hisaoka, Director, Extramural Activities Program, led to the appointment of Dr. J. Buckminster Ranney as the Acting Director of EAP. Dr. John W. Diggs was appointed Acting Chief of the Scientific Evaluation Branch, EAP. Also during the year Mr. Edward Donohue was appointed as Chief of the Grants Management Branch, EAP; Ms. Charlotte Karel was appointed as Chief of the Office of Data Analysis and Reports, EAP; and Ms. Mary Layman as the Budget Analyst in the Office of the Director, EAP.

In summary, the Extramural Activities Program provides for the Director of the Institute and the Directors of the Program Areas, scientific, fiscal, and administrative management support services.

ANNUAL REPORT
October 1, 1977, through September 30, 1978
Research Grants Program
Extramural Activities Program
National Institute of Neurological
and Communicative Disorders and Stroke

The Research Grants portion of the NINCDS Annual Report is intended as an overall summary of administrative, logistical and personnel problems and developments as they pertain to the Extramural Activities Program, research grants. For purposes of cohesiveness, however, other activities (training awards, contracts, etc.) are mentioned although they are discussed in more detail elsewhere.

The research grant, contract, and training programs of the NINCDS are focused on the identification, stimulation, and support of essential research problems aimed at the improved diagnosis, treatment, and prevention of disorders of the nervous system, the neuromuscular apparatus, the ear, and human communication. They include disorders of the young (cerebral palsy, epilepsy, learning disabilities) of adulthood (head and spinal cord injury, multiple sclerosis, brain tumors) and of the aged (stroke, parkinsonism, otosclerosis). The administrative instruments used to accomplish these purposes include research projects, research program projects, clinical research centers, specialized research centers, research career awards, research career development awards, teacher-investigator awards, institutional research fellowship awards, individual research fellowship awards and contracts.

The following Table shows the number of research grant applications considered by the Council at its spring meetings in recent years.

JUNE '72	JUNE '73	JUNE '74	JUNE '75	SEPT.'76*	MAY '77	MAY '78
398	467	428	481	493	643	676

*Comparable to a spring meeting on the basis of the new fiscal year.

The number of applications reviewed has increased 70 percent with rather regular gradual increases from year to year. This is attributed primarily to the effectiveness of the research training programs of the Institute in which the output of fully trained investigators has only in recent years reached its full potential. The very large increase in May 1977 is probably due largely to the fact that for the past year or two it has been possible to fund only 25-30 percent of the approved applications. A large proportion of the unfunded applicants reapply, thus increasing the number of applications to be reviewed.

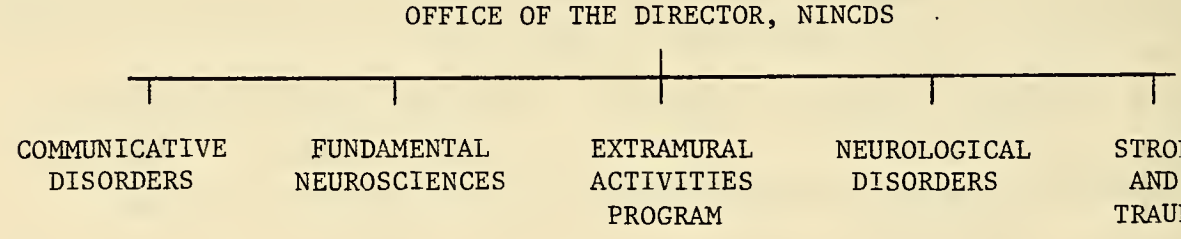
The following Table shows the number of research grants awarded and the total amounts of funds expended (in millions) each year for the past 10 years.

	FY '69	FY '70	FY '71	FY '72	FY '73
NUMBER	1,798	1,267	1,256	1,280	1,056
DOLLARS	$67.8	$48.8	$48.8	$64.2	$62.4
	FY '74	FY '75	FY '76	FY '77	FY '78
NUMBER	1,065	1,221	1,200	1,075	1,223
DOLLARS	$70.0	$86.6	$90.4	$94.9	$108.9

*Exclusive of Impounded Funds **Exclusive of Transistion Quarter

The large decrease in number of grants and in dollars between FY '69 and '70 was because all research on vision was removed to form the National Eye Institute. Since that time there has been a gradual increase in the funds available but the number of grants has somewhat levelled off, presumably because of inflation and the increasing cost of doing research.

During FY '76 the entire extramural effort of the Institute was reorganized and moved (March 1976) from the Westwood Building to the Federal Building. The reorganization into four scientific program areas and one administrative and supervisory Extramural Activities Program is illustrated below:



The Extramural Activities Program provides supervisory, coordinating and service functions for the other programs as well as for extramural activities in general. The top staff consists of:

Director
Deputy Director (Research Grants)
Assistant Director for Manpower Programs
Assistant Director for Contract Research

The responsibilities of these people are apparent from their titles in that they carry out an overall coordinating and supervisory function in regard to the implementation of recommendations of the NANCDS Council and Contract Advisory Committees, and the processing and issuance of proposals and awards

in the respective areas. The Director, in consultation with the Director of NINCDS, in addition works closely with the other Program Directors on questions of policy. The rationale for the reorganization is that the Director of each scientific program should have available all of the mechanisms (grants, contracts, training awards) and he should use them more or less at his discretion to best accomplish the objectives of his program. It is further felt that the Program Director, in consultation with an Advisory Committee, should exert a fair degree of control and direction on the program to best meet its specific objectives. It was decided that this arrangement would function most effectively if the grants activities and the contract activities were housed together. Therefore, the grants staff were moved from the Westwood Building to the Federal Building in March 1976.

The problems which have arisen are exactly those which have been faced by all other Institutes organized in this way. The fundamental problem is to avoid having the extramural effort of the Institute break up into four separate and distinct programs, each going its independent way scientifically and administratively. It is well known that research in one area is frequently applicable to problems in another area. Therefore, it is essential that the programs be interrelated through staff discussions and adequate liaison.

In general, rather specific allocations of funds are made to each program. In the case of grants, the Program Directors have agreed that the best proposals above a certain priority cut-off should be supported regardless of what their relevance to a particular program appears to be at the moment. An effort is made to reserve a modest amount of funds which the Program Directors may use to support other approved projects which they feel are especially important to their program.

In summary the reorganization was based on the philosophy that:

1. A Program Director should exercise an appreciable degree of direction and control of his program.

2. This can be best accomplished by each Program Director having at least the two main mechanisms of project support (grants and contracts) which should be utilized more or less interchangeably to attain the objectives of the program.

3. The joint utilization of grants and contracts would be most effective if the respective staffs are housed in close proximity.

The primary logistical problems have been:

1. Attendance at NINCDS and NIH meetings, practically all of which are outside of the Federal Building, and involve transportation and parking problems.

2. Delays in receiving materials such as applications and summary statements which affect all of us, especially the Office of Program Support and Awards (OPSA).

One of the primary technical problems concerns the coding of competing grant applications for Program Areas by the Office of Data Analysis and Reports (ODAR). Under the new schedule of deadlines, Study Section and Council meetings, the applications for the next Council meeting (September) are received just before the current (May) Council meeting, a time when ODAR is especially busy with priority lists and other Council activities. Incoming applications must be arranged on the shelves numerically within each Program Area. Therefore, they must be coded by Program Area before they can be arranged. However, the coding by ODAR comes at a time when ODAR is overwhelmed with work for the immediate Council meeting. As a result the coding, and the ready availability of applications to program staff may be delayed, perhaps even until after Study Section meetings which now come at almost the same time as Council meetings. Like many other problems, this one results primarily from the very cyclic nature of many EAP activities. Consideration is being given to some type of reorganization within ODAR to resolve this problem.

Table I is attached which shows the number of awards and the amounts of funds expended for each type of award within each program area.

TABLE I

NUMBER OF AWARDS AND DOLLARS* EXPENDED BY PROGRAM AREA AND TYPE OF AWARD

PROGRAM AREAS

TYPE OF AWARD	CD		FN		ND		ST		TOTAL	
	No.	Dollars	No.	Dollars	No.	Dollars	No.	Dollars	No.	Dollars
Research Grants	202	$13.824	271	$17.311	403	$28.655	132	$10.202	1008	$ 69.992
Program Projects and Clinical Centers	17	6.821	9	3.145	26	12.531	28	12.060	80	34.557
Contracts	14	1.460	19	2.300	23	7.910	15	1.886	71	13.556
New Training Grants	17	1.010	8	.705	18	1.399	2	.051	45	3.165
New Fellowships	45	.709	56	.812	106	1.681	10	.154	217	3.356
Teacher-Investigator Awards	8	.235	-	-	26	.787	6	.204	40	1.226
Research Career Development Awards	15	.509	22	.703	46	1.561	7	.227	90	3.000
Research Career Awards	1	.033	-	-	3	.095	1	.031	5	.159
TOTALS	319	$24.601	385	$24.976	651	$54.619	201	$24.815	1556	$129.011

*Dollars in millions

October 1, 1977 - September 30, 1978

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Assistant Director Contract Research Programs Report
Extramural Activities Program
National Institute of Neurological and
Communicative Disorders and Stroke

Research contracts are employed by the Institute for areas of research which are not receiving sufficient investigative attention and Institute staff are prepared to provide the management required by the contract mechanism. The Institute's programs have panels for the review of contract concepts, and the NINCDS Contract Review Board reviews contract awards.

The number and dollar value of research contracts for FY '78 are:

	Number	$ in Thousands
Communicative Disorders Program	14	1,460
Fundamental Neurosciences Program	19	2,300
Neurological Disorders Program	23	7,910
Stroke and Neural Trauma Program	15	1,886
Office of Biometry and Epidemiology	14	1,400
Intramural Research Program	27	3,097
	112	18,053

The Assistant Director Contract Research Programs is also the NINCDS focal point for the P.L. 480 Special Foreign Currency Program. The NIH Special Foreign Currency Program supports collaborative research projects in countries where the U.S. Treasury Department has a balance of local currency excess to the needs of the U.S. These countries are:

Burma	India
Egypt	Pakistan
Guinea	Poland

The scientific content of the P.L. 480 research is complementary to other NINCDS research and covers both clinical and laboratory studies. Each of the P.L. 480 projects must have a U.S. scientist to serve as a collaborating Project Officer who interacts with the foreign scientist in varying degrees. The role of the U.S. Project Officer varies from that of an active collaborator participating in the research to that of an informed sponsor. The Project Officer must each year review the expenditures and the progress report and advise the Institute whether the progress has been satisfactory.

ANNUAL REPORT
October 1, 1977 through September 30, 1978
Assistant Director for Manpower Programs' Report
Extramural Activities Program
National Institute of Neurological
and Communicative Disorders and Stroke

The Institute has four training programs. Two, the Individual National Research Service Award Program and the Institutional National Research Service Award Program, are funded from $7.322 million available in the FY '78 budget for training. The other two, the Research Career Development Award Program and the Teacher-Investigator Development Award Program, are funded from FY '78 funds available for research grants.

Normally awards for training are made following the three meetings of our National Advisory Council. FY '78 was unusual in this respect because we made awards following only two meetings of the Council, January and May. Sixty-eight Individual National Research Service Award applications that were recommended for approval in September, 1977 were funded out of FY '77 funds and are not included in the tabulations below.

This report concerns specifics of the four training programs:

Individual National Research Service Award Program

This program receives the highest funding priority. The Institute has always given priority to supporting individuals who submit their own postdoctoral research training applications and following the January and May Council meetings, we funded all applications which received priorities of 300 or better. During the reporting period, NINCDS made the following number of new and continuation awards, according to NINCDS program area:

Program	New Awards		Continuation & Renewal Awards		Total	
	Number	Amount*	Number	Amount*	Number	Amount*
Communicative Disorders	27	$ 441	23	$ 328	50	$ 769
Fundamental Neurosciences	50	621	20	311	70	933
Neurological Disorders	43	703	87	1.332	130	2.035
Stroke and Trauma	7	109	6	90	13	198
Total	127	1.874	136	2.061	263	3.935

*Amounts in thousands

The NINCDS Training Board has agreed that Individual NRSA applications recommended for funding are those having the highest priority. Thus the cut-off for funding, according to priority, is the same for each NINCDS program area. The funding of any application below the cut-off, which a Program Director considers important to his program, would be discussed individually with the Assistant Director for Manpower Programs.

Institutional National Research Service Award Program

From FY '78 funds, the Institute provided continuation support for 36 institutional NRSA programs that had future years of committed support and made nine new awards. NINCDS made awards to support the following programs, according to NINCDS program area:

Program	New Awards		Continuation & Renewal Awards		Total	
	Number	Amount*	Number	Amount*	Number	Amount*
Communicative Disorders	2	52	15	958	17	1.010
Fundamental Neurosciences	3	235	5	530	8	765
Neurological Disorders	2	276	16	1.225	18	1.501
Stroke and Trauma	2	111	0	0	2	111
Total	9	674	36	2.713	45	3.387

*Amounts in thousands

These 45 awards, for the year July 1, 1978 through June 30, 1979, will provide stipend support for 205 individuals, 44 predoctoral trainees and 161 postdoctoral trainees.

The NINCDS Training Board selects the specific training grant applications to be recommended to the National Advisory Council for special consideration for funding.

Research Career Development Award Program

During the period covered by this report, NINCDS made the following number of new and continuation Research Career Development Awards, according to NINCDS program area:

Program	New Awards		Continuation & Renewal Awards		Total	
	Number	Amount*	Number	Amount*	Number	Amount*
Communicative Disorders	7	232	11	394	18	626
Fundamental Neurosciences	7	200	17	578	24	778
Neurological Disorders	9	290	39	1.364	48	1.654
Stroke and Trauma	6	198	1	29	7	227
Total	29	920	68	2.365	97	3.285

*Amounts in thousands

Of the approved applications, those recommended to the Advisory Council for special consideration for funding are those selected by each NINCDS Program Director.

Teacher-Investigator Development Award

During the past year, we made a number of changes in the TIDA Program. One change was to add the word "Development" to the name of the program. Other changes were to place a stronger emphasis on the purpose of the program. Although this has always been a program for the preparation of individuals to engage in clinical research, the guidelines were changed to stress this specific emphasis. Since many of the TIDA applicants are in their final year of residency training, and have essentially no research experience, reviewers were sometimes unwilling to recommend five years of support for an untried applicant. Thus, we made a change in the review of the application for the -04 year of support. Although committed to the candidate for five years of support, when the -03 year award is made, we will send out the application for the -04 year. In the -04 year application, the candidate is required to specify, in detail, his accomplishments during the initial two years and to specify, in detail, the training plan he would follow during the -04 and -05 years. The -04 year application would be reviewed by a Program Project Review Committee and an award would be made only if the candidate could report an appropriate amount of progress during the first two years of support and only if he/she had an adequate training plan for the final two years of the award. One other change was to include a $4,000 per year research allowance which would enable the individual to purchase badly needed supplies (chemicals, animals, glassware, modest pieces of equipment, etc.) for his/her training program.

During the reporting period, NINCDS made the following number of new and continuation awards, according to NINCDS program area:

Program	New Awards		Continuation & Renewal Awards		Total	
	Number	Amount*	Number	Amount*	Number	Amount*
Communicative Disorders	2	61	5	150	7	211
Fundamental Neurosciences	0	0	0	0	0	0
Neurological Disorders	11	406	15	380	26	786
Stroke and Trauma	2	67	2	64	4	131
Total	15	534	22	594	37	1.128

*Amounts in thousands

ANNUAL REPORT
October 1, 1977, through September 30, 1978
Contracts Management Branch
Extramural Activities Program
National Institute of Neurological
and Communicative Disorders and Stroke

The Contracts Management Branch (CMB) consists of the Contracting Officer, who is Chief of the Branch, three Contract Specialists, and two supporting staff members.

During the fiscal year 1978, the CMB was responsible for some 100 research contracts and Interagency Agreements, totaling $18.0 million in value. In addition, there are 75 research contracts in various stages of being closed-out administratively.

The CMB expects to award approximately 18 new contracts during FY 1978. Added to this workload is 60 renewals of existing contracts and over 100 actions not involving funds but modifying contracts in some other manner.

The CMB has been coordinating the training of NINCDS Project Officers in accordance with the Department-wide requirement for training of Program personnel serving in this capacity.

ANNUAL REPORT
October 1, 1977, through September 30, 1978
Grants Management Branch
Extramural Activities Program
National Institute of Neurological
and Communicative Disorders and Stroke

During Fiscal Year 1978, the Grants Management Section and the Office of Program Support and Awards were combined to form the Grants Management Branch. As a result of this reorganization, the Office of Program Support and Awards was redesignated the Grants Processing Section.

Several changes within each section have occurred since the reorganization. For example, the responsibility for closing out terminated grants has been transferred to the Files Unit in the Grants Processing Section. Also, the use of the Grants Processing Worksheet was initiated during FY '78. This worksheet was designed to aid both Health Scientist Administrators and Grants Management Specialists in their pre-award review of a grant application.

In the Grants Management Section there has been an effort for greater involvement in both the pre- and post-award phases of a grant. Examples of such involvement include the administrative review of administrative increase requests, the identification of consortium activity and the review of the contracts required to support such consortium administratively, fiscally, and scientifically, and a closer monitoring of expenditure reports which has resulted in some additional savings for the various programs.

ANNUAL REPORT
October 1, 1977, through September 30, 1978
Scientific Evaluation Branch
Extramural Activities Program
National Institute of Neurological
and Communicative Disorders and Stroke

The Scientific Evaluation Branch (SEB) was established in 1975 and assigned responsibility for the technical merit and scientific review of Contracts, Program Projects, Clinical Research Centers, Institutional National Research Service Awards and Teacher-Investigator Development Awards. The Communicative Disorders Review Committee, Neurological Disorders Program Project Review A Committee, Neurological Disorders Program Project Review B Committee, and Special Review Committee are components of the SEB and provide the initial technical merit and scientific review of the proposals for the Institute. Summary statements are prepared by the SEB for the Institute staff, Contract Review Board and the Institute National Advisory Council.

The SEB has established liaison with the Intramural Program of the Institute, the Communicative Disorders Program, Fundamental Neurosciences Program, Neurological Disorders Program, and the Stroke and Trauma Program to facilitate the technical merit and scientific review of contracts, grants and fellowships. The SEB maintains a continuing liaison with leaders of the scientific community for the purpose of identifying the most qualified persons to serve on SEB panels and committees.

ANNUAL REPORT
October 1, 1977, through September 30, 1978
Office of Data Analysis and Reports
Extramural Activities Program
National Institute of Neurological
and Communicative Disorders and Stroke

In the past year the Office of Data Analysis and Reports (ODAR) was affected by several major personnel changes. The chief of the Office and the computer programmer left and were replaced during this period. To cope with an increasing number of requests for programming services by the program area scientific and administrative staff, the Office recruited a programmer trainee who works under the direct supervision of the senior programmer. The trainee provides real assistance in many of the simpler aspects of programming and this aid will become even more valuable in the future. A considerable programming effort is necessary to convert the format of computer records maintained by ODAR to the same length as Division of Research Grants' records. This task is underway and when completed should simplify data retrieval involving the use of both current and historical data.

The Office continues to publish Data Books (currently active) and Summary Books (fiscal year). In addition, this year the Office issued the first edition of a new volume, NINCDS Index to Research Grants and Contracts. This Index will be revised and published annually. While keeping abreast of requests for special and recurring reports using our existing automated data system and manual files, we are cooperating with the Office of Program Planning and Evaluation in its efforts to install and operate a data base management system for the Institute.

Several employees have received training in various job-related areas, Computer Programming, Medical Terminology I and II, IRS (Inquiry and Reporting System), Effective Communications, the Role of OMB in Grants.

In recognition of their performance the employees of the ODAR received a Group Special Achievement Award in February.

ANNUAL REPORT
October 1, 1977 - September 30, 1978
NEUROLOGICAL DISORDERS PROGRAM
NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE
NATIONAL INSTITUTES OF HEALTH

The Neurological Disorders Program supports research in the developmental disorders, convulsive and neuromuscular disorders, demyelinating and sclerosing disorders, disorders of adult life, and infectious diseases of the nervous system. The program supports 570 research grants, program projects, and specialized research centers with an expenditure for FY-1978 of $43.629 million. Research in the developmental disorders and in the convulsive disorders was also supported under the contract mechanism with a dollar value of $7.91 million for FY-1978. Statistically, the program has not changed significantly since its inception July 1, 1975. This has not been due to lack of research opportunities, but rather reflects fiscal limitations in a period of high inflation coupled with a relatively stable budget. During 1978, the Neurological Disorders Program received approximately 600 approved applications for research support. At this point in the fiscal year, 218 of these have been awarded. Our award rate for FY-1978 of 37% contrasts favourably with 16% for 1977 in the Neurological Disorders Program. We have thus managed to prevent the further erosion of the biomedical research base in these disorders of such import to the health of the nation, and have provided for a modicum of recovery. It is hoped that this progress will continue. A funding rate of at least half of those applications approved by rigorous peer review is necessary to attract into research careers (both clinical and fundamental) in the neurological disorders the most talented of the nation's young physicians.

The Developmental Neurology Branch (DNB), NDP, underwent extensive reorganization during Fiscal Year 1978 as a result of (1) the completion of primary and secondary analysis and interpretation of data and publication of findings derived from the NINCDS Collaborative Perinatal Project; and (2) a determination to establish new research initiatives in mental retardation, cerebral palsy, autism, and CNS birth defects. To facilitate research efforts and management of these new initiatives, the Director, NINCDS, has approved the creation of four new sections within the DNB. These are as follows: Section on Mental Retardation and Learning Disorders; Section on Cerebral Palsy and Other Motor Disorders; Section on Autism and Behavioral Disorders; and Section on Birth Defects and Genetic Disorders.

Three of the five existing Comprehensive Epilepsy Programs were reviewed during this fiscal year and have been extended for two years. This year also saw the addition of sodium valproate to the armamentarium of anti-convulsant drugs. The Epilepsy Branch contributed significantly to the scientific data needed for FDA approval. A request for applications has been issued in a continuing effort to stimulate research interest in novel chemical structures with anticonvulsant activity. The program has

established a new grant supported center for research in multiple sclerosis concerned especially with investigation into the putative viral etiology of MS. This year we also set up a research center in the peripheral neuropathies, especially diabetic neuropathy, at the Mayo Clinic in Rochester. The program held a workshop to define and provide criteria for the differential diagnosis of the Guillain-Barré-Landry Syndrome. These will be published soon in the archival literature. New program efforts have been devoted to research in Reye's Syndrome. We supported an International Conference on Reye's Syndrome in Halifax which provided important suggestions for future directions. The book from the Workshop/Conference on Alzheimer's Disease/Senile Dementia and Related Disorders, edited by Drs. Katzman, Terry, and Bick, is expected in October 1978. The second follow-up conference which will focus on behavioral, treatment, and public policy issues in these disorders will be supported in part by the program, and is scheduled for December 6-8, 1978. The reports of both the Huntington's Disease and the Epilepsy Commissions have been exhaustively reviewed by the program. Both of these provide us with an opportunity and a challenge in implementation of their recommendations, which should have a significant impact on the national research thrust in these two disorders. Program professional staff have been extensively involved with several of the Research Panels of the Long Range Research Strategies effort in the development of their reports. Our program interfaces with the Convulsive and Neuromuscular Panel, the Disorders of Early Life Panel, the Pain Panel, the Inflammatory, Degenerative and Demyelinating Disorders Panel, and the Neurological Basis of Behaviour Panel.

Increasingly complex regulations and "red tape," especially in the area of recruitment, have frustrated our efforts to recruit high-level scientific personnel. Despite extensive and continuing efforts, we have not yet been able to appoint a leader for the demyelinating and sclerosing diseases, nor a head for the autism branch. Restrictions on travel funds have made it difficult to provide adequate monitoring of contract supported research and have limited our efforts in program development in areas of importance such as the dementias and the disorders of early life.

Mr. George Durall, Administrative Officer, Neurological Disorders Program, retired effective June 30, 1978 after many years of providing exemplary administrative support services to the Neurological Disorders Program. Mr. Durall's retirement precipitated the consolidation of two of the three NINCDS administrative offices housed in the Federal Building which previously provided administrative services to four different program areas. Mr. John Jones has assumed the responsibility as principal Administrative Officer for the consolidated office. Mrs. Betty Thorowgood has been assigned responsibility for day-to-day administrative activities in managing personnel, travel, space, procurement, budget, etc. for the NDP. Mr. Christopher Leyes has been assigned similar responsibilities for the remaining three program areas.

As a consequence of the reorganization of the Developmental Neurology Branch, efforts are now underway to transfer DNB employees into the new sections and to identify, recruit and place section chiefs. In addition, the Perinatal Research Section within the DNB has been renamed the Collaborative Perinatal Section with appropriate changes in its functional statement to reflect changes in its research objectives. As a result of this reorganization and change in research initiatives, several employees of the DNB with specialized expertise have become excess to the personnel needs of the Branch and the NDP. Earnest efforts are now underway to provide suitable employment for these employees elsewhere in the Institute.

The NDP was allotted a net increase of 4 permanent full-time employment positions during Fiscal Year 1978. It is anticipated that the NDP's on-board strength will match its assigned employment ceiling of 87 positions by September 30, 1978. However, some difficulties have been encountered in recruiting qualified personnel to fill those vacancies which require highly specialized scientific expertise which could preclude the filling of these positions by September 30, 1978.

The NDP and the Institute continue to be the major benefactors of the efforts of a host of loyal, dedicated and conscientious federal employees. This is best exemplified by the award of nine (9) Quality Step Increases, two (2) Group Cash Awards, two (2) Individual Cash Awards and five (5) promotions to employees within the program during FY-78. In addition, Dr. Katherine Bick, Assistant Director, NDP, was awarded the NIH Director's Award for her superior accomplishment in organizing the joint NINCDS, NIA, and NIMH Workshop/Conference on Alzheimer's Disease/Senile Dementia and Related Disorders.

Finally, the NDP administrative support and management staff has experienced a marked increase in FY-78 of NIH, DHEW, and Presidential directives which are designed to enhance fiscal management of government resources, but which have unfortunately created a by-product of increased "red tape" and paperwork thus severely hampering the staff's ability to provide effective administrative services.

CONTRACT NARRATIVE
Neurological Disorders Program
October 1, 1977 - September 30, 1978

CLINICAL NEUROLOGY INFORMATION CENTER AT THE UNIVERSITY OF NEBRASKA
(NIH-NINCDS-72-2300)

Title: The Operation of a Clinical Neurology Information Center

Contractor's Project Director: Walter J. Friedlander, M.D.

Current Annual Level of Funding: $160,000

Objectives: To operate a specialized Information Center on Clinical Neurology. This Center will be an international focal point for information relating to those diseases of interest to NINCDS, especially information relating to diagnosis, treatment and prevention of diseases of the brain and central nervous system. The Center is producing reviews of various clinical problems of interest to the Government, bringing together the relevant clinical knowledge as it applies to the problem. These reviews may focus on an entire disease problem as a whole, or on any distinct part of a disease entity. The Center will identify sources of information relevant to clinical neurological problems, including indexing services, abstracting services, periodical journals, books, monographs, etc.

Major Accomplishments: The Center's most innovative product and one that has been received enthusiastically by the approximately 1200 scientists who receive it is the Concise Clinical Neurology Review. This publication emphasizes a one sentence (terse) abstract of each paper in a cluster of terse statements on a single topic. A number of these topics are covered in a single issue of the bulletin. The bibliographic citations are referenced by a number and appear together in a second part of the bulletin. Approximately 375 papers are selected for inclusion each month, based on a review of 940 serials. This publication seems to fulfill a need not otherwise met in neurology. It is produced once every two weeks and has a subscription rate of $28 per year.

Proposed Course of the Contract: This program is under the surveillance of an NINCDS project officer and its performance is under continued review.

A N N U A L R E P O R T

October 1, 1977 - September 30, 1978

Neurological Disorders Program

National Institute of Neurological and Communicative Disorders and Stroke

National Institutes of Health

Extramural Grant Activities

DISORDERS OF EARLY LIFE AND OTHER NEUROLOGICAL DISORDERS

This program covers several neurological areas pertaining primarily to the developmental, sensory and metabolic aspects of the nervous system. Statistically this program supports 235 research projects, active as of July 1, 1978, with a total investment of about 19 million dollars. In fiscal year 1978, a total number of 234 research grant applications were assigned to this program. Out of these 74 were funded giving a funding ratio of 1:3.

Developmental program is directed toward understanding the normal neurological events in the development of CNS and effects of internal and/or external stimuli, stresses, or organic factors which influence these normal sequences as determined by structural, organizational, neuronal connections, communication, and recognition. Also, the following broad areas of research are covered under this program: Vitamins - Role of thiamine in brain disorders, folic acid deficiency and brain development, Vitamin B6 in Down's Syndrome; Nerve Growth Factor - Isolation, characterization, and functions of NGF, role of NGF in neuronal differentiation and regeneration; CSF and Hydrocephalus - CSF formation and absorption in hydrocephalus, pathogenesis of hydrocephalus, role of choroid plexus in regulation of CSF; Synaptology - Synaptic transmission, synaptic relations in sensory system, synaptic connectivity in motor system, nerve-muscle synapse, nervous system development and synapse formation; Cell Surface Events - Cell adhesion and contact, effect of lectin-like protein on cell recognition; Membrane - Membrane connections and drug action, cell interconnection and transport across membranes.

Sensory Modalities include studies on spinal somesthetic pathways, sensory properties of electrical brain stimulation, neural control of ingestive behavior, development of perceptual capacities, etc. Pain and related aspects include a considerable portion of this program. For example, effects of local and general anesthesia are being investigated at the sub-cellular level with respect to membrane conductivity, permeability and transport. Also included in this are the studies of hemispheric specialization of cognitive mode, laterality of handedness, motor function, attention and learning, state-dependent control of somatic reflex activity, neuronal control of discrete behavioral processes, etc. A detail report on pain will be presented later in this report.

Learning and Memory: One of the most intriguing and challenging areas of research involving the nervous system is the way information is received, stored, transferred and retrieved, either on short term or long term basis. Mainly two lines of attack have been proposed. One involves spread of learning from one brain region (hemisphere) to another region as detected by neurophysiological stimulation and recording methods and delineation of factors that influence the direction of EEG asymmetry in perception and learning. The second mode of attack involves biochemical approaches. During the past few years evidence has been presented suggesting that catecholamines are significantly involved in memory processes. There is some indication that experimentally induced amnesias can be reversed by manipulation of catecholamine systems which may have some potential treatment of the various amnesic states

in man. Also, closely related to this approach is a demonstration that certain proteins have a critical role in the functional mechanisms of learning and retention of information. A current hypothesis is that proteins may have neurosecretory function in the brain. In clinical terms, the results might suggest that certain types of senile dementias, mental retardation and chronic alcoholism, where there is memory loss, may arise from a decrease in the capacity of the CNS to make specific proteins. As indicated above electrophysiological and biochemical approaches are being used but thus far have added little to our understanding of basic mechanisms in man. Intensive investigation in exploiting these and other approaches is underway.

Inherent and induced metabolic disorders: Lysosomal storage diseases are emerging as an important and rapidly expanding group of inherited human disorders of metabolism. Most of these diseases have progressive nervous system deterioration as their major component. Onset of neurological signs in these disorders range from infancy to adult life, and all are severely disabling or fatal diseases. Data on population genetics and incidence are fragmentary due to the recent discovery of most of these diseases and because biochemical methodology needed to differentiate between clinical subtypes has only become available within the past few years. Most lysosomal diseases, are inherited as autosomal recessive traits. The insidious nature of this pattern of inheritance permits the carrier genotype to become widespread in a population before recessive patients appear in significant numbers. The benefits and limitations of screening, prenatal diagnosis and selective abortion for prevention of these diseases are apparent. Progress in therapy of the lysosomal diseases has progressed rapidly. Results from patient trials have provided encouragement and sufficient justification for mounting an intensive effort in the development of safe and effective methods for treatment of these diseases. This program supports fundamental as well as clinical research on lipid and other inherited diseases. Some of these diseases are Nieman-Pick, Gaucher's, Krabbe's, Fabry's, metachromatic leukodystrophy, etc. Gangliosidoses (e.g., GM_1, GM_2, GM_3) form an integral part of this research area.

A detailed report on Lysosomal Storage Diseases follows: Also included in this program are related areas pertaining to myelin, cholesterol, phospholipids, keto and hydroxy fatty acid metabolism.

Malnutrition and Toxic Substances: There is strong evidence that nutritional and environmental entities cause encephalopathy which are probably due to the alterations following damage to the CNS. These studies are concerned with morphological and biochemical changes in isolated microcirculatory fractions in these disorders.

Neuroendocrine Disorders: Studies are being conducted on the influence of thyroid hormones on developing brain with the ultimate aim of understanding mental retardation occurring in hypothyroid conditions. The effects of neonatally administered glucocorticoids and thyroxine on postnatal cell formation are being examined since both treatments lead to cell deficits in developing brain.

Lysosomal Inherited Storage Diseases

Introduction *

Lysosomal storage diseases are emerging as an important and rapidly expanding group of inherited human disorders of metabolism. Most of these diseases have progressive nervous system deterioration as their major component. Onset of neurological signs in these disorders range from infancy to adult life, and all are severely disabling or fatal diseases. Data on population genetics and incidence are fragmentary due to the recent discovery of most of these diseases and because biochemical methodology needed to differentiate between clinical subtypes has only become available within the past few years. The most reliable information of this type comes from studies of Tay Sach's disease. Data from mass screening programs and projections based on birth of diseased children agree closely and place the carrier frequency of this disease among Ashkenazi Jews at 1 in 30. Of greater significance to the U.S. population as a whole is that while only 30 percent of patients with this disease are non-Jews, current estimates predict that between 500,000 and 800,000 Jews and non-Jews living in the United States are carriers of this disease. World figures are projected to be 5-7 times this number. These figures are remarkable but not entirely unexpected since this disorder, and apparently most lysosomal diseases, are inherited as autosomal recessive traits. The insidious nature of this pattern of inheritance permits the carrier genotype to become widespread in a population before recessive patients appear in significant numbers.

The benefits and limitations of screening, prenatal diagnosis and selective abortion for prevention of these diseases are apparent. Progress in therapy of the lysosomal diseases has reached a pivotal juncture. Results from patient trials have provided encouragement and sufficient justification for mounting an intensive effort in the development of safe and effective methods for treatment of these diseases.

Although much remains to be learned about the pathogenesis of lysosomal enzyme deficiency diseases, current knowledge is suffciently advanced to warrant focusing increased attention and effort on the final and most important phase in the evolution of research on these diseases; enzyme replacement therapy. Results of *in vitro* tissue culture studies and preliminary *in vivo* trials in man and animals encourage the notion that exogenous lysosomal hydrolase administered parenterally, can be taken up and utilized by enzyme deficient tissues to correct a metabolic defect. While this experience justifies optimism and encourages continued pursuit of promising replacement therapy, several fundamental questions must be answered and certain technical hurdles surmounted before this goal can be fully achieved.

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Common Characteristics of Storage Diseases

Genetics

One of the common features of these storage diseases is that they are all hereditary. With the exception of Fabry's disease (and possibly adrenoleukodystrophy and Menke's disease which are not strictly lipid storage diseases), they are transmitted as autosomal recessive defects. Statistically, law of Mendelian inheritance is followed. Two heterozygous individuals will produce one normal, 2 heterozygotes and one affected child. This observation is supported by the fact that in the heterozygotes the defect, as determined by enzymatic activities, falls in between the values of normals and those of affected offsprings. Heterozygotes, even with 50% of genetic defect lead normal lives.

Fabry's disease, on the other hand, is transmitted as a recessive characteristic by the X-chromosome. This means that only the mother need be a carrier to have an affected (in this case hemizygous) male child since males have only 1 X-chromosome. Half of the carrier's sons will have the disorder, half will not be involved. Fifty percent of her daughters will be heterozygotes. The clinical picture of Fabry's disease is a little more complicated than other lipid disorders since some of the female heterozygotes have manifestations of the disease although usually of much less severity.

Detection of heterozygotes: Clinical detection of carrier state can be made by measuring enzyme activities when leukocyte preparation from venous blood are reacted with suitable substrates. The test is reliable and provides a convenient procedure for estimating the rate of progress of the disease, because the determination carried out with white cells accurately reflects the level of enzyme activities in all body tissues. Similar reliable tests can be made using cultured skin fibroblasts. These fibroblasts can be stored at liquid nitrogen temperature, thawed, and can be regrown in tissue culture medium still retaining all the characteristic features of parent-cell line. Use of leukocytes and fibroblasts have obviated the necessity of diagnostic procedures involving organ biopsies. There are, however, a few exceptions where the correct diagnosis cannot be established using intact fibroblasts or leukocytes, e.g., in I-Cell disease and in Batten disease.

Genetic counseling: The combination of amniocentesis, the ability to grow fetal cells in tissue culture, and knowledge of the specific enzymic defects in lipid storage diseases has provided genetic counselors with the ability to diagnose these storage diseases in the second trimester of pregnancy. The feasibility of these procedures has been demonstrated repeatedly. For example, in one clinical center, using a method for the prenatal diagnosis of Tay-Sachs disease by amniocentesis and enzyme assay, over 25,000 individuals throughout the United States, Canada and other nations have been tested for their carrier status. Over 100

pregnancies at risk for Tay-Sachs disease have been monitored. Approximately one-fourth of these have been terminated electively after the fetus was found to have Tay-Sachs disease. Also 43 pregnancies at risk were monitored. Out of these 10 affected fetuses in utero were detected and 33 were unaffected. The diagnosis was confirmed in all instances save one in which tissue was unavailable. Thus far, no errors have been made in these series.

Biochemistry

The main core of nearly all accumulated lipids in the storage diseases is called ceramide. Ceramide is composed of mainly three components; two long chain saturated fatty acids joined through an amino acid-serine. The first product is sphingosine. It is a long chain amino alcohol synthesized in all of the body tissues by the combination of two simple precursors, palmitic acid (C16) and serine.

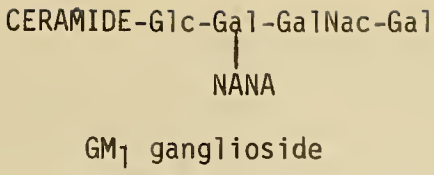
The second step in the formation of ceramide is an addition of another long chain saturated fatty acid usually stearic acid (C18) attached to the nitrogen atom of carbon 2 of sphingosine.

Alcoholic group of ceramide can be substituted with a variety of compounds to form different sphingolipids. For example, in Niemann-Pick disease, lipid that accumulates is ceramide attached to phosphorylcholine. This is called sphingomyelin which can be defined as ceramide-phosphoryl-choline. The defective enzyme is sphingomyelinase which is unable to cleave the terminal phosphoryl-choline moiety.

Cerebroside is a generic term for monohexosylceramide, i.e., alcoholic group is substituted by a sugar hexose such as galactocerebroside, glucocerebroside found in Krabbe's disease and Gaucher's disease respectively. Sulfatide is galactocerebroside with an additional sulfate group attached to galactose found in metachromatic leukodystrophy.

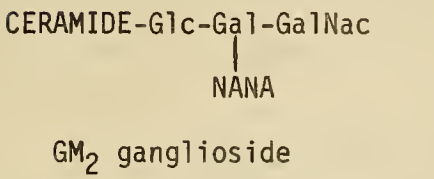
Ceramide oligohexosides are sphingolipids in which a series of hexoses are linked together. Unlike cerebrosides, all ceramide oligohexosides in the brain have a glucose moiety linked to ceramide. For example, in Fabry's disease the accumulated compound is ceramide-glucose-galactose-galactose (ceramide trihexoside).

Gangliosides are defined as sphingolipids that contain sialic acid. Sialic acid is a generic name for N-acylneuraminic acid, and the Acyl group of sialic acid in gangliosides of brain is always acetyl form, N-Acetylneuraminic acid and is abbreviated as NANA. There are about 12 kinds of gangliosides that have been identified in the brain. All have ceramide oligohexosides as the backbone with one or more NANA moieties attached. Out of all these gangliosides, GM_1 and GM_2 and their asialo forms are intimately involved in gangliosidoses.



GENERALIZED GM

GM_1 ganglioside



TAY-SACHS
SANDHOFF
JUVENILE

GM_2 ganglioside

CERAMIDE-Glc-Gal-Gal-GalNac

SANDHOFF

globoside

Morphological Features

While much is known about the biochemistry of these storage diseases, the knowledge about the morphological basis, as to how the lipids accumulated lead to neuronal dysfunction is woefully lacking. It is surmised that the substances accumulated may be cytotoxic. For example, in Krabbe's disease the hypothetical presence of psychosine is implicated in the selective loss of oligodendroglia. Unfortunately, no direct evidence is available that psychosine or other accumulated stored lipids lead to neuronal alteration resulting in neuronal dysfunction.

Recently, studies have been conducted on the tissues from several different forms of human gangliosidosis in an attempt to characterize abnormalities from the standpoint of neuronal geometry. The studies showed the presence of new 'dendritic-like' structures covered with many spines between the cell body and initial axonal segment of pyramidal neurons. Electron microscopic examination of the surface features of these large neuronal processes, designated "meganeurites" showed that the spines are postsynaptic components of morphologically identifiable synapses. In classical Tay-Sachs disease (infantile GM_2 gangliosidosis) meganeurites of extraordinary shape were found in virtually all pyramidal neurons revealed by the Golgi method. It is proposed that meganeurites and meganeurite synapses may contribute to the onset and progression of neuronal dysfunction in storage diseases by altering electrical properties of the neuron and modifying integrative operations of somadendritic synaptic inputs.

Metabolites Accumulated

In each storage disease a specific lipid or a class of related lipids has been shown to be accumulated in nearly all the body organs and in blood and enzymes responsible for their terminal degradation are either absent or inoperative. However, in Krabbe's disease the accumulation of galactocerebroside has not been demonstrated, even though galactocerebrosidase is absent. Similarly, in Gaucher's disease, only spleen, lymph nodes and bone marrow show accumulation of glucocerebroside; CNS involvement has not been implicated.

Enzymatic Abnormalities

Each storage disease is characterized by a specific (one) enzyme which is unable to cleave the terminal moiety of stored lipid. That means that the synthetic processes involved in the synthesis of stored compounds are normal, it is the final step in the degradative process which is impaired. There are, however, certain exceptions to this general statement. For example, in the I-Cell disease, absence of two enzymes is suggested; beta-galactosidase and alpha-fucosidase. Similarly, in adrenoleukodystrophy evidence of two-disease entity has been presented. Also multiple sulfatase deficiency in metachromatic leukodystrophy has been observed.

Enzyme Replacement Therapy

The theoretical possibility of correcting inherited lysosomal diseases by enzyme replacement was recognized soon after the pathogenetic basis for these diseases was first advanced. During the past decade significant progress has been made toward this worthy goal. For example, patients with Tay-Sachs, Fabry's and Gaucher's diseases were injected with enzyme purified from human urine or placenta. The following results were attained:

Most of the exogenous enzyme administered to an infant with GM_2 gangliosidosis was concentrated in the liver but was not found in brain or cerebrospinal fluid. A precipitous reduction (50%) in plasma globoside occured 4 hours post-infusion. Other plasma sphingolipids rose to levels above pre-injection values. There appeared to be a 2.4 fold increase in liver hexosaminidase above that which could be accounted for by exogenous enzyme activity, suggesting possible mutant enzyme activation. The patient's neurological disease was not apparently altered.

Patients with Fabry's disease were infused with exogenous ceramidetrihexosidase with resulting reduction in serum ceramidetrihexoside to normal levels. Evidence is presented to support the conclusion that hydrolysis of this substrate occured at an extra-circulatory site, probably within lysosomes.

Patients with adult Gaucher's disease were infused with exogenous glucocerebrosidase, with resulting decrease of levels of glucocerebroside in liver and erythrocytes.

In addition to some of the examples cited above, several trials involving human patients and experimental animals with other storage diseases have also been undertaken. Although, in principle the technique of correcting these disorders with fibroblasts has been fully established, its clinical application in diseased subjects is far from perfect. It has been observed that the injected enzyme is readily metabolised in the liver and very little, if any, reaches the brain. Thus, the major problem in enzyme replacement therapy in lipidosis is the existence of blood-brain barrier.

Blood-Brain Barrier: The blood brain barrier in vertebrates is thought to be created by the specialized structure of the capillary-glial complex. There are two components to the blood brain barrier: 1) a passive component, which acts as a physical barrier and consists of endothelial cells which are sealed by tight junctions and which contain few intracellular vesicles capable of transporting material from blood to brain; and 2) an active component which is responsible for selective permeability and which is composed of the enzyme systems of endothelial and adjacent astocytes which acts to facilitate transfer of substances in one or both directions. This component has many characteristics of transporting epithelia.

A number of experimental treatments, including mechanical trauma, thermal lesions, embolism, hypercapnic hypoxia, enzymatic disturbances, hyperosmotic solutions, extreme stress, radiation, electric shock, infection, intoxication, acute hypertension, hyperbaric oxygenation, urea and other solutes, can lower the blood-brain barrier with localized fluid and proteins into the intracellular spaces of the brain parenchyma. The great majority of these procedures, although useful in studying various physiopathological aspects of the blood-brain barrier, do not offer immediate possibilities of clinical application.

Recently, emphasis has been placed in devising methods of enzyme delivery to the target cells which offer the greatest potential for circumventing the impediments offered by the blood-brain barrier. These methods must take into account the stability of enzyme in extracellular fluids and lysosomes and the efficiency of enzyme uptake by target and other cells. This form of therapy requires that in addition to being efficacious in amelioration of lysosomal disease, the treatment must not complicate the primary disease and should not induce secondary complication.

Enzyme encapsulation: Recently, a significant number of reports have appeared which provide encouraging evidence that encapsulation of enzyme in lipid vesicles (liposomes) may overcome many of the difficulties encountered in perfecting enzyme replacement therapy. Liposomes consist of lipid bilayers alternating with aqueous compartments, arranged in closed concentric spheres. Since these artificial liposomes are composed of lipid and aqueous layers, they have both hydrophilic and lipophilic properties and as such can entrap water soluble as well as lipid soluble compounds. Using this technology the feasibility of administering enzyme (beta-galactosidase) in GM_1 cats has been tried.

Preliminary results indicated that liposomes are non-toxic when administered intravenuously. Encapsulated B-galactosidase remained stable in liposomes for a period greatly exceeding that of naked enzyme. Liposomes were concentrated in several organs (including brain?). Uptake of liposomes by feline leukocytes and skin fibroblasts greatly exceeded that of naked enzyme. Although the results seem encouraging, direct delivery of intact enzymes to brain cells by this method seems a remote possibility. It is however, possible to deliver smaller peptides or other activators of reasonably smaller molecular weight which may pass through blood-brain barrier to activate a dorment or mutant enzyme in the brain.

Activators and inhibitors: As indicated above, the enzyme replacement therapy as such offers a remote possibility for curing lysosomal storage diseases. Use of activators or helper protein which can cross blood-brain barrier may be the next logical step to be followed. This approach has been tried in Krabbe's and Gaucher's disease. Several compounds structurally related to galactocerebroside or ceramide have been synthesized. One group of homologous compounds particularly N-decanoyl-2-amino-methylpropanol acted to stimulate the galactosidase in rat brain. As much as 62% stimulation could be achieved in vitro, presumably by combination of the amide with some sensitive site on the enzyme molecule. A test with brain from a child who had died of Krabbe's disease showed that the activator worked with this enzyme too, as well as with the normal human enzyme. This finding raises the possibility that Krabbe's disease could be treated by administration of an appropriate, more effective enzyme stimulator.

Use of inhibitors in blocking or slowing down the synthesis of cerebrosides has equally good potentialities in treating these storage diseases. A series of inhibitors for glucosyltransferase, the enzyme that synthesize cerebroside from ceramide has been developed. All contain a ketone or epoxy group that apparently binds covalently with the enzyme thus blocking or slowing down cerebroside synthesis. These compounds are being tested in animals.

Some tests with potential inhibitors showed good activity with N-decanoyl-3-phenylamino-3-propanol. It acts noncompetitively, persumably at some sensitive site. In culture media with fibroblasts from Gaucher patients, it produced normal appearing cells, presumably by slowing the rate of glucocerebroside accumulation. Thus this compound may have a potential value in the treatment of patients with Gaucher's disease.

Specific enzyme inhibitors may also be useful in producing animal model for specific storage disease. For example, conduritol-beta-epoxide when injected into mice, caused no appreciable increase of glucocerebroside in spleen and in liver, but caused 3-4 fold elevation in the brain. At the same time following a single injection, glucocerebrosidase was inhibited. The activity at 5 hours was found to be almost zero in four organs. The enzyme activity gradually returned almost to normal level in 16 days.

In addition to these synthetic stimulators or inhibitors, naturally occurring activator has been purified from human liver. It is a glycoprotein. The physical properties of the activator are: heat-stable, nondialyzable; molecular weight about 21,000; isoelectric point (pI), 4.1. The purified activator stimulates the hydrolysis of GM_1 by B-galactosidase, GM_2 by B-hexosaminidase as well as ceramide trihexoside by alpha-galactosidase A or B. The hydrollysis of sphingoglycolipids by glycosidases depends upon the amount of activator added. An antibody against the activator was developed from rabbits. The specificity of the antibody to the activator has been established.

Specific storage diseases are discussed in the following section.

Niemann-Pick Disease

Niemann-Pick disease is an inheritable lipid storage disease generally characterized by the tissue accumulation of sphingomyelin and believed to be due to deficiency of the enzyme sphingomyelinase. However, the disease is actually quite heterogeneous and at least five phenotypes have been suggested. In addition to sphingomyelin, bis-(monacylglyceryl) phosphate, bis-(MAG)P, an acidic phospholipid normally present in only trace amounts, has been reported to accumulate in some cases of Niemann-Pick disease. Furthermore, there have been a number of reports of patients with lipid storage disease, some resembling Niemann-Pick phenotypes, in which bis-(MAG)P concentration was greatly increased but in which sphingomyelin levels were normal or only slightly increased. These studies strongly suggest that the tissue accumulation of bis-(MAG)P may be related to human disease.

The accumulation of an acidic phospholipid has been identified as bis-(MAG)P in the livers of some patients with classical Niemann-Pick disease (Crocker Type A), in patients with "uncertain diagnosis" (early death, neurologic involvement with storage cells resembling a lipidosis), in a patient with "adult Niemann-Pick disease" (Crocker Type C, D or E) in which sphingomyelin levels were normal, and in a patient with late infantile amaurotic familial idiocy. Accumulation of bis-(MAG)P has been reported in a patient with hyperlipidemia associated with hepatosplenomegaly and lipid storage cells in lymph nodes, liver and spleen. Although this case resembled Niemann-Pick disease clinically in some aspects, liver sphingomyelin levels were normal. Liver bis-(MAG)P elevation with intermediate levels of sphingomyelin and sphingomyelinase were recently reported in another case of adult Niemann-Pick disease. Finally, human "Niemann-Pick-like syndrome" has been induced by the coronary vasodilator, 4,4'-diethylaminoethoxyhexestrol, in which bis-(MAG)P accumulates in liver, spleen, muscle and lymph nodes. This disease could be reproduced in rats by feeding 4,4'-diethylaminoethoxyhexestrol.

In the past few years much additional progress has been made in understanding the biochemical basis for some of the Niemann-Pick lipidoses, especially Types A and B. However, the nature of the defect in types C, D and E is still undetermined; sphingomyelin metabolism is apparently relatively normal in these cases. Bis-(MAG)P metabolism may be the abnormality of pathophysiologic importance in these diseases. Studies are being conducted to elucidate the relationship between disordered bis-(MAG)P metabolism and the pathophysiology of Niemann-Pick disease(s) as well as some unclassified lipidoses. Finally,

phospholipds are important structural components of biomembranes and are important in the integrity and proper function of many membrane-bound enzymes, and although lipidoses of the kind described above are rather rare, it is probable that work on the biosynthetic and catabolic pathway of phospholipids will have a more general significance in terms of the biogenesis and properties of biomembranes.

Interaction of enzymes with lipid substrates: Since lipids are water insoluble their interaction with enzymes is a phenomenon of heterogenous catalysis resulting from the fact that the true substrate is not a molecule of the lipid but a colloidal dispersion. The nature of dispersion depends upon the type of lipid and the method used. Prenatal or postnatal diagnoses of the lipidoses are becoming more widespread, but the existence of variants of the diseases and interfering factors while assaying the enzyme in reliable diagnosis are not recognized. This is especially true when lipid substrates are used. Conflicting data were reported on the nature of the enzymatic defect of a disease, e.g., on the utilization of lactosyl ceramide in Krabbe's disease. This disease was erroneously identified as lactosylceramidosis and was proposed as a new disease. It is common experience that enzymatic reactions in which the substrate is a lipid frequently yield unreliable and irreproducible data. Currently, water-soluble, synthetic substrates are employed in diagnosis of lipid storage diseases with result that unreliable data are obtained. To mention a few: the physical state of the water-insoluble substrate, the membranous structure of the enzyme, the presence of non-specific absorption of the substrate or the enzyme.

Because of the difficulty in working with water-insoluble substrates, the pre- or postnatal diagnosis of the lipidoses are done with artificial, synthetic water-soluble substrates. However, several diseases cannot be diagnosed this way (i.e., Niemann-Pick's disease). Furthermore, this procedure might lead to the selection of wrong isoenzymes which happen to have a high affinity to the synthetic substrates. Such an erroneous selection of a wrong enzyme, may be a matter of life and death for an embryo or infant suspected of having a lipidosis. It is therefore imperative that a thorough understanding of the nature of the interaction of enzymes with lipid substrates be acquired in these diseases. It is precisely this approach to which a considerable part of research efforts is being directed. This approach has already yielded a significant contribution to the theoretical as well as the methodological aspects of lipid enzymology.

Gaucher's Disease

Gaucher's disease is characterized by the accumulation of glucocerebroside primarily in spleen, liver and bone marrow, and is due to a defect in the lysosomal enzyme, B-glucosidase, which is responsible for cleaving terminal glucose molecule from glucocerebroside. The disease is transmitted as an autosomal recessive trait and follows Mendelian inheritance pattern. Three forms of Gaucher's disease have been identified; infantile, juvenile and adult. The deficiency of enzymatic activity corroborates with increasing age of onset of the disease, i.e., lowest in infantile increasing to about 50% of normal control in the adult form. Although primary defect is in lipid metabolism, except in infantile form, CNS involvement is not clear.

Enzymes involved in glucocerebroside metabolism have been identified and characterized with respect to properties, developmental changes in rat brain, and distribution among cell types and organs. Assay procedures were developed for these enzymes, involving whole tissue and natural substrates. Many new compounds, resembling the natural substrates of these enzymes or the products of enzyme action, were synthesized and tested for their effects on the enzymatic rate. Several of these compounds acted as abnormal substrates for glucocerebroside (and galactocerebroside synthesis). A number of these compounds proved to be good inhibitors, either competitive or noncompetitive. Some of the synthetic compounds show promise for inducing disorders in animals that resemble the natural human genetic disorders, Gaucher's and Krabbe disease. Others offer promise in the amelioration of these diseases. These findings are presented in the introductory part of this report under the caption "Stimulators and Inhibitors".

Enzyme replacement therapy: It has been suggested that the glucocerebrosidase deficiency characteristic of this particular disorder may be amenable to treatment through supplementation with concentrated doses of purified normal enzyme. This approach seemed particularly appealing since involvement of the nervous system often does not occur and because the majority of the immobilized lipid is deposited within the reticulo-endothelial system where exogenous enzyme might be expected to be localized. The successful isolation of glucocerebrosidase from human placental tissue provided the opportunity for initiating these studies. The enzyme was infused into two patients with Gaucher's disease and a dramatic clearance of glucocerebroside from the liver as well as the circulation was observed.

An examination of the lipid levels in these same patients several months following enzyme infusion revealed the levels of glucocerebroside in blood were still substantially below the preinfusion level a year after it was administered. This provides a hypothetical 'long term' model of lipid accumulation in Gaucher's disease. These considerations at the molecular level provide added encouragement for further investigation of enzyme replacement in hereditary inborn errors of metabolism.

Future studies: Characterize the purified enzyme in terms of properties. Examine the nature of the bond with substrate and activating materials, such as bile salts, emulsifier, and helper-proteins. Look for activated and inactive enzyme forms. Look for naturally occurring activators and inhibitors, including helper-proteins. Use the purified enzyme to make antibodies. Label the antibody with an electron-dense material and locate the enzyme within cells by electron microscopy. Use the antibody to determine the reason of low enzyme activity in Gaucher's disease. Try to develop a large-scale isolation method for enzyme particularly from human urine or tissue, and see if it can be used in the treatment of Gaucher's disease.

Metachromatic Leukodystrophy (MLD)

Pathology of Metachromatic Leukodystrophy: The first indication of a biological role for an arylsulfatase was observed with a deficiency of arylsulfatase A in tissue of patients with metachromatic leukodystrophy (MLD). MLD is a genetically determined disorder characterized by accumulation of cerebroside

sulfate (sulfatide), particularly in the nervous system, which results in progressive neurological degeneration. Although the sulfolipid is a carbohydrate linked sulfate ester rather than an arylsulfate, a cerebroside sulfatase fole for arylsulfatase A was implied. It was shown that purified porcine kidney arylsulfatase A did indeed have cerebroside sulfate sulfohydrolase activity. It was also demonstrated that extracts of tissues from MLD patients were unable to hydrolyze cerebroside sulfate. In order to elicit sulfatidase activity by the normal enzyme, it was necessary to supplement it with a heat stable complementary factor. Thus the requirements for the hydrolysis of the physiological substrate appeared to be more complex than those for synthetic substrates.

Forms of MLD: Actually MLD is not a single genetic disease; a variety of mutant enzyme forms associated with this disease have been shown. For example, MLD is arbitrarily divided into three classical forms (late infantile, juvenile, and adult) differentiated by the age of onset of clinical symptoms. While it is difficult to clearly show any reliable level of arylsulfatase A in tissues or cultured cell extracts with most cases of MLD, it seemed unlikely that enzyme activity could be totally lacking in all forms of the disease. By a test system utilizing intact tissue culture cells, it has been demonstrated that cells derived from patients with later stages of the disease retain cerebroside sulfate hydrolyzing capability. In fact, the level of activity in this system was directly correlated with the age of onset of clinical symptoms. In general, an enzyme deficiency state could be due to decreased normal enzyme production, increased degradation, or the formation of a mutant form. By utilizing antibodies against normal human arylsulfatase A, it has been possible to show that a mutant enzyme protein is in fact present in cultured MLD cells and MLD tissue. While there is less antibody reactive protein in MLD cells than in normal, it is considerably more abundant than anticipated from residual enzyme activity alone. In fact evidence has been presented for antigenically active material in tissues even from late infantile MLD patients where essentially no residual enzyme activity can be detected.

Other conditions showing an arylsulfatase A deficiency have also been noted, such as multiple sulfatase deficiency disease and an atypical form of MLD. One patient's cells had 10% of normal enzyme when assayed with synthetic substrate, but no activity was found when assayed with cerebroside sulfate. It is thus apparent that not only a variety of mutant forms of human arylsulfase A can be expected, but some may differ from the normal only in a subtle manner. A thorough characterization of the normal human arylsulfatase is therefore a necessary prelude to unraveling this complex system of human genetic defects.

Comparative characteristics of arylsulfatases A: Arylsulfatase A has been prepared from several animal tissues. It has also been purified from human sources. It was isolated at a high state of purity from placenta, however, the final product was exceedingly unstable and essentially no studies were performed on this purified enzyme. Very recently enzyme has been purified from urine. Comparitive studies indicated that the activities of the enzymes from the three sources were essentially identical toward two synthetic substrates, 4-nitrocatechol sulfate and 4-methylumbelliferyl sulfate, and the physiological substrate, cerebroside sulfate. Observations on other properties of the human enzymes are rather limited as yet, but by several parameters the

human enzymes were similar to the ox enzyme. The amino acid composition of the human liver enzyme was similar to that of the ox enzyme in that proline content was unusually high, there was a preponderance of hydrophobic amino acids and there was an excess of acidic amino acids. The human enzymes have a pH of 4.7 compared to 3.4 for the ox enzyme so the excess of acidic amino acids was not as great in the human enzyme.

Studies with fibroblasts: Pioneering studies led to the identification of the metachromatic material in neural tissue as cerebroside sulfate, the deficient enzyme as arylsulfatase A, and the proof that this enzyme functioned as a cerebroside sulfate sulfohydrolase. Later, it was shown that the disease could be readily diagnosed by enzymatic assay of leukocytes. It was observed that cultured fibroblasts derived from a patient affected with late infantile MLD expressed the arylsulfatase A deficiency. The expression of the enzyme defect by cultured fibroblasts implied that MLD was amenable to prenatal diagnosis. Case reports of examination of five pregnancies at risk for MLD have appeared and many other unreported cases. At least four fetuses were found to be affected and termination of pregnancy was elected in each case.

Opinion is divided on whether leukocyte or fibroblast arylsulfatase A activity level is a reliable index of heterozygosity for MLD. The cumulative data on a statistical basis showed an activity ratio of 2:1:0 for control subjects, obligate heterozygotes and patients affected with MLD. This is consistent with a gene-dosage effect. However, in experimental set-up (leukocytes: 65, 7 and 4; fibroblasts: 34, 10 and 14) there was considerable overlap between the control and heterozygote groups, so that positive identificaton of the genotype of any isolated individual has been tenuous. Nevertheless, investigations carried out on several high risk families have shown clear segregation patterns within the family, so this approach may serve some utility. The greater difficulty in heterozygote diagnosis for MLD as compared with Tay-Sachs disease may be a reflection of the greater genetic heterogeneity involved.

Enzyme replacement therapy: Soon after the basic enzyme defect had been recognized, enzyme replacement therapy was tried in MLD patients. The treatments were ineffective, but there were a number of experimental limitations which are basically unavoidable with such trials. It was felt that it would be desirable to carry out preliminary feasibility studies in a controlled model system, and fibroblasts in culture offered such a system. A crude preparation of arylsulfatase A was isolated from human urine. MLD fibroblasts incorporated this enzyme added to the growth medium and retained activity for extended periods. When enzyme loaded cells were challenged with cerebroside sulfate, the pattern of incorporation and release of inorganic sulfate was identical to that of normal cells. Alternatively, if the cells were first allowed to accumulate cerebroside sulfate, than transferred to medium containing enzyme, the accumulated sulfatides were cleared. Thus, the feasibility of enzyme replacement, at least in MLD fibroblasts, was established. While there is considerable distance between curing a flask of fibroblasts and a clinically effective therapy, the tissue culture model offers a valuable tool for basic testing.

Future investigations: The long term goals are to understand metachromatic leukodystrophy (MLD) at the molecular level. The enzyme deficient in this

genetic disorder is arylsulfatase A and an understanding of its physiological function(s), natural history, and physico-chemical properties is central to this goal. There is increasing evidence that there is a family of allelic mutations affecting this enzyme which in turn leads to a heterogeneity of clinical manifestations. Thus, the identification and characterization of the biochemical differences between the gene products is necessary before the relationship between biochemistry and pathology can be established. Findings from these studies will find utility in early type specific diagnosis, heterozygote identification, genetic counseling, antenatal diagnosis, and provide a rational basis for developing and testing specific approaches for therapeutic and/or prophylactic intervention.

Fabry's Disease

The metabolic defect in Fabry's disease is due to a lack of the enzyme, ceramide trihexosidase (alpha-galactosidase), which cleaves the galactosyl residue next to the terminal N-acetylgalactosamine residue in globoside. The result of this defect is accumulation of ceramide trihexoside, galactosyl-galactosyl-glucosyl-ceramide in visceral organs. Since the sequence of the saccharide chain isolated from Fabry's patients was shown to be galactosyl-galactosyl-glucosyl-ceramide, it is logical to assume that the defective enzyme in Fabry's patients is a galactosidase responsible for the hydrolysis of the terminal galactosyl residue in this glycolipid. The anomeric specificity of the galactosidase missing in Fabry's patients was not settled for a long time. In order to know its anomeric specificity, the anomeric configuration of the terminal galactosyl unit in ceramide trihexoside must be known. By using specific alpha-galactosidases, it has been proven unequivocally that the ceramide trihexoside isolated from the kidneys of Fabry's patients, normal human kidney, and other tissues contained terminal alpha-galactosyl linkages. Thus, basic biochemical studies on the alpha-and-B-galactosidases led to the correct understanding of biochemical etiology of Fabry's disease--the deficiency of a specific alpha-galactosidase. It should be pointed out that the substrate specificity of various glycosidases is far more complex than is generally realized. For example, several alpha-galactosidases isolated from various sources such as from Mortierella vinancea, Diplococcus pneumoniae, Calvatia Cyathiformis, and coffee beans, are active only in cleaving the terminal galactose present in the oligosaacharides of the raffinose family, but fail to cleave the terminal alpha-galactosyl residue from glycoproteins and glycolipids. Further studies showed that the alpha-galactosidase capable of hydrolyzing the terminal alpha-galactosyl residue in glycoproteins and glycolipids is the only one isolated from Fig Latex. Since this enzyme cleaves the terminal alpha-galactose from ceramide trihexoside very effectively, its potential use for enzyme replacement therapy in Fabry's disease is stressed.

Alpha-galactosidase isolated from human liver: Since alpha-galactosidase is the key enzyme which catabolizes ceramide trihexoside, alpha-galactosidase was prepared from human liver and placenta in highly purified form. This preparation showed 2 components, A & B. In order to elucidate the possible different physiological roles of the two isozymes, their substrate specificities were investigated. It has been reported that only alpha-galactosidase A can hydrolyze melibiose and ceramide trihexoside. In contrast with these reports, it was found that not only alpha-galactosidase A, but also B catalyzes

the hydrolysis of both ceramide trihexoside and melibiose. However, hydrolysis of ceramide trihexoside by alpha-galactosidase A or B takes place only when the activator is added to the reaction mixture. With the same units of enzyme, the hydrolysis of ceramide trihexoside by alpha-galactosidase A proceeded at a faster rate than the reaction catalyzed by alpha-galactosidase B. The physiological role of alpha-galactosidase B is still obsure. Although it has been reported that Fabry's disease is due to the deficiency of alpha-galactosidase A, the role of alpha-galactosidase B remains to be clarified.

Activator requirement for glycosidases to hydrolyze glycolipids: It was found that highly purified alpha-galactosidase (or B-galactosidase) of human liver required the heat stable co-factor to hydrolyze ceramide trihexoside (or GM1 ganglioside) respectively. This activator is glycoprotein in nature. Chemical composition of activator indicated 17 amino acids, N-acetylgalactosamine, mannose, galactose, and N-acetylneuraminic acid (sialic acid). In order to develop a sensitive method for detecting the activator in various tissues, rabbits were immunized to produce the antibody against the activator. By using the immunological method, existence of the activator in various organs was determined. Since the activator has also been isolated from the liver, differences between the activator and the bile salts were examined. Results indicated that the activator and the bile salts are not related at all. This activator was also extracted from human kidney and brain. These findings on the activator requirement for the glycosidases to hydrolyze sphingoglycolipids have revealed new information about the complexity of the catabolism of sphingoglycolipids.

Glycolipids accumulated: Kidneys of patients with Fabry's disease accumulate both ceramide trihexoside; galactosyl-galactosyl-glucosyl-ceramide and ceramide digalactoside; galactosyl-galactosyl-ceramide. By using alpha-galactosidase isolated from Ficin, studies were made on the structure of ceramide digalactoside extracted from kidneys of a Fabry's patient. It was found that fig alpha-galactosidase liberates the terminal galactose from ceramide digalactoside whereas Jack bean alpha-galactosidase has no activity towards intact ceramide digalactoside. The results established the structure galactosyl-galactosyl-ceramide for this glycolipid. It is interesting to note that both glycolipids which accumulate in Fabry's disease have the terminal alpha-galactosyl residue. These results further support the biochemical etiology of Fabry's disease as being a deficiency of alpha-galactosidase.

Enzyme replacement therapy of Fabry's disease using tissue culture as a model: Fabry's disease is an X-linked inherited catabolic disorder characterized by the accumulation of large amounts of ceramide trihexoside and ceramide digalactoside. The structure of these two sphingoglycolipids has been established as described above. The biochemical abnormality of Fabry's disease is due to a genetic defect in alpha-galactosidase. Fibroblasts cultured from the skin of Fabry's patients exhibit both the chemical abnormality and enzymic deficiency and accumulated a 4- to 6- fold excess of ceramide trihexoside. To demonstrate the correction of this phenomenon, cells previously labeled with (C-14)-glucose were grown in a medium containing a highly purified alpha-galactosidase preparation obtained from Ficin. The results indicated that, in spite of its instability in culture, alpha-galactosidase was rapidly taken up from the medium by the cultured cells. Furthermore, it catabolized the stored ceramide trihexoside

in the cells. These findings support the reports of therapeutic endeavors by renal transplantation and plasma infusion in Fabry's disease and suggest the extension of such studies to other related disorders where the cultured skin fibroblasts are chemically abnormal, namely Gaucher's disease, Lactosyl ceramidosis, and GM_2-gangliosidosis.

Future Investigations: Isolation of homogeneous glycohydrolases from various sources, especially of those capable of hydrolyzing the sphingoglycolipids which accumulate in various types of sphingolipid storage disease, characterization of physical and chemical properties and the specificities of the enzymes toward various natural substrates, isolation and characterization of the glycoprotein activator from various tissues which stimulate the hydrolysis of sphingoglycolipids and to study its role in controlling the catabolism of sphingoglycolipids, use glycosidases as a tool to study the structure of the complex carbohydrate chain in various sphingoglycolipids, investigate the possibility of using glycohydrolases for enzyme replacement therapy in sphingolipid storage diseases.

Mannosidosis

Unlike most lysosomal storage diseases of humans, mannosidosis is relatively common disease of Angus cattle. In a population of 4 million cattle in New Zealand, it is estimated that about 10% are heterozygous carriers of this recessive trait. The disease is characterized by the storage of oligosaccharides containing N-acetylglucosamine and mannose in a repetitive sequence. The enzyme deficient is alpha-mannosidase which is unable to cleave the terminal mannose from stored oligosaccharides. The disease has been defined in pathological and biochemical terms as a basis to developing a control program in cattle and as a model for studying certain aspects of human storage disease.

Purification and study of acid alpha-mannosidase have indicated mol. wt. between 300,000 and 350,000. In plasma, it exists in a higher mol. wt. either due to greater polymerization or a carrier molecule. Evidence is available that native alpha-mannosidase exists in a polymeric form and that the basic sub unit has a mol. wt. approx. 10,000. Complete purification has not yet been achieved.

Techniques for detecting cattle heterozygous for the mannosidosis genotype including factors affecting normal levels have been investigated. Plasma alpha-mannosidase levels are highly accurate in detecting heterozygotes. As age and environmental factors influence normal levels of enzyme, results of other individual tests are less accurate. Consequently, further experiments using leukocytes, lymphocytes utilizing reference enzymes are currently being evaluated. In heterozygous individuals, plasma and leukocyte enzyme levels are 37% those of normal individuals.

Basic work including the ultrastructural pathology of mannosidosis, tissue culture techniques and enzyme kinetics has been done as a prerequisite to experiments on enzyme replacement.

Enzyme replacement therapy by transplantation is being studied in an experiment involving a placental chimeric calf with mannosidosis born co-twin to a normal individual of opposite sex.

Future studies: To evaluate various techniques for identifying individuals heterozygous for a lysosomal enzyme deficiency. To purify acidic alpha-mannosidase and to study its molecular structure, various forms and properties. To study the storage product in mannosidosis and develop a quantitative test for use in enzyme replacement studies. To investigate some of the basic problems in enzyme replacement therapy using a stepwise experimental approach in expendable animals.

I-Cell Disease

I-Cell disease or mucolipidosis II is a childhood disorder. The disease is believed to be transferred in an autosomal recessive manner and is characterized by a severe psychomotor retardation, early cessation of growth in stature, minimal skeletal deformities, mild or no hepatic enlargement, absence of excessive excretion of mucopolysaccharides in urine and the presence of numerous cytoplasmic granular inclusion bodies in cultured skin fibroblasts. Death occurs between the ages of two and nine.

Microscopic examination: Cultured fibroblasts derived from I-Cell patients contain abundant refractile cytoplasmic inclusions. These inclusions became an index for this disease and the object of extensive studies. Electron microscopy revealed that these inclusions bodies are composed of multivesicular membranes and display morphology similar to altered lysosomes. A small number of inclusion bodies were also observed in some fibroblast cells of fathers of affected children. Subsequent studies of liver, brain (i.e. neurons), peripheral nervous system (i.e. Schwann cells, axons), kidney and skin derived from I-Cell patients demonstrated the presence of inclusions similar to those found in cultured fibroblasts.

Chemical identification of the inclusions: These inclusions stain positive with periodic acid-Schiff reagent and Sudan Black. Extraction of the inclusions with chloroform: methanol prior to staining with Toluidine Blue results in a weak metachromatic product. These investigations suggested that the storage material may be both glycolipid and mucopolysaccharide. Lipid determinations on cultured fibroblasts revealed that I-Cell patients contained threefold higher total lipid than control fibroblasts. This increase could not be attributed to any specific class or species of lipid. The relationship of the accumulation of lipid to the basic defect is even more puzzling since lipid contents of brain, liver and spleen from autopsied samples of I-Cell patients were within normal limits.

Identification of deficient enzymes in body tissues: Biochemical studies of I-Cell tissues and cultured fibroblasts are not in complete agreement. The most consistent finding to date is a marked decrease in the activity of B-D-galactosidase in autopsied samples of liver, brain, kidney and spleen obtained from I-Cell patients. For example, in liver the B-D-galactosidase activity was 8-25% of normal while the activity of the enzyme in gray matter was 25% of normal levels.

Enzymes in fibroblasts: In contrast to the results obtained with tissues, experiments using cultured fibroblasts demonstrated very low levels or virtual absence of B-D-galactosidase and alpha-L-fucosidase activities, while the activities of the N-acetyl-B-D-hexosaminidase, B-D-glucuronidase, arylsulfatase A, alpha-galactosidase and alpha-D-mannosidase were greatly reduced (5-25% of normal control values). In addition, B-D-glucosidase, acid phosphatase and B-D-xylosidase showed normal or slightly increased levels of activity in I-Cell cultured fibroblasts. Non-lysosomal enzymes such as lactic acid dehydrogenase and malic acid dehydrogenase were also at normal activity levels in I-Cell cultured fibroblasts. Mixing experiments employing fibroblasts or frozen tissues derived from I-Cell patients and control subjects resulted in intermediate levels of the affected enzyme activities. These data suggest that the reduced activities in the skin fibroblasts and tissue samples are not due to the presence of endogenous inhibitors.

Enzymes in culture medium: The supernatant medium from I-Cell cultured fibroblasts revealed two- to nine-fold increase in the extracellular levels of B-D-galactosidase and B-D-glucuronidase and appeared to be dependent on the type of medium used. B-D-galactosidase remained at normal levels in the supernatant media of I-Cells culture, while a 9-fold increase in specific activity occurred at 15 days after sub-culture.

Enzymes in body fluids: Increased levels of lysosomal hydrolase activities were found in the extra-cellular-fluids of I-Cell patients. Plasma contained increased enzyme levels which varied from two- to 90-fold depending on the enzyme in question (e.g. arysulfatase A activity was increased 90-fold). Cerebrospinal fluid and urine showed smaller increases in levels of enzyme activity (e.g. 7- and 3-fold respectively for arylsulfatase A). Examination of both the culture and medium and extracellular fluids of I-Cell patients revealed the absence of nonlysosomal enzymes: transaminase, creatinine kinase and lactic acid dehydrogenase. The excessive lysosomal activities in extracellular fluids suggested that there may be a significant cellular leakage in vivo of lysosomal hydrolases and that the observed reduction in the activities of multiple lysosomal hydrolases in cultured cells could be an expression of the primary defect in I-Cell disease.

The studies indicated above suggest that the deficient enzymes (B-galactosidase, alpha-fucosidase and possibly arylsulfatase A) are normally produced in vivo (body fluids) and in vitro (fibroblasts) of I-Cell patients since the activities of these lysosomal hydrolases can be demonstrated in body fluids and in culture medium in which I-Cell fibroblasts are grown.

Since most of these hydrolases are membrane bound, an apparent leakage is indicative of impairment in the membrane structure.

The enzymes leaked are apparently normal, since they react with the same substrates as those isolated by the conventional methods, it is safe to conclude that no co-factor or membrane component is required to alter their enzymatic activities..

Future studies: Prior to elucidating any mechanism for the pathogenesis of I-Cell disease, a study is needed to characterize several of the affected

enzyme activities. Crude dialyzed preparations of B-D-galactosidase, and alpha-L-fucosidase obtained from various I-Cell disease source will be characterized electrophoretically, kinetically, and thermally (activity profile with increasing temperature). The enzymes excreted by I-Cells are suggested to be more resistant to heating at 50° and more stable to freezing than the corresponding normal controls. Thus far, a detailed study has not been carried out involving the above properties of either the reduced enzyme activities in I-Cell cultured fibroblasts and tissues, or the enhanced enzyme activities present in I-Cell conditioned medium. The properties of these hydrolases, determined by the above experiments, may indicate that there are structural differences between these enzymes in the disease and normal states.

GM_2 - gangliosidosis
Tay-Sachs disease, Sandhoff's disease and Juvenile GM_2 gangliosidosis

It is currently believed that the neuronal cytoplasmic storage which occurs in the GM_2 gangliosidosis is GM_2 ganglioside, and the defective enzyme which is unable to degrade this compound has been identified. There is a generalized absence of hexosaminidase A in Tay-Sachs disease and near absence of hexosaminidases A and B in juvenile GM_2 gangliosidosis. In the above mentioned studies it was demonstrated that: (a) the enzymic defect persists in cultured fibroblasts over many cellular generations, (b) soluble endogenous inhibitors did not account for the enzymic deficiency, (c) heterozygotes had intermediate reductions of activity, (d) the defect was present in all tissues studied, (e) prenatal diagnosis of each disorder was possible by enzyme assay of amniotic cells obtained by amniocentesis, (f) both hexosaminidase A and B catalyze the hydrolysis of the terminal N-acetylgalactosamine residue of asialo-GM_2 and globoside, while only hexosaminidase A catalyzes the hydrolysis of the N-acetylgalactosamine from GM_2.

The mutation in each of the ganglioside storage disease is inherited in an autosomal recessive manner. Cultured fibroblasts from heterozygotes exhibit an enzymatic activity which is approximately 50% of normal levels. These results strongly suggest a strict gene-dosage effect for the expression of the mutant gene leading to the synthesis of a mutant protein with deficient enzyme activity. The existence of a structural gene mutation must be proven by studies involving both normal and disease tissue. In the GM_2 gangliosidoses several laboratories have used either highly purified or homogeneous hexosaminidase A and or B to obtain antisera to each protein. These studies have demonstrated that (1) hexosaminidase A and B cross-react immunologically, (2) cross-reacting material against hexosaminidase A and B is present in tissues from patients with Sandhoff's disease, and (3) cross-reacting material against hexosaminidase A is not detected in tissue samples from Tay-Sachs disease.

Hexosaminidases: In spite of an explosion of knowledge about the ganglioside storage disease in the recent years, the precise mechanism whereby the genetic mutation leads to deficient enzyme activity in each disease is not yet known. The following hypotheses have been postulated to account for the GM_2 gangliosidoses.

Hypothesis 1: Hexosaminidase B is normally converted to hexosaminidase A by a sialyl transferase, and the defect in Tay-Sachs disease is the absence or alteration of the enzyme, transferase. Thus, hexosaminidase A would not be present. In Sandhoff's disease, the mutation would result in synthesis of inactive hexosaminidase B. Since the transferase would be active in Sandhoff's disease, hexosaminidase A would be synthesized but inactive. However, using this model, it is extremely difficult to explain how hexosaminidase A activities in heterozygotes are maintained at one-half normal levels, unless both the concentrations of hexosaminidase B and the activity of the transferase were rate-limiting.

Hypothesis 2: Both hexosaminidase A and hexosaminidase B possess common and unique polypeptide chains, e.g. hexosaminidase A: aa, cc and hexosaminidase B: bb, cc. The defect in Tay-Sachs disease is a mutation in the "a" polypeptide, whereas the defect in Sandhoff's disease is in the "c" polypeptide. This model predicts that cross-reacting materials are present in Tay-Sachs tissues which correspond to mutant hexosaminidase A and cross-reacting materials are present in Sandhoff's tissues which correspond to mutant hexosaminidase A and B. This model also suggests that the "a" portion of hexosaminidase A is needed for ganglioside GM_2-B-galactosaminide activity and that the replacement of this "portion" in Tay-Sachs disease may be sufficient to reverse the defect, since the "c" "portion" abounds.

Metabolites accumulated: As stated above, hexosaminidase A is deficient in the classical form of Tay-Sachs disease. This conclusion was based on the measurement of B-N-acetylhexosaminidase activity with artificial substrates such as p-nitrophenyl B-N-acetylglucosamine or 4-methylumbelliferyl B-N-acetylglucosamine. In spite of this, there is still no direct proof that hexosaminidase A is the actual enzyme which catabolizes the Tay-Sachs ganglioside GM_2. There are apparently several variant forms of GM_2 gangliosidosis. The biochemical characteristics of patients with classical Tay-Sachs disease are the large accumulation of GM_2 ganglioside in nervous tissue, a many-fold increase of GM_2 in extraneural organs and little or no increase of asialo GM_2 and globoside in visceral organs. Total hexosaminidase activity is increased in serum and tissues, but hexosaminidase A activity is lacking.

Patients with Sandhoff's disease with clinical record of classical Tay-Sachs disease, show a transient enlargement of the spleen and liver. In these patients the GM_2 content of the brain was essentially the same as in classical cases of Tay-Sachs disease, but with an extensive storage of asialo GM_2 and globoside in neural and visceral organs. The brain and other organs of these patients were completely devoid of both hexosaminidase A and B activities. In view of the complete absence of hexosaminidases in this variant form of Tay-Sachs disease, mucopolysaccharides and glycoproteins might be expected to accumulate however, no such finding was reported.

The Tay-Sachs disease (Juvenile form) is characterized by the accumulation of GM_2 ganglioside with slight increase of both hexosaminidase A and B activity in brain tissue. The storage of GM_2 ganglioside in the Juvenile variant is particularly hard to explain. The accumulation of GM_2 ganglioside cannot be explained simply as the result of an enzyme defect, since both hexosaminidase A and B are normal or even enhanced in the brain.

Mechanism of enzymatic reaction: The role of B-N-acetylhexosaminidase in the degradation of the B-N-acetylgalactosaminyl residue in GM2 ganglioside and in other hexosamine- containing complex carbohydrate chains has not been elucidated. It has been found that the terminal B-N-acetylgalactosaminyl residue in the intact Tay-Sachs ganglioside is resistant to B-N-acetylhexosaminidase isolated from normal human brain, liver, kidney and urine. It was found that none of the B-N-acetylhexosaminidase preparations were able to cleave the terminal N-acetylgalactosaminyl residue from the intact ganglioside. Although hexosaminidases seem to be the key enzymes which can unlock the etiology of GM2 gangliosidosis and related diseases, very little is known about the nature of the various hexosaminidase isoenzymes in different tissues.

Although the etiology of sphingolipid storage disease has been shown to be the congenital defect of a specific glycosidase, the precise role of the glycosidase in the disease process remains to be established. For example, classical Tay-Sachs disease is known to be due to the lack of hexosaminidase A. Yet enzyme B is not lacking in this disease and enzymes A and B are equally active in vitro. (A and B enzymes are two isozymes for hexosaminidase. Enzyme A has an isoelectric point around pH 5 and enzyme B around pH 7.) Why enzyme B is not functional in vivo remains to be explained. Furthermore, hexosaminidase isolated from human tissue or other sources hydrolyzes terminal B-N-acetylgalactosaminyl unit in Tay-Sachs ganglioside, GM2, with great difficulty. It is apparent, therefore, that the biochemical basis of this disease as well as other lipid storage diseases is not as simple as had been thought. More studies are needed to elucidate the actual relationship between the glycosidases and disease. The fact that hexosaminidase is not able to cleave the intact Tay-Sachs ganglioside, but is capable of hydrolyzing its asialo derivative, suggests that the N-acetylneuraminic acid residue hinders the GalNAcGal linkage. By using a space-filling model, it was found that GalNAcGal linkage and the NANGal linkage are indeed sterically hindered. The possibility of the sialic acid residue to form an inner ester may also be considered. Little attention has been paid to this aspect in the past. Further research will provide an unusual opportunity for a careful examination of the glycan specificity of the various glycosidases as well as the effect of the aglycan moiety on the specificity of glycosidases.

GM1 Gangliosidosis and Globoid Leukodystrophy
Generalized gangliosidosis (GM1 gangliosidosis Type I and II)
Krabbe's disease (globoid leukodystrophy; GLD)

There are three distinct lipid storage diseases in which a deficiency of beta-galactosidase has been determined to be the primary defect. In generalized gangliosidosis (GM1 Type I) the lipid accumulated is GM1 ganglioside and in juvenile gangliosidosis (GM1 Type II) in place of GM1, its asialo derivative is stored in brain and viscera. Globoid leukodystrophy (GLD) on the other hand is a disease which does not involve gangliosides; its suspected lipid metabolite is cerebroside (galactocerebroside and/or psychosine?). Since in all these three diseases the enzyme which is either deficient or inoperative is beta-galactosidase, they have been grouped together under the same section.

In addition to these three recognized genetic diseases B-galactosidase deficiency, variants which have milder or more severe symptoms are constantly

being identified. These are collectively called mucopolysaccharidoses, and include Hurler's, Hunter's and Sanfilippo syndromes. In addition to decrease in B-galactosidase noted in the tissue from patients with mucopolysaccharidoses, decrease in the activity of two isozymic forms of B-galactosidase was also observed when the activities were measured with synthetic substrates. The evidence for the primary defect of B-galactosidase activity in these three mucopolysaccharidoses is slim and probably reflects some as yet unknown compensatory mechanism on the part of the diseased tissue. Indeed, if it were the primary lesion in these disorders, it should be manifest in fibroblasts grown in tissue culture. It is interesting, however, that only B-galactosidase activity appears to decrease in certain tissues in these genetic diseases, while the activities of other lysosomal enzymes are normal or increased.

Distinguishing features of three types of diseases involving beta-galactosidase: Of the main three lipid storage diseases, a deficiency of B-galactosidase activity has been demonstrated with natural and synthetic substrates for GM_1 Type I and Type II and only using natural glycolipids substrate in Krabbe's disease. One of the unique distinguishing features in Krabbe's disorder is the lack of overt accumulation of galactocerebroside, despite the catabolic block in the terminal degradative enzyme, B-galactosidase. On the other hand, in addition to genetic block in B-galactosidase, children with generalized gangliosidosis do accumulate GM1 ganglioside, whereas in Juvenile gangliosidosis, in addition to GM1, its asialo derivative is also accumulated in brain and viscera. It is interesting to note that even though GM1 ganglioside is accumulated in the brain, enzyme preparations from cerebral gray matter of these subjects (Type I & II) showed normal or above normal activity of B-galactosidase when reacted with galacto-, lacto-, and gluco-ceramide, whereas preparations from livers of these patients demonstrated virtual absence of B-galactosidase. In practice the activity of this enzyme is normally visualized using flourescent substrate, 4 MU-B-gal. Therefore, it appears that GM_1 ganglioside-B-galactosidase activity is mimicked by synthetic substrates, (4 MU-B-gal and p-NP-B-gal). The use of these two synthetic substrates has been made in the diagnosis of these two (Type I & II) ganglioside storage diseases a routine assay in many laboratories when a suitable enzyme source is to be tested.

Krabbe's disease can be differentiated from Type I & II GM_1 gangliosidosis by using natural and synthetic substrates: The main characteristic of Krabbe's disease is deficiency of B-galactosidase activity towards its natural substrate, galactosyl-ceramide. Further work indicated that these patients were also deficient in catabolic activity towards psychosine (acylated galactose-ceramide), monogalactosyl-diglyceride and lactosyl-ceramide.

On the other hand, using synthetic substrates, 4-MU-B-galactose and P-nitrophenyl B-galactose, the enzyme preparation from Krabbe's patients showed no defect in B-galactosidase activity.

In general, it may be concluded that Krabbe's disease can be demonstrated by inactivity of enzyme preparation towards its natural substrates and by positive action when reacted with synthetic substrates. In contrast, in Type I and II diseases, B-galactosidase is active in gray matter and can be shown when reacted with both synthetic and natural substrates.

Pathogenesis of Globoid Cell Leukodystrophy (GLD): A series of studies has largely clarified the underlying genetic cause of both human and canine globoid cell leukodystrophy (GLD), Krabbe's disease) and has contributed to better understanding of its pathophysiology. However, there are still some unanswered questions regarding the precise pathogenetic mechanism of the disease. As stated above, one of the unique characteristics of GLD is the lack of overt accumulation of galactocerebroside, despite the metabolic block in its immediate terminal degradative step. Except for the unlikely possibility that excess galactocerebroside can be removed from the brain, the only possible explanation would be the cessation of galactocerebroside synthesis by whatever the mechanism. It has been suggested that the almost complete loss of oligodendroglia in the brain of patients might explain the termination of galactocerebroside biosynthesis. However, later finding of the absence of specific galactocerebroside accumulation in the normal kidney suggested another possibility that there might be a metabolic regulatory mechanism which shuts off galactocerebroside biosynthesis in the presence of the degradative block. Furthermore, the finding of the simultaneous deficiency of psychosine degradation provided the possibility of this cytotoxic compound to be responsible for the devasting oligodendroglial cell loss.

Possible Role of Psychosine in the Pathogenesis of GLD: As stated above, a unique feature of the GLD is the lack of overt accumulation of galactosylceramide in the brain despite the block in its degradation. This appears to result from the very rapid disappearance of oligodendroglial cells during the course of the disease. In order to explain this unique biochemical pathogenesis of GLD, a hypothesis was proposed, now referred to as the psychosine hypothesis. This was based on the findings that psychosine (galactosylsphingosine) is also a substrate of galactosylceramidase and that it is highly cytotoxic. The hypothesis postulates that galactosylsphingosine, rather than galactosylceramide, might be the compound responsible for the unusually rapid demise of the oligodendroglia. The results so far have been consistent with this hypothesis. Furthermore, it received a strong support when recently for the first minute amount of psychosine in GLD brains was found 10-100 times that of normal brain. Even at this concentration, the absolute amount is extremely small. Nonethe-less, there appears some credibility to the role of psychosine in the pathogenesis of GLD.

Animal models for GM_1 gangliosidosis: Recently, a feline model of GM_1 gangliosidosis has become available. The following analogous characteristics with human GM_1 gangliosidosis Type II have been established: Feline GM_1 gangliosidosis is an autosomal recessive trait involving altered catabolism of GM_1 ganglioside. The disease in cats is indistinguishable from human biochemical characteristics. An *in vitro* fibroblast culture system has been developed which serves as a basis for *in vitro* studies of this disorder. Preliminary investigations of renal transplantation and parabiosis failed to support the thesis that renal transplantation offers promise as a method for providing active enzyme to mutant cats. A highly purified GM_1 ganglioside B-galactosidase has been isolated from normal cat brain and liver, and used for biochemical characterization of this lysosomal hydrolase including comparisons with mutant (immunologically cross-reactive) enzyme. Purified enzyme has also been used in preliminary studies of the fate, distribution and metabolic effect of exogenous enzyme administration to mutant cats.

Further investigations are being conducted to: Elucidate the factors which influence uptake and metabolic utilization of exogenous enzyme by mutant tissues, develop "packaging" techniques or molecular modification of exogenous enzyme to facilitate stability, uptake and hydrolytic function. Investigate lysosomal hydrolase administration techniques which may overcome therapeutic limitations imposed by the blood-barrier, define any deleterious physiological consequences of repeated administration of purified exogenous lysosomal hydrolases, and to evaluate the ability of cells to recover from prolonged dysfunction, following correction of the metabolic defect by enzyme therapy.

A canine model of human Krabbe's disease is also available, in which B-galactosidase deficiency has been confirmed, including detection of an intermediate, heterozygous activity levels in the histopathologically normal brain. Three genotypic levels of B-galactosidase activities are found in human serum, leukocytes and fibroblasts. These three activity levels have been confirmed in dogs, but in canine serum from normal heterozygous and affected, all had similar activity levels. With the exception of this difference in serum activity, all other catabolic deficiencies in nervous tissue and other pathological processes are virtually identical to human Krabbe's disease.

Utilizing dogs as animal models of human disease, the following aspects of this disease are being investigated: Determination of morphological features which enable certain white matter regions to resist lesion formation compared to susceptible regions, and determination of the nature and extent of the enzymatic deficiences responsible for white matter degeneration observed in Krabbe's disease.

Heredopathia Atactica Polyneuritiformis

(Phytanic Acid Storage Disease - Refsum's Disease)

In 1946, a new inherited neurological syndrome was identified called Heredopathia Atactica Polyneuritiformis. It is also called phytanic acid disease or Refsum's disease named after its discoverer. It was shown that patients with this syndrome accumulated in tissues and serum an unusual branched-chain 20-carbon fatty acid, phytanic acid (3,7,11,15-tetramethylhexadecanoic acid). Clinical studies established that there was little or no endogenous biosynthesis of Phytanic acid; phytol and phytanic acid were identified as dietary constituents. The normal pathway for phytanic acid degradation was shown to involve an initial alpha-oxidative attack, leading to the formation of alpha-hydroxy-phytanic acid, followed by decarboxylation to yield the (n-1) fatty acid pristanic acid, and finally a series of successive beta-oxidations to CO2.

Pathogenesis: The pathogenesis of the neurologic syndrome remains uncertain but there is reason to believe that phytanic acid accumulation, directly or indirectly, is responsible. The possibility was considered that accumulation of phytanate might be a reflection of a more general defect (e.g. a general defect in fatty acid alpha-hydroxylation) and that the neural changes related not necessarily to phytanate accumulation per se. However, analyses of skin lipids from patients with Refsum's disease showed normal concentrations of straight-chain alpha-hydroxy fatty acids. Furthermore, several important differences between the straight-chain alpha-hydroxylation system of brain and the alpha-hydroxylation system for phytanic acid catabolism have been described. For example, the brain system is

microsomal where as the phytanic acid oxidation system is mitochondrial; attempts to demonstrate phytanate oxidation in brain have been negative; the brain system for straight-chain oxidation is stimulated by ferrous ion while the phytanic acid oxidation system in liver is inhibited. Because of the close structural relationship between the side chain of vitamin E and the structure of phytanic acid, the possibility that the pathogenesis related to a defect in vitamin E function or metabolism had to be considered. However, direct study failed to reveal any defect in vitamin E metabolism or in vitamin E levels. The best evidence that the pathogenesis relates to phytanate accumulation comes from the improvement seen clinically when phytanate levels are reduced in diet.

Pathogenetic role of Phytanic Acid: Accumulation of phytanic acid in heredopathia atactica polyneuritiformis not only means that the clinical signs are a direct result of such a product, it also indicates evidence of a metabolic block. The fact that although liver, heart, peripheral nerves, CSF, and even parts of the retina contain an excess of phytanic acid, the brain contains only small amounts, and the so-called blood-brain barrier must be relatively impermeable to phytanic acid. It has been shown that changes are present in liver, heart, and kidney not only with regard to phytanic acid content, but also in levels of cholesterol. Death of patients may well be associated with the cholesterol accumulation rather than with an increase in an unusual fatty acid.

High levels of phytanic acid (serum phytanic values above 100 mg/100 ml) may cause acute toxic symptoms in patients as well as in experimental animals. The subject loses weight and fails to thrive. Increasing fatigue and death appear to occur at serum levels ranging between 200 to 250 mg/100 ml.

Animal studies: Several loading experiments with phytanic acid have been carried out in animals in order to provoke symptoms and signs resembling Refsum's disease. Large amounts of phytanic acid caused toxic effects, but did not produce any clinical signs or histological manifestations resembling heredopathia atactica polneuritiformis from the nervous system. However, analysis of the brain lipids showed incorporation of only trace amounts of phytanic acid, in contrast to what has been found in nervous tissue from patients. It seems clear that phytanic acid accumulates much more easily in the nervous tissue of patients than in brain and nerves of experimental animals on long-term phytanic acid feeding. The mechanism behind the different degree of incorporaton is unknown. However, it is reasonable to assume that this difference explains why it has been impossible to mimic the nervous system symptoms in animals. Hence, these experiments do not speak against a causal relationship between the accumulation of phytanic acid and the nervous lesions.

Genetics: The overall rarity of phytanic acid storage disease together with its occurrence in more than one child in a sibship strongly indicated a Mendelian recessive inheritance pattern and the almost equal occurrence in males and females indicated an autosomal character. Further studies established the existence of a biochemically identifiable carrier state. Fibroblast cultures derived from unaffected siblings or from parents of patients with phytanic acid storage disease were shown to oxidize added phytanic acid at a rate approximately 50% of that seen in control fibroblast cultures. There was little or no overlap between the results in presumed heterozygotes and those in controls. The availability of a method for identifying the carrier state may prove helpful in

certain clinical situations. For example, siblings of clinical cases can be studied to ascertain whether they do or do not have the defect early in life. Since intervention with appropriate diet appears to arrest progression, identification of cases as early as possible has potentially important implications. With the development of safe techniques for amniocentesis it is now possible to identify either the homozygous or the heterozygous state in utero.

Nature of Enzyme defect: As stated earlier, the normal pathway for phytanic acid degradation follows an unusual initial alpha-oxidative attack, leading to the formation of alpha-hydroxyphytanic acid, followed by decarboxylation to yield the (n-1) fatty acid, pristanic acid which is further degraded by a series of successive beta-oxidations resulting in acids such as acetic, butyric, dimethylacetic, etc., and finally to CO2. It has been shown that patients with phytanic acid storage disease and skin fibroblast cultures derived from them showed a marked reduced rate of conversion of phytanic acid to CO2. It was also shown that the rate of oxidation of pristanic acid, the second identified intermediate in the degradative pathway, was quite normal both in vivo and in cell culture. Further investigation indicated that the enzymatic error lies specifically in the production of alpha-hydroxyphytanic acid. This follows from the fact that the rates of oxidation of pristanic acid and of alpha-hydroxyphytanic acid are normal in patients who show a marked defect in the rate of oxidation of phytanic acid itself. Further support comes from the fact that fibroblasts in culture likewise oxidize pristanic acid and alpha-hydroxyphytanic acid normally while showing a severe defect in phytanic acid oxidation, Disassociation between the oxidation of phytanic acid and of its hydroxyl derivative has now been demonstrated in 8 different cell lines representing 8 different kinships. Thus, there is no evidence for a clinical syndrome with phytanic acid storage other than on the basis of a defect in alpha-hydroxylation since the straight-chain alpha-hydroxy acid levels in patients appear to be normal.

Over the past several years something like 100 serum samples from all over the United States and from abroad have been analyzed because of a clinical suspicion of Refsum's syndrome. Only three new examples of phytanic acid storage disease have been encountered -- one sibship in England, one in France and one here in the United States. Combined with the extensive screening that has been done at other centers (especially in Oslo and the previous screening done at the National Institutes of Health), these findings justify the conclusion that this is in fact a rare mutation and that its infrequent occurrence does not simply reflect a low index of clinical suspicion. Certainly, at all major neurologic centers the possibility of phytanic acid storage disease appears in that differential diagnosis of every patient with unexplained peripheral neuropathy and/or unexplained retinitis pigmentosa.

Treatment: Soon after it was established that phytanic acid was not endogenously synthesized but had its origin in the diet, it was possible to show a decrease in serum phytanic acid levels in patients placed on diets containing low levels of phytol and phytanic acid (fat and chlorophyll free diet). Two patients were studied on this special diet. These studies showed, in addition to the expected fall in plasma phytanic acid levels, improvement in nerve conduction velocity, improvement in some tests of muscle strength, restoration of peripheral sense modalities in some areas, reversion of ECG to a normal pattern and subjective clinical improvement.

As an emergency measure, patients with very high levels of phytanic acid may be treated by plasmapheresis. Once a week over a period of several months, 400 ml of plasma was removed, the blood corpuscles being reinjected into the patients. This technique was found to be an excellent adjuvant to the dietary treatment with a low-phytol low-phytanic acid diet. Excellent correlation between the reduction in serum phytanic acid levels and the clinical improvement in muscular strength and ataxia was seen.

Future studies: Assuming that accumulation of phytanate itself initiate the changes that lead to nerve dysfunction, it is proposed to explore the effects of incorporation of phytanate on cell function and structure. The possibilities that are considered include: 1) that incorporation of phytanate into myelin alters its structure such that it is unstable and undergoes dissolution more rapidly than it can be repaired: 2) that incorporation of phytanate into glial cells (or Schwann cells) relates to pathogenesis by interfering with repair processes or by inducing release of lytic enzymes: 3) that incorporation of phytanate into certain specific lipid components rather than overall phytanate incorporation is relevant to pathogenesis e.g. phospholipids or complex lipids in critical sites in membranes may be altered by incorporation of phytanate so that the membrane or subcellular particle is altered functionally.

Batten Disease

(Neuronal Ceroid-lipofuscinosis NCL)

Batten disease (late infantile and juvenile lipidosis) and other disorders of lipofuscin accumulation cause long term, progressive mental disability and blindness in infants and children, and sometimes in adults (Kufs' disease). By a series of histochemical and chemical studies the NCL has been defined as a group of heritable degenerative diseases with maximum effect on the brain. These diseases were considered as familial amaurotic idiocies resulting from lysosomal accumulation of gangliosides. To the contrary, it is now shown that the neuronal ceroid-lipofuscinoses are due to the accumulation of autofluorescent lipopigments resulting from an enhanced rate of peroxidation of polyunsaturated fatty acids. The hypothesis was advanced that the degradation of tissue peroxides is deficient, an assumption seemingly supported by the observation of a markedly reduced activity of a p-phenylene diamine (PPD)-mediated peroxidase. However, certain conceptual difficulties have arisen because the enzyme is deficient in at least three different human types of neuronal ceroid-lipofuscinosis, the acute Jansky-Bielschowsky and the chronic Spielmeyer-Sjogren type, both inherited as autosomal recessives, and also in the adult form, which is inherited as an autosomal dominant. Furthermore, the same enzyme is deficient in English Setters with neuronal ceroid-lipofuscinosis. In other words, an identical enzyme defect which satisfactorily explains the pathogenesis is encountered in presumably different genetic defects producing different clinical but similar pathomorphologic phenotypes.

Genetics: The disease follows strictly Mendelian recessive inheritance characteristics. This statement is verified by the fact that the activities of heterozygotes have shown PPD-peroxidase activites values in between those of normal controls and of homozygous affected. The same Mendelian ratio has been observed in experimental animal, English Setters.

Para-Phenylenediamine (PPD) Mediated Peroxidase: It has been reported that a p-phenylenediamine-linked peroxidase is deficient in the granulocytes (leukocytes) of patients with Batten disease, associated with early and excessive neuronal lipofuscin accumulation and with mental deterioration and blindness. A deficiency of the same peroxidase activity has also been found in the leukocytes of dogs with neuronal ceroid-lipofuscinosis. In addition, there was a deficiency of granulocyte myeloperoxidase, demonstrated histochemically with benzidine, which probably represents the same enzyme demonstrated with p-phenylenediamine. Normally, myeloperoxidase is present in the azurophil granules of neutrophilic polymorphonuclear leukocytes, and it is a lysosomal enzyme. Eosinophil granules also have peroxidase activity, but this appears to differ from myeloperoxidase and is believed to be a "true" peroxidase. Myeloperoxidase or PPD-linked peroxidase is reported to be similar to thyroid peroxidase biochemically found in Batten disease. Although other workers have had some difficulty confirming the findings in Batten disease, the fact that a deficiency of a p-phenylenediamine-linked peroxidase has also been detected in dogs with neuronal ceroid-lipofuscinosis supports the suggestion that a defect in this peroxidase activity is related to Batten disease.

Substrate for PPD-Peroxidase: A difficulty arises from the fact that physiological substrate for the PPD-mediated peroxidase is unknown. The procedure which reveals the activity of this peroxidase with substrate p-phenylenediamine is only 20% and 5% as efficient as a hydrogen donor as are methoxybenzidine and guiacol, respectively. Nevertheless, with these two substrates the enzyme defect cannot be demonstrated and it appears that the physiological substrate for the PPD-mediated peroxidase represents a group of heterogenous proteins, as for example, catalase.

Leukocytes: It has been observed that the PPD-peroxidase deficiency can be demonstrated only when leukocytic extract is prepared in a special manner; i.e. after freezing and thawing of white cells. Only with the soluble fraction of peripheral white cells can the deficiency of PPD-mediated peroxidase be demonstrated when compared to the similar preparation from normal individuals. There is no deficiency of this enzyme in these patients when whole leukocytes or the soluble and membrane components are combined. There is no satisfactory explanation for the deficiency of PPD-peroxidase in a soluble fraction. Presence of inhibitor in white cells of affected animals and human beings has been ruled out.

Metabolites accumulated: The hypothesis is that the pathomorphologic and clinical manifestations of the neuronal ceroid-lipofuscinoses (NCL), namely the canonically conjugated formation of autofluorescent lipopigments and progressive nerve cell death are the result of an increased rate of peroxidation of unsaturated fatty acids. The lipopigments isolated from NCL patients consist of more than 50% of an acidic fatty acid polymer. On the other hand, there is no accumulation of any one glycolipid or phospholipid. In fact, these complex lipids have concentrations smaller than those obtained from whole brain homogenates. The same applies to neutral lipids and fatty acids. Only one lipid is consistently increased in these pigments, namely lysobisphosphatidic acid, the nature of this increase remains unknown. Although the elevated concentration of this presumed intermediary is, on the relative basis, quite high, its absolute concentration is so low that

it seems highly improbable that lysobisphosphatidic acid would represent the primary non-degradable compound leading to the accumulation of lipopigment.

Recently evidence contrary to the one given above has been presented. Cystosomes filled with intensely fluorescent material in the form of curvilinear bodies have been isolated from the cerebral cortex of a 7 year old child who had died from late infantile form of Batten disease. Forty three percent of material extracted suggested presence of retinoyl polyenes linked to a small peptide. The results indicated that the fluorescent component of neuronal storage material is a retinoyl complex and is not derived from peroxidized polyunsaturated fatty acid as has been previously thought.

Experimental treatment with antioxidants: In order to test the hypothesis that Batten disease may be associated with an increased rate of unsaturated fatty acid peroxidation, dogs, English Setters with this disease were fed controlled diet containing antioxidant drug. These trials revealed that vitamin E in daily doses of 400 mg per day at the age of 7 months or earlier delayed the onset of this disease by approximately 2-3 months, however, this treatment did not improve the life span. Also vitamin E given with the onset of symptoms was ineffective. If the treatment is initiated at the earliest age, it is possible to produce 100% homozygous affected offsprings by mating affected animals which had been pretreated with vitamin E.

Treatment of human NCL patients showed that EEG abnormalities are occasionally improved or at least do not deteriorate at the same rate as in untreated patients. Also, the intellectual performance is maintained at a stationary level for years, but visual deterioration is not retarded. It should be pointed out that others who have used this treatment have not been able to confirm these results.

Experimental enzyme therapy: Enzyme therapy in genetic diseases has not produced encouraging results in a number of lysosomal diseases, however it appears that not all possibilities have been fully explored. Experience with kidney exchange transplanation between two litter mates, one affected, the other one a heterozygote, has not produced any tangible results.

On the other hand, among four homozygous affected dogs who received various combinations of splenic and bone marrow transplants at the time of birth, one has shown consistently normal peroxidase activity whereas the other three have typical values of affecteds. Since homozygous children at risk can be identified before the onset of the disease, the projected studies may lead to a more direct and curative treatment than the antioxidant regimen, provided that the biochemical studies implicate PPD-peroxidase deficiency as the probable etiology of NCL.

Adreno-Leukodystrophy ALD

(X-linked Schilder's Disease)

Schilder's disease is characterized by two clinical abnormalities. One related to adrenal insufficiency and other related to cerebral dysfunction. There is also a possibility that other abnormalities hitherto unidentified may also be

involved. Several cases of Adreno-leukodystrophy have been identified, all occurring in males.

The disease is X-chromosome linked (as in Fabry's disease) which means that only mothers are carriers of recessive trait resulting in one-half of male affected children, the other half will be normal.

Symptoms: The neurological symptoms have usually manifested themselves between the ages of 5-15 years. The initial symptoms are often behavioral and academic apathy. Decreasing vision with subsequent development of optic atrophy and loss of pupillary reaction to light. A spastic ataxic gait soon becomes evident with eventual quadraplegia and decorticate posturing. Focal or generalized seizures may occur late in the illness. Peripheral neuropathy and fasiculations have not been observed. Death has been the inevitable outcome, usually between one and five years after the first neurological finding. Electroencephalographic findings have revealed initial delta foci in the posterior hemispheres. The area of the delta activity increases as the disease progresses. Isotope brain scans have revealed an early increased uptake of isotope in the parieto-occipital lobes in two cases. Elevated cerebrospinal protein is a common finding, sometimes with an increase in gamma globulin. The combination of an abnormal brain scan, elevated CSF protein and EEG slowing has occasionally led to a mistaken diagnosis of brain tumor.

Electron microscopic studies of the adrenal gland and of a small peripheral nerve fasicle present in the adrenal capsule from ALD patients were conducted. In the zona fasiculata, striated cells were present, and the striations proved to be predominantly linear, membrane-like accumulations with the cytoplasm. These accumulations often consisted of rows of paired, electron-dense leaflets each 25Å thick, separated by a clear space which varied between 20Å and 70Å. Similar profiles were found in the cytoplasm of some of the Schwann cells surrounding myelinated axons in the small capsular nerve. The membrane-like appearance suggested these accumulations might be composed of lipoprotein and lipid. The presence of similar changes in the schwann cells and adrenal cells further support the hypothesis of a related metabolic defect in adrenal and nervous system.

Biochemical studies: There have been three biochemical studies of brain biopsy tissue from ALD and none has identified a specific error in lipid metabolism. Some workers have demonstrated a near-normal lipid content in severely affected areas of white matter while others have shown chemically abnormal myelin with increased cholesterol esters. These findings reflected a non-specific process of myelin destruction. A recent investigation of cholesterol metabolism in fibroblast cultures from patients with ALD, revealed the uptake of cholesterol and cholesterol palmitate to be similar to control fibroblast cultures, however the ALD cultures accumulated greater amounts of both substances on continuous exposure. These data are stated to be consistent with ALD being a generalized defect in cholesterol metabolism.

Adrenal insufficiency: The pathological changes in the adrenal have received little attention. Adrenal atrophy has usually been described as severe. Some of the patients have not received steroids, hence the adrenal atrophy has not

been considered secondary. No significant abnormalities of the pituitary or adrenal medulla have been described, and the zona glomerulosa of the adrenal cortex has been relatively preserved. Changes consisting of a loss of cells without an inflammatory infiltrate have been described in the zona fasciculata and reticularis.

In some patients the clinical history of adrenal cortical insufficiency has been present for as long as seven years prior to the onset of neurological disease. The earliest recorded onset of adrenal insufficiency is at 3 years. Serum electrolyte abnormalities have been rare. Melanoderma, in combination with reduced response to ACTH infusion, has indicated that the adrenal disease has been primary in these patients. Initiation of steroid therapy has not prevented the development of the demyelinating process, nor modified the relentless course once the process has started.

Rational for future studies: The simultaneous destruction of adrenal cortex, and CNS myelin in a hereditary disease has suggested that ALD is a lipidosis. This notion has been reinforced by the presence of ultrastructurally similar cytoplasmic inclusions in adrenal cortical cells, Schwann cells, testis and brain macrophages. Since adrenal cortex, testis and CNS myelin contain large amounts of cholesterol, a logical beginning for the biochemical studies is the examination of cholesterol metabolism in fibroblast cultures from patients with ALD. Another promising biochemical investigation centers around the isolation of the inclusions and their chemical characterization.

Ultrastructural and light microscopic analysis of the nervous system and endocrine changes will be of great help in understanding the evolution of the disease. The rationale for the intense study of the demyelinating process and the immunological status of these patients stems from the resemblance of the central nervous system changes to those of multiple sclerosis. Both ALD and multiple sclerosis are inflammatory demyelinating conditions and there is evidence that immunologic factors have a role in the demyelinating process of MS. An investigation for a possible immunologic component in ALD may yield results that explain the radically different light microscopic appearance and clinical courses of the CNS and adrenal process.

Cerebro-hepato-renal syndrome

(Zellweger Disease)

Zellweger disease is characterized by the absence of peroxisomes and impaired capacity of mitochondria in electron transport system. This is a rare, fatal and heritable disease. Zellweger disease seems, therefore, the first inheritable cirrhosis apparently due to, or associated with abnormal mitochondrial functions. This conclusion resulted from biochemical and morphologic studies of initial enzyme histochemical staining reactions performed on liver obtained by conventional biopsy techniques.

Despite morphologic absence of peroxisomes, total catalase activity was normal in the one liver examined. Is catalase in Zellwegers syndrome soluble rather than peroxisome-bound? The cytochemical staining procedure which differentiates soluble from peroxisome-bound catalase will be utilized to study this important

question. Because the liver of these children is cirrhotic, techniques developed for subcellular fractionation of homogenates of needle biopsies cannot be utilized. Similar enzyme histochemical studies will be performed in the heterozygote parents of the children when possible.

These studies should provide clues regarding the function of peroxisomes and the relationship between absent peroxisomes, normal catalase activity, impaired mitochondrial electron transport and inheritable cirrhosis.

Antenatal diagnosis of the cerebro-renal syndrome has been attempted with cytochemical studies of mitochondrial oxidative activities on unfixed cultured cells obtained by amniocentesis. In the initial screening test, cells were incubated for succinoxidase and other mitochondrial oxidative activities with either menadione for phenazine methosulfate, as intermediate electron acceptor. In the cerebro-hepato-renal syndrome, mitochondria have severely reduced capacity to oxidize succinate with menadione. In two instances, staining reactions were normal; normal children were born. Whether the motochondrial abnormality in this syndrome can be utilized in antenatal diagnosis is, therefore, not yet known. Additional cultures will be utilized to test this possibility.

La Fora form of Myoclonic Epilepsy

This fatal disorder, afflicts adolescents and young adults. A characteristic, nonlysosomal, mucopolysaccharide inclusions, are present in the brain, and in skeletal muscle, which are considered to be altered peroxisomes. Heptocytes are swollen with PAS-positive material and abundant SER compresses other organelles, restricting them to the periphery of the nucleus and the cell. This appearance is described as diagnostic test for LaFora's disease.

Metabolite accumulated: Electron microscopic studies showed that the fibrillar ultrastructure of LaFora bodies is hydrolyzed both by alpha amylase and by gamma amylase. These findings established the fibrillar structures, (rather than the matrix itself), are the polyglucosans. Amorphous densities also disappeared following these amylolytic enzymes, suggesting that they, too, were polyglucosan in nature.

Muscle fibers: Highly distinctive light and electron microscopic changes were found in muscle fibers in LaFora disease. For example, an abnormal, prominent stippling pattern occurred in muscle fibers with the NADH-tetrazolium reductase stain. The stippling corresponded to small membrane-bound packets of densely osmiophilic granules. Both larger and smaller granules were entirely removed after one hour of alpha-amylase digestion. Approximately 5% of packets had collections of fibrils strongly resembling those found in the cerebral LaFora bodies. The unique morphological findings in muscle permit one to diagnose this disease of the central nervous system without having to resort to biopsy of the brain. The histochemical reactions of areas of polyglucosan storage in muscle show a strong reaction with diaminobenzidine peroxidase at pH 9, an activity inhibited by 6-aminotriazole, suggesting that catalase was giving this reaction. These observations suggested that in muscle, a peroxisome-like organelle accumulate as the storage material. A clear relationship between peroxisomes and LaFora bodies in the central nervous system remains to be established.

Skin changes: Diagnostically skin changes were found in one patient, in whom the diagnosis was histologically proven. Skin lesions were restricted to the medial surface of the ear lobule, where a number of pores were filled by small blebs.

Serum studies: Serum studies on three patients during life showed they had an absolute decrease in alpha-2 globulins, and an absolute increase in gamma globulins. In contrast, parent and other normal controls did not show these changes.

Urine analysis: Urine studies have shown two sets of changes. In one study, in which LaFora's disease was histologically proven, three affected family members showed an increase in one or two fractions. These were consistent with true glycosaminoglycans (acid mucopolysaccharides) of low sulfate content.

PAIN

The term "Pain" is used in a variety of situations. It is sometimes applied in a physical sense and sometimes to the effect produced by mental processes. Physical circumstances to the perception of pain can be described as those situations in which body or any part of the body comes in contact with the objects which under normal conditions are innocuous, but under changed conditions become painful, hurtful, unpleasant, or even unbearable. For example, a bullet placed in the palm of your hand is harmless. The same bullet at an acclerated velocity can produce not only pain, it may even be fatal. Similarly, other altered physical states of objects can produce unpleasant sensation, e.g., acceleration in heat, electricity, wind velocity, etc.

Pain can also be caused by the metabolic disturbances, either inherent, induced or by the environmental factors. Psychological processes also play a considerable influence in eliciting or supressing pain. However, these influences are rather hard to assess in strictly scientific terms. Pain is a problem of major importance for two general reasons; in its chronic form it is a clinical problem which touches nearly every aspect of life. As a neurobiological problem, it is important to understand not only what actually pain is, but to know what causes pain, how it is percieved, how the stimulus causing pain is transmitted and where, if any, are pain receptor centers located. Closely related are problems where apparently physical stimulus may cause pain but the underlying biochemical mechanism may be so affected so as to either accentuate or even retard the threshhold of pain perception. The investigation of pain mechanisms goes well beyond adding essential details to our knowledge of sensory processing and somesthesis. It leads into the studies of molecular mechanisms of receptor activation, somatic, automatic and neurendocrine reflex organization; the neural basis of motivation, affect and accompanying emotional states; the neural events providing a necessary condition for learning and the determinants of attentional control over sensory and motor processes.

Current Knowledge of the Neural Mechanisms of Pain

Peripheral nerves: Clinical and experimental observations have provided strong evidence that receptors which generate impulses in certain small-diameter myelinated (A-delta) and unmyelinated (C) fibers mediate normal pain sensation. Noxious stimuli which threaten to damage tissue are required to activate these nociceptors. There is evidence that some nociceptors are activated by a substance released from the stimulated tissue. The biochemical and biophysical processes by which this is accomplished are currently under active investigation.

First central synapse: Impulses from nociceptive afferents activate physiologically and anatomically distinct cells in the dorsal gray matter of the spinal cord (dorsal horn) and in the trigeminal sensory nucleus of the brainstem. The former mediates sensation from the body and visceral organs while the latter mediates facial and oral sensations. In both structures, some central neurons appear to respond exclusively to noxious stimuli while others respond to a wider range of stimuli, showing maximal responses to noxious events. The different roles played by these different types of

neurons and the chemical substances mediating their synaptic activation are not yet established.

Pathways for pain in the CNS: A number of central structures and pathways are activated by the impulses ascending from the nociceptive spinal cord and trigeminal nucleus neurons. Some impulses travel a direct route via axons projecting to sets of somesthetic integrative thalamic neurons located between the brainstem and the cerebral cortex. At least 3 separate groups of thalamic neurons receive these direct spinothalamic or trigemino-thalamic projections. Other spinal and trigeminal nociceptive impulses travel one or more indirect routes as they ascend to the brain. These impulses activate neurons within the deep central gray matter (reticular formation) of the spinal cord and brain stem; these reticular formation neurons in turn send ascending projections to the thalamus and hypothalamus and some send descending projections back to spinal cord and brainstem sensory and motor neurons. The functional roles of the direct and indirect pathways are not yet established, but the evidence at hand suggests that the direct spinothalamic and trigemino-thalamic pathways are important for discriminating spatial, temporal and intensive features of noxious stimuli while the indirect pathways subserve motivational, autonomic, and other non-discriminative aspects of the pain experience. The role of the cerebral cortex in pain is not known.

Intrinsic mechanisms for pain modulation: At each point of synaptic activation along the pain pathways, the ascending flow of impulses is subject to modulation by the activity of other neurons. The activation of spinal dorsal horn and trigeminal nucleus neurons by nociceptive afferents is modified by concurrent activity in other peripheral afferent fibers. Similarly, fibers descending from certain neurons of the brainstem reticular formation and cerebral cortex can suppress the activation of central cells by noxious stimuli; in some instances, this suppression can be related to behaviorally manifest analgesia. There is evidence that at least some of these intrinsic pain control systems may mediate the analgesic effects of opiates and provide the neural substrate for the analgesic effects of the endogenous morphine-like peptides recently found in the brain. These exciting discoveries have significance well beyond the area of pain research and, among many other possibilities, they provide the opportunity to study pain mechanisms by producing controlled analgesia by a variety of methods.

Significant advances in our understanding of pain mechanisms have been made in recent years. The new knowledge acquired has often contributed to other areas of neuroscience and has provided a new basis for the consideration of new therapeutic approaches. There is little doubt that, with the proper resources, future research will supply the new knowledge which is necessary for continued progress in basic and clinical neuroscience.

Specific Clinical Pain Problems

The specific clinical problems are those in which pain, either chronic or frequently recurring acute pain, is the dominant complaint. Some of the examples are given below:

Headache and other cranio-facial pain: At least 40 million U.S. citizens are estimated to suffer from chronic recurring headaches of various types throughout a significant fraction of their adult working lives. The mechaism by which pain is produced in the major types of headache is not known. The pain-sensitive structures include muscle, the dura, and dural blood vessels, but little is known of the physical and chemical conditions required for the activitation of the nociceptors in these structures. In two other major types of cranio-facial pain, paroxysmal trigeminal neuralgia (tic douloureux) and temporomandibular joint pain-dysfunction syndrome, the mechaisms are no clearer. As a result of the lack of understanding of the pathophysiology of these conditions, therapy is often inconsistent and inadequate for many patients; this leads to trials of numerous drugs and ultimately to surgical procedures which may compound the problem by providing another source for pain as a result of postoperative complications.

Low back pain: The causes of this condition are legion, ranging from benign (though disabling) muscle strain and spasm to cancer involving the vertebrae and surrounding tissue. Most low back pain, like headache, is not fatal, but it affects an estimated 14 to 15 million individuals in the United States and is responsible for an estimated 4 to 5 billion dollars in direct medical costs of various kinds. An estimated 93 million work days may be lost each year because of low back pain. A lack of understanding of the pathophysiology of this disorder depends upon our present ignorance of the interactions of the biomechanics of the spine and the supporting pain-sensitive tissues.

Pain due to cancer: Many people fear the pain of cancer more than death. An estimated 345,000 people in the United States die of cancer each year; of these, the majority require relatively prolonged management of chronic or recurring pain. Potent narcotic analgesics are effective and available, but, when the disease is protracted, the development of tolerance may be a problem. Ablative neurosurgical operations on the brain or spinal cord are certainly not without risk of mortality or morbidity; many afford effective but only temporary relief and some are associated with an undesired change in the patient's personality. Further refinement of these procedures or continued development of methods for focal electrical stimulation in the brain may lead to more physiologic and specific means of long-term pain control.

Pain of other causes: Chronic arthritic pain affects and estimated 20 million people in the United States, but there is little or no information on the number of individuals suffering chronic or recurrent pain from innumerable other disorders such as trigeminal neuralgia, painful neuropathies from nerve injury or diabetes, phantom limb pain, pain due to CNS disease (central pain), or chronic, intractable pain of undetermined etiology. The heterogeneity of this group emphasizes the diversity of conditions in which pain is a major diagnostic and therapeutic problem. In each instance, inadequate knowledge of the pathophysiology of chronic pain is a major impediment to the development of consistent and effective forms of therapy.

Endogenous Peptides - Endorphins: A most important step in the recent history of neuroscience research has been the discovery of specific opiate binding sites in the central nervous system and the subsequent discovery and chemical

identification of endogenous peptides which are apparently the natural ligands for those opiate receptors. Two pentapeptides were isolated from mammalian brain which have specific binding to the opiate receptors of the brain and differed from each other only in the 5th amino acid position: "Leucine-" and "methionine-enkephalin". Subsequently, other larger peptides were found in the brain and pituitary with similar opiate binding capacity. The generic term "endorphins" was coined to encompass all such endogenous morphine-like compounds. The structure of methionine-enkephalin was found to be contained within a 91-amino acid pituitary hormone, beta-lipotropin, discovered some years earlier. Methionine-enkephalin occupied positions 61 to 65 of this longer peptide chain. It has now been shown that the entire 31-amino acid fragment comprising positions 61 to 61 of beta-lipotropin is itself a potent opiate receptor ligand, this peptide has been labeled "C-fragment" or "beta-endorphin". According to some workers beta-lipotropin may be a pro-hormone which becomes enzymatically cleaved in the pituitary or brain to form beta-endorphin and, in turn, methionine-enkephalin. On the other hand, it has been found that the quantity and distribution of beta-endorphin and enkephalin in the brain is unaffected by hypophysectomy. Also, no pro-hormone or other precursor has yet been discovered for leucine-enkephalin; so the possibility remains that beta-endorphin and the enkephalins, all of which have been found to exist intraneuronally in brain, are synthesized de novo and do not result from cleavage of still larger peptide chains.

These data on endorphins and those related to nociceptive modulation have links as follows: some are well confirmed while others need corroboration. Opiate binding sites, as well as enkephalin and beta-endorphin cell bodies and/or terminals have been found to be distributed in the brain in close proximity to each other and to medial brain stem sites known to support stimulus produced analgesia and the effects of minute injections of morphine on nociception. Beta-endorphin and the enkephalins have been found to have variable effects upon intraventricular injection or administration directly into the periaqueductal gray matter. Although the reason for the differences is not known, it seems possible that negative results are related to the fact that the enkephalins (unlike beta-endorphin) are enzymatically destroyed rapidly in the brain. Analgesic brain stimulation in the rat and in man has been reported to release measurable amounts of enkephalin-like material into brain tissue and cerebrospinal fluid. It has been reported that baseline endorphin levels are lower than normal in chronic pain patients, and that normal levels are restored by such analgesic treatments as transcutaneous electrical stimulation and stimulaton of the medial brain stem.

It seems that there is considerable evidence suggesting the existence of a natural or endogenous antinociceptive or pain-inhibitory system having a specialized anatomy and a neurochemistry involving endogenous peptides. Yet reason and historical perspective compel caution. Many of the observations still need to be replicated and extended. We have already seen that multiple paths of modulation exist, some apparently related to opiate ligands and some not. Similarly, a causal link between dorsal horn neuronal inhibition and behaviorally defined analgesia is far from established, and a great deal of the circuitry and synaptic neurochemistry of the modulation paths has yet to be disclosed. Moreover, there are several inconsistencies. In particular,

there is evidence that opiate drugs and endorphins can exert a direct antinociceptive action at the spinal level. Moreover opiate binding sites and endorphins are found in great density in those exact regions of the dorsal horn where pain inhibition is thought to take place.

Very little is known about the natural, physiological mechanisms which could act as accesses of control over the supposed endogenous pain-inhibitory system. There are data to suggest that endorphins are not released tonically under normal conditions but only when sustained discomfort or stress is experienced. It has also recently been reported that naloxone blocks placebo analgesia in placebo responsive human subjects. Whether or nor any relationship exists between this finding and the reports of naloxone blocking acupuncture analgesia remains to be determined. Evidence examining the possible naloxone reversibility of some forms of analgesia is inconclusive. Until reliable data are available indicating the conditions under which the endogenous systems producing analgesia-like effects is entrained, it can scarcely be considered a true functional entity.

It is established that opiate binding sites and endorphins are located in brain areas apparently unrelated to pain inhibition. There are questions about the effects of opiates on sensory pain perception in clinically effective doses; therefore, opiate binding is not a certain link to the sensory aspects of pain. There are observations implicating the endorphins in such diverse pathological syndromes as psychosis, epilepsy, and dyskinesia or rigidity. There are suggestions that different opiate binding sites not only are anatomically separated but also differ from each other pharmacologically. Thus it is evident that endorphins cannot be assigned a role exclusively in antinociception.

Treatment of Pain

There are four main modes of therapeutic approaches employed in the management for control of pain.

(1) Pharmacological Treatment: Alleviation of pain with minimum side effects produced by the drug is the main objective of this treatment. Drugs which are useful in the treatment of pain are classified into those which act peripherally at the tissue-receptor interface or on the receptors themselves and those which act at the level of the central nervous system. Some analgesics may act both peripherally and centrally; the site of action is not known for all analgesics.

Peripherally Acting Analgesics: The best example of a peripherally acting analgesic is aspirin. There is evidence that it has some central action as well. This common drug has the advantage of being both anti-inflammatory as well as analgesic. By virtue of its anti-inflammatory action, aspirin relieves a primary cause of pain by reducing the hyperemia, edema, and local tissue effects produced by the primary disease process and thus eliminates several potential sources of receptor activitation.

Acetaminophen is less effective than aspirin in reducing inflammation and perhaps also in reducing receptor activation which accompanies tissue damage.

The mechanism of action of acetaminophen is not as well established as it is for aspirin. Although it is not a gastrointestinal irritant at high doses, it is also generally less effective than aspirin. Severe liver damage may occur as a result of overdose with acetaminophen.

Butazoladine, phenylbutazone and indomethacin are potent anti-inflammatory compounds which are used primarily in arthritic conditions or where pain can be relieved by directly attacking the inflammatory process. These are effective drugs but do not necessarily have a direct analgesic action in the absence of an inflammatory process. They are also potentially dangerous compounds because of their potential hematopoetic toxicity.

Phenoxybenzamine has been used effectively in some cases of causalgic pain due to peripheral nerve damage. This compound is an alpha adrenergic blocker and thus pharmacologically produces some of the effects of a sympathectomy. The difficulty with this compound is that the side effects (hypotension, lethargy, and decreased sexual activity) are often not well tolerated.

Propranolol is a beta adrenergic blocker used primarily in hypertension and cardiac arrhythmia. This drug has been found to be effective, however, as a prophylactic agent in many cases of migraine headache or other vascular headaches. This compound does not have primary analgesic effects; why it is sometimes effective in migraine and vascular headache is not known.

Centrally Acting Analgesics: The most effective and widely used central analgesics are the opium alkaloids of which morphine is the typical example. Most of the other commonly used narcotic agents are derivatives of morphine produced by various chemical substitutions on the morphine molecule or other alterations of its basic structure. The common examples are heroin, merperidine, codeine, and the weak narcotic agent propoxyphene. The more potent narcotics act at the central nervous system level to produce both an elevation of pain threshold and a euphoria which is particularly prominent in the case of patients suffering severe pain. Both the euphorigenic and sensory modifying actions of the narcotics are essential components of the analgesic action. The site of action of these compounds is not yet known but there is strong experimental evidence that their primary site of action in relieving pain is at the level of the reticular formation and deep structures closely associated with the reticular formation. The recent discovery of opiate receptors in the brain provides further evidence that these compounds act primarily at the level of the central gray substance and reticular formation in the upper brain stem; opiate receptors are also found in high concentration in the striatum, but there is no clear clinical or experimental evidence that this latter structure plays any significant role in pain or in pain modulation.

Morphine and the other opioid narcotics referred to above are capable of significantly depressing respiration, stimulating various smooth muscle structures, producing a deep narcosis, and predispose the patient to physical dependence and possible addictive use of these drugs. The addiction problem is undoubtedly related to the strong euphorigenic action of these compounds.

Nitrous oxide is another potent centrally acting compound which possesses strong euphorigenic and analgesic effects. The site of action of nitrous oxide is not known, but there is evidence that it may act on structures similar to those affected by morphine.

Anxiolytic agents such as diazepam and hydroxyzine are often useful in attenuating the motivational and emotional aspect of pain. In addition, these minor tranquilizing compounds, especially hydroxyzine, may strongly potentiate the action of morphine by mechanisms as yet unknown. Major tranquilizers, such as the phenothiazines, may also potentiate the action of morphine and they may be used in conjunction with the weaker narcotics such as propoxyphene. This latter combination has been found particularly useful in the treatment of painful neuropathies.

Lithium has been found useful in the prophylactic management of cluster migraine. There is no evidence at present that lithium has a primary analgesic action of its own. Its mode of action in the prevention of cluster headache is unknown.

Other centrally acting compounds which may be of particular benefit in pain due to disease of the nervous system include carbamazepine, phenytoin, and other anticonvulsant medications. These drugs are frequently successful in controlling tic douloureux and various types of central pain.

Future Trends: Development of peripherally acting compounds which can selectively attentuate pain without interfering with other sensor modalities. The identification of specific algogenic substance(s) in tissue and mechanism by which they activate nociceptors. The identification of those regions of the nervous system which are primarily concerned with mediating the experience of pain. Development of drugs which specifically interfere with transmission along the pathways or to selectively trigger descending pain modulatory systems. Identification, distribution and elucidation of function of endogenous opioid peptides (enkephaline) and to use these peptides in the alleviation of pathological pain.

(2) Physical methods: The variety of physical and physico-chemical therapeutic techniques in current use for the relief of pain include: diagnostic and therapeutic nerve blocks; physical therapy; electrical stimulation of skin; and acupunture.

Nerve blocks for pain therapy: Nerve or analgesic blocks, in which a local anesthetic or neurolytic agent is injected in or around a site of nerves or pain-sensitive structures have been used to control acute and chronic pain for about a century. Experienced clinicians can block virtually any nerve in the body which contains pain pathways. The bases for the efficacy of nerve blocks in patients with pain is the interruption of specific sensory pathways for pain, sympathetic motor nerves, and somatic motor nerves. Blocking pain pathways relieves pain promptly and interrupts the sensory side of abnormal reflex mechanisms which may be contributing to the physiopathology of the pain syndrome. Blocking sympathetic pathways eliminates the increased vasomotor, sudomotor and visceromotor hyperactivity which often contributes to the physiopathology of certain pain syndromes such as causalgia, reflex

sympathetic dystrophies and visceral pain. Blocking somatic motor nerves relieves muscle spasm which is often associated with musculoskeletal disorders and contributes to the painful stimulation. Low concentrations of local anesthetics block the A delta and C fibers without significantly affecting motor functions.

Physical therapy: The empirical use of heat and cold, especially heat, to relieve pain is probably as old as mankind. Studies in recent years have shown that when heat, either in the form of infrared radiation or ultrasound, is applied to the skin overlying a major nerve or painful area, the pain threshold increases. For chronic pain states, it is best to use high temperatures--43-45^{o} C.--applied to the site of pathology. This usually produces relief. For acute pain of bursitis or herniated discs, it is preferable to apply milder temperature ranges for superficial relief. In many conditions it is more appropriate to apply cold. Physical exercise and postural manipulations are also used to treat or prevent pain. They are very effective when lumbar lordosis is a cause or aggravating factor in low back pain. The patient with exaggerated lordosis and accompanying increased forward (downward) thrust of the pelvis develops contracture or shortening of the spinal extensors and hip flexors. The abdominal muscles and hip extensors, which normally act as a force couple to tilt the pelvis backward (upward), may be weak. Flexion exercises are designed to stretch the spinal extensors and hip flexors, and to strengthen the abdominals and hip extensors. The patient is taught to press the pelvis backward to decrease the lumbar lordosis, first while in a supine position and then while sitting, standing and walking. Exercise is also indicated in treating patients with chronic pain due to other musculoskeletal disorders, particularly those affecting the limbs and neck. These include chronic myofascial pain syndromes, postoperative pain and disability, chronic reflex sympathetic dystrophy, and patients with chronic pain behavior due to operant mechanisms. Traction is most frequently applied to the cervical spine. It can provide significant relief of pain and relieves pressure on compromised nerve roots. Moreover, the pain relief often far outlasts the duration of traction. Why this should be so is not completely understood, but is probably due to separation of the vertebrae which, while minimal, increases the size of the intervertebral foramina. Studies have shown that maximal separation requires the application of traction for atleast 25 minutes at a force sufficient to achieve vertebral separation. It is helpful to use heat massage to relax the involved muscles and prevent voluntary contractions which can overcome traction of up to 55 pounds. The rationale for manipulation is the least well established of the physical treatment modalities. That fact, combined with the knowledge that manipulation is the therapy of choice of non-medical practitioners such as chiropractors and osteopaths helps explain why the medical profession is reluctant to employ this technique, other than in setting fractures or in regaining some motion in frozen shoulders. While the sheer numbers of Americans seeking chiropractic treatment every year suggest that some symptomatic relief for pain is obtained, scientific evidence to support the claims of osteopathic physicians and chiropractors is lacking.

Transcutaneous electrical stimulation: For hundreds of years, patients, physicians, and charlatans have played with electricity as a form of therapy for all kinds of illness, including pain. The mode of action of electrical

stimulation is unknown. The fact that a significant number of patients can be benefitted justifies its use as a clinical tool. Electrical stimulation of the skin is now a safe and inexpensive method of alleviating pain in some patients. This technological advance is the result of the development of compact, lightweight, battery-operated, solid-state devices. The major problem with transcutaneous electrical stimulation (TES) is patient selection. No valid predictors of long-term efficacy have been found. Some generalizations are possible, based on studies over the past decade: Electrical stimulation is not effective in patients with markedly abnormal or absent sensation in the region stimulated. Pain relief is usually reported only when paresthesis is elicited in the area of pain. Approximately three-fourths of an unselected group of chronic pain patients will report initial pain relief with TES, but only one-fourth will report significant pain relief lasting more than three months. Some of this pain relief may be ascribed to non-specific effects (placebo), but some is clearly related to the sensory effects of stimulation.

Acupuncture: In China, the use of acupuncture for the treatment of acute and chronic pain dates back several millennia. While there have been modifications in the concept, in the number of acupuncture points, and even in techniques over this period, it has never been abandoned. Today, therapeutic acupuncture in China is used extensively to relieve nearly all types of painful conditions. Despite the long and widespread use of acupuncture and claims of its great efficacy in treating pain and other non-surgical disorders, no strong evidence is in support of this claim.

(3) Surgical control of pain: Interruption of those pathways in the nervous system which are concerned with pain is a logical approach to the relief of intractable pain. This is especially true when powerful analgesic-narcotic drugs are ineffectual, either initially or as a result of the development of tolerance. Surgery for pain has in the past been considered a last resort, not because of intrinsic shortcomings, which exist, but because of the risk associated with surgery and the realization that severing central pathways is an irrevocable step: Nerve cells and fibers in the brain and spinal cord cannot regenerate. That surgery can provide relief from some of the most severe and disabling of human pains is well established. The paroxysmal bursts of intense searing facial pain of tic douloureux, for example, can be permanently relieved by surgery. Moreover, the newest techniques provide pain relief while sparing other facial sensibility. The persistent, progressively severe pain which often parallels the spread of cancer can be controlled for periods of six months to a year by interrupting the main pain pathway in the spinal cord. Thus, this procedure (spinothalamic cordotomy) provides pain relief exactly when it is most needed.

In the search for better methods of controlling pain, progressively higher levels of the nervous system have been explored in efforts to relieve pain or the anguish, fear, and suffering which accompanies pain. It is at this point that the issue of psychosurgery arises, for surgical intervention may affect the personality or other uniquely human quality of the individual. Improvements in the techniques of psychosurgery, whereby impairments in personality are completely or almost completely avoided, while anguish and suffering secondary to pain are controlled, might clearly benefit some patients. Such surgery would eliminate the psychological influences on the patient even

though pain, as such, might not be modified. Such procedures must always be evaluated in the context of the severe, incapacitating and at times overwhelming magnitude of the pain problem confronting the patient and the family. Psychosurgical procedures for pain must be evaluated in an effort to reach a balanced and objective position. Benefits far outweighing the shortcomings can be derived from specific psychosurgical procedures in a small number of selected cases, provided that ethical safeguards are assured the patient and treatment is undertaken by highly qualified surgeons.

(4) Psychological techniques: The psychological techniques currently employed to relieve acute and chronic pain include: 1) psychotherapy; 2) biofeedback; 3) hypnosis; and 4) operant conditioning and related methods of behavior modification. These generally unrelated modalities are all considered "psychological" techniques because pain relief is achieved primarily by altering psychological, motivational or cognitive factors by psychologically induced changes in autonomic or somatic functions, or both.

Future Outlook in Pain Research: Few areas of research endeavor touch upon so wide a range of significant problems in basic and clinical neuroscience. The biophysical and biochemical mechanisms of a unique class of receptors are to be investigated. Noxious stimuli are potent activators of a broad range of somatic, autonomic and neuroendocrine reflexes, the organizations of which are in the very early stages of investigation. Our understanding of sensory physiology, and the neural coding systems requires continued research on pain mechanisms. The activation of nociceptive afferents offers a uniquely effective way of engaging the neural systems which subserve attention, motivational and affective states, and provide a necessary condition for learning. Pain is a major, dominant component of a wide variety of clinical disorders and, especially in its chronic form, it presents a crippling burden to the society for the patient's care. There continue to be significant advances in the assessment, treatment, and management of pain based in large part upon an improved understanding of pain mechanisms. But many of the current treatments for pain are of doubtful value and some carry a significant risk of morbidity, if not mortality. Promising avenues of approach need to be explored. The present deficiencies in pain management can be attributed to lack of basic knowledge about the pathophysiology of pain, the failure to communicate what is known to the health care community, and the misapplication of current knowledge in the clinical setting.

DISORDERS OF AGING

Introduction

Aging is a gradual change with the passage of time of the structure and function of an organism and its components. It could be said that aging begins at the end of the development period. This subprogram is concerned with the neurological components of age dependent disease.

Over 23 million people in the U.S. are over 65 years old and with the extended human longevity, it is expected that this number will be increased up to 20% by the year 1990.

A substantial number of elderly citizens will develop presenile dementia, an earlier mental deterioration than would be expected in relation to their age. Furthermore, in the patients with an Alzheimer's type of dementia, the life expectancy will be reduced by about 5 years. This represents a staggering national health problem in terms of socioeconomic costs, loss of productive lives, and premature departure of our dearest.

Although they are clinically separable entities, quantitative research data tends to support the notion that Huntington's chorea, Alzheimer's disease and senile dementia represent points on a spectrum instead of unrelated disease entities. One exception to this list of presenile dementias is Jakob-Creutzfeldt disease. Recent findings concerning transmissibility of this disorder implicate slow virus as an etiologic factor and therefore it will be discussed under the infectious diseases subject. Another progressive and disabling "disease" of the nervous system usually beginning in the fifties is Parkinson's disease. Tremor symptoms appear initially in one limb and encompass other parts of the body within a few years. Encephalitis, brain tumors, head injuries and toxic agents may cause Parkinsonism, but the real etiology of this disease is still unknown.

In the fiscal year 1978 there were 54 active grants (including 5 clinical centers) for which support was $4,259,000. Seventy five applications were received of which 10 new and 12 competing renewals were funded with an expenditure of over $860,000.

The list of pathological findings in the aged brain is long and by far incomplete. There is loss of synapses and neurons. Neurofibrillary degeneration, senile plaques, amyloid deposits are examples of the changes observed, to name only a few. Electrophysiology of the brain is changed. Neurotransmitter metabolism is altered. Almost all parameters studied in the senile brain indicated some differences as compared to the controls; immunological aspects, brain proteins, nucleic acids, and enzymes. Some of these studies appear to implicate more extensively the aging process of the nervous system: cell loss (decrease in cell number with age in certain cortical areas), formation of senile plaques (Anyloid B made of immunoglobulins light chains and degenerated cells), paired helical filaments (PHF) found in neuronal cytoplasm (a protein probably similar to the one found in normal neurotubules and neurofilaments), and neurotransmitters: acetylcholine (ACh), monoamine oxydase (MO), homovanillic acid (HVA), nor-epinephrine (NE), and dopamine.

Approximately half of the program is involved with studies of Parkinsonsim. The remainder of the program is concerned with studies of Alzheimer's and Related Dementias and with studies on the Disorders of Aging that are not specifically targeted toward the dementias. A small portion is concerned with Huntington's Disease. The neurological disorders related to aging include studies of neurological mutants which lead to advanced aging, immunobiological changes dependent on the aging process, and analysis of changes of neuro-endocrine function and behavior. Pathological changes in neurofilament proteins not specifically associated with Alzheimer's are included in this category and there are several grants involved with the study of long term memory formation and with changes in the biochemical parameters of aging brain, with particular reference to membrane functions and the efficacy of membrane transport with age.

In Parkinson's Disease we have eight grants studying the disease in humans; these include four Clinical Research Centers; thirteen grants are studying the pathological process as it occurs in animals. In processes theoretically relevant to disease, we find the studies of the dopaminergic metabolism and ultrastructural and histochemical studies of synaptic transmission in the basal ganglia. At the present time, the Huntington's Disease program is a limited one. Two of the projects are involved with the study of disease in humans; the other two involve animal models. In the dementias, we have an almost equal effort devoted to disease in humans and to the pathogenic process as it occurs in animals. The animal studies here deal with the role of heavy metals and environmental toxins in producing the pathological changes in membranes, the changes in neuroendocrines, and changes in the neuropsychological status of the organism.

In these disorders the research emphasis is on the etiology of these disorders and reflects the unfortunate fact that with the exception of Parkinsonism there is no treatment. In Parkinsonism, the advent of L-DOPA in the late fifties was a therapeutic breakthrough and the work carried on in New York with the use of small incremental doses of L-DOPA to avoid some of the side effects was the key. The use of peripheral decarboxylase inhibitors greatly reduced undesired side effects. The current work involves the search for dopamine agonists which may produce more specific effects on the defective receptors without the side effects. The major side effects still concerning researchers in Parkinsonism are the "on-off" effect and the psychological disturbances that accompany long-term usage of L-DOPA.

An imbalance between acetylcholine and dopamine mediated activity is suspect in the syndrome of neuroleptic-induced tardive dyskinesia. Preliminary treatment data suggested modifying effect on the movements of the patients with tardive dyskinesia, and this study is now extended with grant support to investigate further the effect of dopamine and acetylcholine agonists and antagonists. Dopaminergic mechanisms are analyzed in cats, and a study in monkeys investigates electrophysiological and pharmacological aspects of the role of the basal ganglia in the motor function.

In Huntington's Disease, the grants support mostly the research on etiology. We have one grant which taking from its etiologic studies, has a potential for the development of a diagnostic test for the at-risk individual. This is the individual who has the genetic background to have the disease but who is still too young for the gene to have become manifest. Most of the work in the dementias and aging is concerned with etiology.

It is possible to consider the disorders of aging in a broad sense as neurotransmitter deficiency diseases. The lack of dopamine in Parkinsonism, the lack of GABA, as well as the synthetic enzyme for GABA, in Huntington's Disease, is clearly documented by many studies. Some studies of dementia have reported a lack of the synthetic enzyme involved in the production of acetylcholine, choline acetyl transferase. This catalogue does not tell us the cause for these neurotransmitter deficiencies. In fact, people have argued that the deficiency is due to cell loss and is not a cause but a result of some still unknown process. However, I think one can look at it another way and consider that if the disease can be characterized by the loss of a specific enzyme or substrate (the neurotransmitter), regardless of the cause of that loss, then you can make a rational attempt at a therapeutic replacement, without necessarily having a knowledge of the etiology.

In Parkinsonism, the research support is divided almost equally between the histochemical, biochemical, and pharmacological approaches, i.e., the localization of neurotransmitter within specific cells and cell types, the quantitative determination of a neurotransmitter in a given region of the brain, and the selection of dopamine mimics which will act only on those synapses which are deficient in Parkinson's Disease. These can be thought of in two groups, the apomorphines and related compounds and the ergolines, drugs related to and derived from ergot, such as bromocriptine and lergotrile. Another approach has been to use inhibitors of monoamine oxydase B, which will result in more of the neurotransmitter being present.

Behavioral studies in Parkinson's Disease are important in terms of the psychiatric disturbances seen in some patients on L-DOPA and related compounds. The fundamental studies are related to understanding the separate categories of dopamine receptors and of the separate types of monoamine oxidase inhibitors.

We have learned, for example, that dopamine sensitive cyclic nucleotides are involved in the neurotransmitter function at the synapse. And by knowing the distribution of these transmitters on the cellular level with histochemical techniques, either by looking at them directly, using fluorescence, or by studies of the enzymes involved in their synthetic pathways, then we can perhaps begin to understand more effectively what is going on in these disorders. Recently there has been an increasing interest in the study of the peptide transmitters: substance P and enkephalins, which are naturally occurring substances which use the same receptors as opiates.

Huntington's Disease is often thought of as a mirror image of Parkinsonism, or on the opposite end of the spectrum of the dopamine defect. Dopamine loading has been used to test for the at-risk Huntington's individual. It is not widely used because of the risks and the ethical questions. Huntington's Disease is a disease of rather limited distribution. The current estimates are

in the range of 5 affected individuals per 100,000. It is the result of an autosomal dominant gene which becomes apparent in early middle age and produces the symptoms. The inheritance pattern is not perhaps as clear-cut as it sounds. There may be other genes which modify the expression of the gene. In the juvenile form of Huntington's there appears to be some sex-linkage, in that it is more likely to be inherited from the paternal line. The disease is marked by a degeneration of the caudate nucleus and the cerebral cortex. Unaffected individuals cannot pass the trait to their offspring. Late onset means that at-risk individuals may have reproduced before the symptoms are apparent. Thus, a reliable test for the individual who has the disease is particularly important but raises difficult ethical questions and problems of management. No treatment is possible at the moment; pharmacological management can ameliorate the chorea and psychological support can help with the early behavioral abnormalities and depression. There are exciting new ideas on etiology. Investigators at Albert Einstein have presented the idea that there are abnormal proteins produced in the Huntington's brain and that Huntington's may be a very accelerated form of aging. New approaches involving advanced biochemistry lead us to the idea that there may be membrane changes in cells in Huntington's patients which are intriguing both in terms of our understanding of the disease and what we do about it. The immunological aspects have begun to be investigated. It is apparent that there is a gene product which can be recognized by immunological techniques.

The Congress created a "Commission for the Control of Huntington's Disease and its Consequences" which worked during 1977-1978. The Commission, in its report, recommended an increase in the appropriations and effort in genetic and neurosciences research which should benefit research in related disorders such as Parkinson's Disease, MS, senility, mental disorders, nuscular dystrophy, and ALS. The Commission felt that the care for all chronically ill patients should become a part of national health insurance, more funds should be appropriated for training, plus a number of other across the board recommendations which would be beneficial to the advancement of the research in this area.

NINCDS is actively interested in the support of and state of the art of research on HD. The institute is sponsoring the "International Symposium on HD" which is to be held in November 1978.

In the dementias, prevalence had been estimated by some investigators to be as much as 3 per thousand. These figures are really guesses because there have been no extensive epidemiological surveys which are sound enough to give us a clear understanding of what is going on. It appears that there is at least some genetic component. It has been described by some investigators as being polygenic, i.e., many genes affect the expression of this trait and that its expression is, of course, age related. In classic Alzheimer's pre-senile dementia there have been a few cases reported which appear to follow the inheritance pattern of autosomal dominance. In the dementias, one sees brain atrophy and granulo-vacuolar changes but the neurofibrillary tangles and senile plaques are diagnostic. The identity and our understanding of these neurofibrillary tangles have been accomplished by the application of sophisticated biochemical techniques by investigators at Albert Einstein and at Children's Hospital in Boston. The anyloid core of the senile plaques has been identified.

These have been quantitatively correlated with the degree of dementia. The etiological factors involved are almost totally unknown. There have been suggestions in the literature that aluminum is involved in the production of these patterns of the tangles and the the plaques. Other kinds of environmental toxins have also been impugned.

The Workshop-Conference on Alzheimer's Disease-Senile Dementia and Related Disorders was held in Bethesda, June 6-9, 1977. This meeting yielded information on the current state of knowledge in each of several neurobiological aspects of the disorders and such etiological and risk factors as genetics, neurotransmitters, aluminum, latent viruses, proteins, immunological factors, and animal models. The proceedings of this meeting are being published as a 600 page volume in their series on Aging by Raven Press and will be available in October 1978.

A followup meeting on the Clinical/Behavioral Issues in Treatment of Alzheimer's Disease, Senile Dementia and Related Disorders will be held in December 1978 under joint sponsorship of NIMH, NINCDS and NIA.

The problem in attacking disorders of such magnitude as dementia has been the difficulty of finding a handle to probe and dissect the disease process. Our goal is to find new approaches to the disease process so that we will be able to ask questions in such a way that we will be able to find answers.

Research on aging and dementia is gaining momentum. The growing interest of the scientific community in this area is evidenced by recently increased number of publications, workshops and symposia. Consequently, we are able to support more research and of better quality in this highly important program area, although still to a limited extent because of budgetary constraints.

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The Congress created a "Commission for the Control of Huntington's Disease and its Consequences" which worked during 1977-1978. The Commission, in its report, recommended an increase in the appropriations and effort in genetic and neurosciences research which should benefit research in related disorders such as Parkinson's Disease, MS, senility, mental disorders, nuscular dystrophy, and ALS. The Commission felt that the care for all chronically ill patients should become a part of national health insurance, more funds should be appropriated for training, plus a number of other across the board recommendations which would be beneficial to the advancement of the research in this area.

NINCDS is actively interested in the support of and state of the art of research on HD. The institute is sponsoring the "International Symposium on HD" which is to be held in November 1978.

In the dementias, prevalence had been estimated by some investigators to be as much as 3 per thousand. These figures are really guesses because there have been no extensive epidemiological surveys which are sound enough to give us a clear understanding of what is going on. It appears that there is at least some genetic component. It has been described by some investigators as being polygenic, i.e., many genes affect the expression of this trait and that its expression is, of course, age related. In classic Alzheimer's pre-senile dementia there have been a few cases reported which appear to follow the inheritance pattern of autosomal dominance. In the dementias, one sees brain atrophy and granulo-vacuolar changes but the neurofibrillary tangles and senile plaques are diagnostic. The identity and our understanding of these neurofibrillary tangles have been accomplished by the application of sophisticated biochemical techniques by investigators at Albert Einstein and at Children's Hospital in Boston. The anyloid core of the senile plaques has been identified.

These have been quantitatively correlated with the degree of dementia. The etiological factors involved are almost totally unknown. There have been suggestions in the literature that aluminum is involved in the production of these patterns of the tangles and the the plaques. Other kinds of environmental toxins have also been impugned.

The Workshop-Conference on Alzheimer's Disease-Senile Dementia and Related Disorders was held in Bethesda, June 6-9, 1977. This meeting yielded information on the current state of knowledge in each of several neurobiological aspects of the disorders and such etiological and risk factors as genetics, neurotransmitters, aluminum, latent viruses, proteins, immunological factors, and animal models. The proceedings of this meeting are being published as a 600 page volume in their series on Aging by Raven Press and will be available in October 1978.

A followup meeting on the Clinical/Behavioral Issues in Treatment of Alzheimer's Disease, Senile Dementia and Related Disorders will be held in December 1978 under joint sponsorship of NIMH, NINCDS and NIA.

The problem in attacking disorders of such magnitude as dementia has been the difficulty of finding a handle to probe and dissect the disease process. Our goal is to find new approaches to the disease process so that we will be able to ask questions in such a way that we will be able to find answers.

Research on aging and dementia is gaining momentum. The growing interest of the scientific community in this area is evidenced by recently increased number of publications, workshops and symposia. Consequently, we are able to support more research and of better quality in this highly important program area, although still to a limited extent because of budgetary constraints.

DEMYELINATING AND SCLEROSING DISORDERS

Demyelinating and sclerosing disorders are among the common neurological disorders of our society, affecting half a million individuals according to some estimates. They characteristically afflict young adults and consequently, their burden on both the individuals and families involved is multiplied in the societal losses. The stark statement that these disorders are of unknown cause and without cure cannot be denied, but all of us who deal with these elusive disorders cannot help but sense an air of ferment and intellectual curiosity about them that was not true even ten years ago. This has been considered by the NANCDS Work Group on the Report of the MS Commission to be one of the plusses resulting from the Commission's activities.

In the fiscal year 1978 there were 70 active grants (including 8 clinical centers) for which support was $7,958,000. Seventy six applications were received of which 13 new and 16 competing renewals were funded with an expenditure of over $1,961,000.

It is estimated that over 500,000 people in the U.S. suffer from MS and related demyelinating diseases. About half (250,000) people are inflicted with MS, and the remainder includes patients with diffuse sclerosis, acute disseminating encephalomyelitis, postinfectious encephalomyelitis, and amyotrophic lateral sclerosis (up to 10,000 new ALS cases are diagnosed each year). In the destructive process of demyelination, the protective sheath normally present around most nerve fibers is destroyed or dissolved. This process is usually followed by a hyperplasia of the connective tissue which leads to the hardening of the NS (sclerosis).

In Multiple Sclerosis (MS), there are areas of demyelination scattered in the brain and spinal cord which lead to such symptoms as double vision, inability to maintain balance, numbness or paralysis of parts of the body, tremor, nystagmus, speech and elimination difficulties. Demyelination is a primary effect, occuring at random, and does not cause deterioration of a nerve fiber.

In Amyotrophic Lateral Sclerosis (ALS), impairment of the function of nerves controlling muscles (motor neurons) in the brain and the spinal cord produce progressive muscle weakness and wasting. It is caused by deterioration of a nerve fiber, and the demyelination is a secondary effect.

Epidemiological studies suggested that ALS and MS may have infectious etiology. Allergic encephalitis, especially experimental allergic encephalitis (EAE), attracted the attention of scientists for several reasons. It can be induced in a laboratory animal by injection of nervous tissue or BP (basic protein, a component of myelin). This model offers a means of identification of the principles underlying immunologically mediated inflammation, and it's a promising experimental model to study MS and other demyelinating diseases of man.

The human demyelinating diseases are of unknown cause(s) and without cure, and occasionally in the initial stages, are difficult to diagnose. The nation's increased awareness, consciousness, moral obligation, and realization of socio-

economic costs led to the organization of an enormous effort directed to combat these diseases.

The financial support of this area of research in the U. S. is relatively large in comparison to other nations. It is however very small when compared to the needs for research as complex as etiology (causation of the disease), pathogenesis (the sequence of biological processes or events from inception to the characteristic lesion of the disease), epidemiology (the conditions governing the natural occurrence of the disease), prevention, therapy (treatment), and development of specific diagnostic methods for MS as well as ALS. An appropriate balance of priorities had to be determined, maintained, and implemented by the institute director, program director, and their staff.

The Report and Recommendations of the National Advisory Commission on Multiple Sclerosis was issued. The Neurological Disorders Program (NDP) of the NINCDS organized a "work group" for the report of the MS Commission. The Congress in the appropriation report to the Institute specified several areas of research emphasis, some of which are basic research (science base) and MS. Some time ago research was divided into basic and applied. Now it is becoming more meaningful to divide it into science base, application, technology transfer and training (SATT). However, for our understanding of expended effort and funds we can visualize the research as targeted (directed toward) and untargeted (but relevant), although, e. g., neuronal regeneration, or neuron cell culture in ALS research has applications to the MS and neurological sciences in general.

A major emphasis in MS research is the search for etiological clues, biochemical and biophysical characterization of normal and pathological myelin, neuron degeneration, axoplasmic flow and transport, viruses causing chronic diseases, and search for their "foot prints", studies of neurons and oligodendrocytes specific antigens and immune host response, therapy (ACTH, steriods, transfer factor, BP) genetic influence on aberrant immune response and immunologic and immunogenetic patient profile, influence of psychosocial factors on exacerbations/neurophysiology, early and more accurate diagnosis (myelin in CSF, cellular immune response, oligoclonal IgG), clinical research, studies of evoked potentials, epidemiological studies, animal models, e.g., EAE in rats and chronic relapsing allergic encephalitis in guinea pigs.

ALS targeted research is focused on normal biochemistry and physiology of neurons, neuronal degeneration, neurotrophic factors, nerve growth factor, DNA repair, hormonal factors, transmitters, axoplasmic flow, receptors, effect of muscle and nerves on sprouting and neuronal function, viruses causing chronic diseases, nervous system antigens and immune response, pharmacology, cell cultures, clinical research on human disease.

The NINCDS Neurological Disorders Program's extramural research support in demyelinating or sclerosing disorders totals 70 grants which represents total awards of over 7 million. The largest number of grants, and most of the funds, support studies on myelin in normal and pathological states and specifically on MS. It includes seven MS Clinical Research Centers. ALS grant and financial support is small in terms of both number of grants and dollar amount. It includes one Clinical Research Center on ALS.

The experimental autoimmune disease of animals, experimental allergic encephalitis (EAE) is a significant component of the program. Where it is not the perfect animal model for human MS, it is highly relevant to the fundamental research in the demyelinating diseases.

Most of the research addresses the etiology of demyelination. The largest effort is on etiology of MS and ALS. In these studies brain autopsy, or biopsy pathological material, is used for cell isolation and biochemical, psysiological, immunological, histochemical analyses to name a few. These studies are a part of wider investigations, such as research on biochemistry of normal myelin, membrane biophysics, pathological processes in animal models. For example, immunoenzymological approach is being used to develop indicators of myelin development and possibly also of the demyelination. Growth characteristics in vitro are studied of cells isolated from PNS and CNS. The effect of macrophage secreted proteases on myelin degradation are investigated as one of possibly many mechanisms of demyelination. Comparative X-ray diffraction studies of mature, immature, and abnormal myelins derived from various species are done. Nuclear magnetic resonance (NMR) spectroscopy is used to study interactions between myelin basic protein (BP) and myelin specific lipids. New techniques and instrumentation are being developed to study membrane structure and membrane models. Demyelinating effect of serum from MS patients and EAE is being analyzed. Study is in progress on mouse encephalomyelitis virus which is an experimental model for ALS. Experiments are conducted to confirm initial findings of putative virus from ALS autopsy material.

Recently developed oligoclonal immunoglobulin assay confirms MS diagnosis in about 85%. However, there is still great interest in developing better and more reliable tests for MS. A highly sensitive radioimmunoassay is used to assess demyelination and myelination process on nucleotide rich material (NRM) unique to MS found in the spinal fluid. Further, an attempt is made to verify presence of neuroelectric blocking factor(s) found in serum obtained from patients with MS.

It was found that CSF from MS patients has myelinotoxic effect, therefore further studies are conducted to assess its diagnostic potential for MS. Parameters of neuronal and cell-mediated altered immune responsiveness are correlated with the clinical course of MS patients in an attempt to develop a more specific diagnostic test for MS. Idiotypic antibodies are being determined in CSF of patients as markers of MS. An attempt is being made to standardize solid phase radioimmunoassay for brain phospholipid protein (PLP) measurement at various stages of MS.

Although the medical profession is not yet able to treat MS or ALS directly the presently used medical management can protect patients from exacerbations, intercurrent infections, ameliorating symptoms. Many different modes of therapy administered are followed by the clinicians. The treatment in some instances arrests a disease progress, but in general it appears to be symptomatic. It was found that the steroid therapy in combination with cyclophosphamide prolongs remissions, and large doses of adrenocorticosteroids have a positive effect on the clinical course and are helpful in the treatment of acute exacerbations of MS.

ACTH has been utilized for treatment of MS patients with debatable results. A study attempts to assess the value of L-tryptophan and electric stimulation for improvement of certain signs and symptoms occurring through out the course of MS. Another study explores immunosuppressive therapy with azathioprine, and prednisone with azathioprine which after a period of time becomes ineffective in the treatment of chronic MS. The immuno-potentiating agent, Levamisole, has been investigated. Also of great importance is the management of the patient's emotional status. Tension, frustration, depression, have an effect on the endocrine system which plays an unexplained role in the disease.

The Institute support of research in endocrinology is moderate. It involves research in hormonal regulation and control mechanisms, hormone binding, hormone receptors, hormone analogs, biosynthesis, inhibitors, and metabolism, peptides, neurotransmitters, and immunoglobulins. However, hormonal studies of MS patients are very limited. Our Institute's financial support data indicates small activity in this area of research in the U. S. Because the currently favored hypothesis is for a viral etiology of MS, a major interest and research effort has been focussed on this area. There is now however considerable evidence to support the view that MS is an auto-immune disease. Consequently we can expect an increased research effort in the area of hormonal regulation of the immune response.

There is no effective treatment against ALS. Some drugs can be prescribed to relieve muscle cramping. Such drugs as belladonna or atropine are used sometimes to reduce excessive salivation.

Research in genetics, specifically on human leukocyte antigens (HLA) found first to be important in transplantation, became also relevant to MS and ALS, and is one of the research approaches carried out by a number of investigators. In MS and ALS patients, it has been determined that there is over-representation of certain HLA determinants with respect to the normal population, and furthermore, the HLA antigens could be further differentially associated with rapid and slow ALS progression.

Even though research is questioning new data and new leads we are not yet at the point of helping the sick. It is expected, however, that in the next few years at least the viral hypothesis for MS and ALS etiology will be resolved and the immunological studies will be advanced substantially.

INFECTIOUS DISEASES

A variety of slow or persistent viruses and viral infections are investigated. The research is targeted or relevant to elucidation of etiology or pathogenesis of e.g., multiple sclerosis, amyotrophic lateral sclerosis, aging.

The program in this area is small in the numbers of grants and the percentage of dollars allocated. In the fiscal year 1978 there were 31 active grants for which support was $1,778,000. Thirty two applications were received of which 8 new and 6 competing renewals were funded with an expenditure of over $812,000.

The infectious diseases program encompasses some of the very exciting work in neurology today, because most of the work here is in the area on slow viruses. The award of the Nobel Prize to Dr. Gajdusek and colleagues for his pioneering work in this area appears to have exciting possibilities and ramifications for many of the enigmas of neurological diseases. It is a tribute to the advance in antibiotic therapy that the categories dealing with infections in the classical sense, encephalitis and meningitis, have become of much less importance in recent years. The studies of meningitis have to do with improved and more rapid diagnosis of meningitis and studies on the sequelae of childhood meningitis. The post-infectious neuritides include studies of immune complex deposition in the central nervous system, including choroid plexus.

The study of the pathogenesis of a slow virus disease as it goes on in animal models is particularly important. The analysis of slow viruses of man, KURU, Creutzfeld-Jakob, has been paralleled by the discovery of animal slow virus diseases. Investigators on the west coast are studying scrapie and also VISNA in collaboration with an extensive program at Johns Hopkins University. Experimental Creutzfeld-Jacob disease has been developed in laboratory animals by investigators at Yale University. Meningitis has been studied in human populations and also in animal models of the disease. One research project studies the processes by which drugs and hormones are transported in the spinal fluid.

Most of the research is centered on etiology, particularly of the slow viruses. The meningitis area involves the development of new tools particularly important in rapid diagnosis of the disorder in children. There are some attempts at treatment.

The research approaches taken by investigators in dealing with infectious diseases are varied. They include histochemistry, immunology, virology, biochemistry, genetics, epidemiology and behavior. Behavioral sequelae of these infectious disorders are being studied in a small number of grants.

An exciting intellectual current exercise in the thinking of neurobiological research is the possibility that the chronic degenerative diseases, particularly many of the neurological disorders, can be related or associated with a viral cause. Despite the lack of spectacular success in direct efforts to isolate these putative causative agents (in MS, e.g.) the discovery by Gajdusek, Gibbs, and their co-workers, of the transmissible nature of KURU and the long latency

time between the ingestion of the agent and the development of the disease have spurred efforts in this area. The mechanisms by which a virus might attack an organism, either a human being or an experimental animal, and cause a disorder which persists, while exhibiting no significant symptomatology at the time of infection, but be reactivated or released from latency many years after the initial insult, is clearly important. The mechanisms by which these viruses can persist are being actively investigated by grantees from NINCDS. There are several mechanisms that have been proposed to explain this persistence of slow viruses: (1) the production of defective interfering viral particles which interfere with the reproduction of the parent virus; (2) the presence of temperature sensitive mutants of the virus which appear to reproduce poorly at normal body temperatures but may cause a clinically undetectable infection; (3) possibility of the viral particle incorporation into the host genome which under specific triggers is activated into producing virus and causing frank disease.

During the past four decades, an obscure disease of sheep called "scrapie" has emerged as an important area of biochemical research. Transmission studies and neuropathological examination of brain tissue suggest that scrapie is a prototype for the spongiform encephalopathies of man, i.e., kuru and Creutzfeldt-Jakob disease. Prior to the transmission of the kuru and Creutzfeldt-Jakob diseases to chimpanzees by intracerebral inoculation of infected brain both of these diseases were classified as "degenerative" abiotrophies of the nervous system.

To date, the chemical nature of the scrapie agent remains obscure. The unusual physico-chemical properties of the scrapie agent and its slow replication in the absence of any detection by host defense mechanism suggest that scrapie is a novel infectious entity. Unlike viruses, the scrapie agent cannot be visualized by electron microscopy and its presence does not provide a detectable immunological response.

This research program is directed toward understanding the chemical structure of the scrapie agent. Because the present assay for scrapie requires determination of an endpoint by titration in mice over 9 months, it is planned to devote effort to the search for a more rapid and less expensive biological assay. A reliable method for the partial purification of the scrapie agent is planned, as well as the use of this preparation to explore chemical and immunological assay systems. The purification and subsequent elucidation of the chemical structure of the scrapie agent promises to bring new concepts and techniques to several areas of molecular biology and medicine.

Herpes simplex virus (HSV) infection of peripheral autonomic nervous system (PANS) and human disease: it has recently been reported that latent infection of autonomic ganglia with HSV can readily be established in experimental animals. This observation, coupled with reports of HSV in organs innervated by autonomic nerves or in secretory products of these organs raises the question of whether latent and reactivated infection of autonomic ganglia may participate in the production of human disease, as well as in the transmission of virus to human contacts. Involvement of local automic ganglia in infection of the female genital tract of mice suggests that these

ganglia may be important in the passage of virus from the infected human mother to the fetus or newborn with resultant neonatal herpes. Autonomic ganglia, like sensory ganglia, may therefore serve as important reservoirs of latent virus which, when reactivated, may result in significant human disease.

Despite recent advances in clinical recognition and definition of diseases of the autonomic nervous system in man, studies concerning the etiology and pathogenesis of autonomic disorders are few and understanding remains fragmentary. Investigation of such disease has failed to keep pace with the burgeoning knowledge of the basic biology of the autonomic nervous system. In particular, the question of whether viruses can infect these ganglia and cause clinical disease has received little attention, and, to date, only a limited number of observations of viral infection of autonomic ganglia either in animals or in man have been reported. It is speculated that viral infection of the autonomic nervous system in man may occur more commonly than previously suspected, and that such infection, either by destroying ganglia, by disrupting autonomic neural activity, or by inducing secondary viral infection within innervated target organs, could lead to acute, intermittent or chronic disease in man.

The murine models of HSV infection of the SCG allow experimental testing of this hypothesis. The events leading to acquisition of ganglionic infection, the effects of the infection on the functional and structural integrity of ganglionic neurons, and the target organ sequelae of acute and reactivated infection can all be explored using HSV as a prototype virus.

The more severe human afflictions caused by HSV can be looked upon largely as diseases of the nervous system. Involvement of the central nervous system (encephalitis) in adults or neonates is a severe affliction leading to death or extensive morbidity. Recurrent epithelial eruptions can perhaps also be considered as manifestations of reactivated infection of sensory (and perhaps autonomic) ganglia. Also characteristic of these infections is their variability; infection may be lytic, latent or reactivated.

Despite recent advances in the biochemical characterization of lytic HSV infection and the development of models of latent sensory ganglion infection the factors determining whether infection of neural tissue is to be lytic, latent or reactivated, i.e., the factors controlling neural cell permissiveness, are poorly understood. Furthermore, using present methods, the cellular basis of latency and reactivation has been experimentally inaccessible.

Because of the extensive background knowledge concerning the anatomy, physiology, chemistry and pharmacology of the PANS, the use of autonomic ganglia to study infection of neural tissue with HSV presents a unique opportunity. These ganglia are readily manipulated surgically or pharmacologically, their metabolic and functional profile can be followed with well characterized biochemical markers, and their in vitro cultivation has reached an advanced state of sophistication. Infection of autonomic ganglia, therefore, can profitably be exploited to study the factors which underly alterations in permissiveness of neural cells for HSV. The results of such studies might be applied to other neural cell populations just as studies of

development of these ganglia, of their neuronal circuitry and of their synaptic chemistry have been used as models of different and more complex neural structures.

One of the goals is to define the pathogenesis of latent and reactivated herpes simplex virus infection of the peripheral autonomic nervous system. Among the aspects of this infection to be studied are: (a) the factors leading to acquisition of autonomic infection, (b) the determinants of latency, (c) the cellular changes responsible for viral reactivation, (d) the neural and target organ sequelae of reactivation, and (e) whether latent infection of autonomic ganglia occurs in man. Three complimentary approaches will be employed to achieve the proposed objective: (1) studies employing murine model of in vivo infection of the superior cervical ganglion of the sympathetic division of the autonomic nervous system, (2) studies employing an in vitro model of latent and reactivated HSV infection to be developed using dissociated cell cultures derived from autonomic ganglia, and (3) explanation of human autonomic ganglia obtained at the time of portmortem examination.

These studies will be undertaken because of their direct importance to human diseases, because infection with HSV serves as a paradigm of viral infection of the autonomic nervous system, and because they open a new approach to studying infection of the nervous system with this virus.

Studies on viral persistence in lymphocytic choriomeningitis virus have been carried on by investigators at the Scripps Clinic and Research Foundation and on measles virus and variants by investigators in San Francisco and at Ann Arbor. The studies of VISNA virus being carried on in collaboration in San Francisco and at Johns Hopkins show quite clearly that VISNA is incorporated into the genome of the host cell and that somehow the expression of this virus is blocked. VISNA is capable of rather rapid change or mutation within the host. At different stages within the host animal, the virus is producing different kinds of protein. This is called "antigenic drift" or "shift".

The sheep despite the fact that they have an appropriate acute cell mediated immune response, become sick and progress in relapsing and remitting phases to death. The investigators hypothesize that this progression of neurological disease in the face of attempts by the host to defend itself may represent the cumulative effect of clinically apparent attacks induced by intermittent release of antigenically altered virus. Thus, the phenomena of lysogeny, the technical term for incorporation of the viral genome into the host genome, and antigenic drift may combine to provide a unique mechanism which explains the acute episodes of remitting and relapsing disease. In addition to human diseases we have mentioned, this kind of mechanism has been proposed for multiple sclerosis, for subacute panencephalitis, and perhaps a little farther afield, but still within the realm of investigative possibilities, are certain forms of senile dementia.

Our understanding of how viruses persist or lead to infections much later in life is an exciting avenue to pursue. If viral etiology of the chronic degenerative diseases of the nervous system prove to be incorrect, nevertheless it is of importance because it generates relevant research effort, focuses attention of the scientific community, and produces new research leads.

CONVULSIVE AND RELATED PAROXYSMAL DISORDERS

The epilepsies constitute the second most common neurological disorder in the United States involving approximately 1% of the population, about 2.4 million people. Many people with epilepsy live relatively normal lives largely due to successful seizure control using drugs. However, about 25% of the afflicted population have uncontrolled seizures. The frequency and intensity of seizures vary considerably. Mortality, either directly or indirectly due to epilepsies, have been identified in more than half of the cases. And half of these are attributed to suicide. An estimate is that life span is generally reduced as much as 10 years in persons with epilepsy.

Epileptogenesis is not understood. Most laboratory research has been performed in animal models in which focal epilepsy has been produced because of the ease and reliability of the procedures. In fact, epilepsy may result from any form of brain trauma. For instance, injury during perinatal or prenatal period may be causal. Head injuries, infection, congenital malformation, tumors, etc., may result in seizures at any point in life. Particularly discouraging to researchers, is the fact that various forms of epilepsy can occur clinically without any evidence of morphological brain abnormality. Researchers have noted a high incidence of family involvement of epilepsy which, it has been suggested, reflects genetically defective enzyme systems. Seizures are probably symptomatic of a variety of genetic diseases, each one having its own form of inheritance. Prescription and dosage of specific anticonvulsants tend to be determined experientially. In a limited number of cases, where seizures appear intractable, surgical intervention has been indicated. In recent years the stress on accurate determination of serum drug levels has improved the efficacy of medication; in addition, these determinations have permitted the detection of toxicity, cross-reactions among drugs, and various metabolic difficulties. An important side effect of accurate serum drug level determination has been the detection of non-compliance, absorption difficulties, and differences in drug metabolism among patients. Diagnosis has been assisted by the development of monitoring techniques using video recording and radio telemetry of the EEG simultaneously. Evaluation of the simultaneous EEG and clinical seizure has provided a more reliable base for rapid evaluation of specific anticonvulsants and dosages.

Phenobarbital and phenytoin are both widely used as anticonvulsant agents. Unfortunately, they have serious side effects especially to pregnant women and the fetus as well as small children. There is a need for new anticonvulsant drugs to replace these. Thus, our program is highlighting the importance of research on the mechanisms of action of anticonvulsive agents. It is hoped that if the direct mechanisms of action of anticonvulsant agents are understood, new drugs can be synthesized which are specific in their anticonvulsant activity and thus produce fewer deleterious side effects.

During Fiscal Year 1978, 90 applications for research support in Convulsive and Related Paroxysmal Disorders were received; 60 were approved and 20 funded. Currently, there are 65 active grants in this category. These include six specialized research centers (epilepsy). The total amount of research support for all these activities is $5,008,051. The grants are equally distributed amongst research in humans with the disease, the disease in animal models, and

those processes believed to underlie various aspects of the disease. Most of the work of course, relates directly to the epilepsies per se, both the etiology of the disease and the treatment. Researchers are particularly active in work on the pathogenesis of epilepsy and the mechanisms of action of anticonvulsant agents. Neither are well understood. The development of animal models for epilepsy continues with an interesting new possibility using autoimmune genesis for this disease. This is a new model which has promise as a model for generalized seizures as opposed to the focal seizure produced using cobalt, aluminum, or other specific agent. Work continues on the neurophysiological, morphological and biochemical abnormalities that may underlie focal epileptogenesis. Intracellular labeling techniques are particularly popular for studying this problem. Some work, using hippocampal slices, suggests that some forms of epileptiform discharge may be intrinsic to nerve cells rather than due to large synaptic potentials. Various neurotransmitter substances are being studied to determine their roles in seizure genesis and termination. Other research continues on the trauma produced by epileptic seizures themselves. The disease is considered to be progressive by many workers in the field. Mechanisms of action of various anticonvulsant compounds are being studied by one investigator using identified single neurons of invertebrate preparations. Findings are that phenobarbital blocks voltage dependent inward movement of calcium that is necessary to activate outward potassium current which repolarizes the membrane. In a related study, another investigator has proposed that diphenylhydantoin's anticonvulsant properties are the result of changes of flux of calcium in nerve endings. Phenytoin reduces the stimulus evoked release of neurotransmitter and enhances spontaneous release from nerve endings bathed in normal Ringer's solution. Interestingly, there are opposite effects when phenytoin is applied to nerve endings bathed in low calcium solutions. From these data, it is concluded that phenytoin reduces calcium flux into the nerve ending, thereby reducing transmitter release. Simultaneously, diphenylhydantoin reduces calcium uptake by mitochondria, thereby increasing spontaneous transmitter release. This work is continuing.

Attention is focussed on the effects of altered ionic microenvironment in epileptogenesis, largely on potassium. Using a variety of preparations, investigators have shown that increased extracellular potassium and decreased extracellular calcium can induce epileptiform reactions. Intracortical increases in extracellular potassium may lead to depolarization of axons and bursting; this funding has been indirectly supported by other data showing that during epileptogenesis there are large increases in extracellular potassium. In addition, a number of investigators have reported decreases in extracellular calcium in cortex during seizures. Although there are suggestions of an interaction between ionically depolarized terminals and extracellular currents that produce axonal bursting, there is no reason to exclude possibilities of depolarization at synapses by alternative substances released by neuronal or other sources. The amount of research on ion flux and the ionic microenvironment in general, provides promise for understanding in epileptogenesis and mechanisms of anticonvulsant agents.

For several years, it has been known that injections of antiserum to complex antigenic mixtures from brain can induce seizures in rats, rabbits, monkeys, and cats. One investigator has shown recently that antiserum against purified

ganglioside is an effective reagent in producing EEG seizures. This has also been done with antiserum to partially purified actomyosin-like protein from brain. Investigators are currently studying the properties of various antisera in their seizure inducing activity. Thus far, the data suggest an immunological foundation for some forms of seizures. Studies of morphological changes in synaptic sites of cortex in injected animals are currently under study. Although there is no strong suggestion that seizures are the result of immune reaction, the techniques for developing models of the epilepsies are promising.

Research in Narcolepsy and Related Disorders

The Neurological Disorders Program also provides research support for a specialized research center (sleep apnea). Disturbances of sleep constitute a major problem in the United States. These are reflected in such entities as, the upper airway sleep apnea syndrome, the insomnia sleep apnea syndrome, narcolepsy with sleep apnea, sleep apnea in children, the Pickwickian syndrome, and some believe, the sudden infant death syndrome. Virtually nothing is known about the primary cause of the sleep apnea syndrome. Some patients are apparently normal when fully awake as assessed by a complete physical examination. However, after the onset of sleep, profound respiratory impairment appears. These are followed by the varieties of clinical descriptions provided above. Thus far, the major thrust in therapy is a tracheostomy. In more than a dozen cases in which the tracheostomy was performed, there were clinical improvements. Research centers on clarification of the pathophysiology of the sleep apnea syndrome. Work on experimental animals is aimed at an understanding of the interactions of sleep, a putative central nervous system defect, and respiratory control mechanisms in the syndrome. Other studies focus on analysis of the cerebral spinal fluid in patients with sleep difficulties. Studies on plasma catecholamines may be of value in explaining cardiovascular changes sometimes associated with sleep apnea. It is well-known that hypoxia does influence secretion of adrenalin, as do other forms of physiological-psychological stress and excessive muscular activity.

Other studies focus on blood levels of the various catecholamines during various psychological states, stress and at varied levels of exercise. Unfortunately, findings to have shed little light. There is promise because some researchers have successfully bred a litter of narcoleptic dogs which may be used in the future as experimental models. With a growing awareness in the scientific community of the importance of sleep disorders, we may expect greater levels of activity of in the future.

MUSCULAR AND NEUROMUSCULAR DISORDERS

Muscular and neuromuscular disorders are directly responsible for disability in patients with many diseases of the central and peripheral nervous system. Research on the pathophysiology, diagnosis, and treatment of these disorders is a major portion of this program's extramural research support portfolio. Included in this category are muscular dystrophy, myasthenia gravis, the peripheral neuropathies, and other disorders of movement. We are fortunate that one of these disorders, myasthenia gravis, is demonstrably controllable.

Unfortunately, the others are less amenable to intervention. During Fiscal Year 1978, 122 applications for research support in Muscular and Neuromuscular Disorders were received; 99 were approved and 50 funded. The program currently provides research support for 134 grants in the amount of $9,001,086. Four of these grants are in the form of specialized research centers (neuromuscular diseases) and one, a program project primarily concerned with the pharmacology of neuromuscular disorders. The greater portion of research support is for basic studies of the neuromuscular system. It is axiomatic that data emerging from these efforts will eventually benefit patients with these diseases. It is not surprising that when we categorize the individual research grants and the separate components of the centers according to their relevancies, most efforts are focused on processes "theoretically relevant to the various diseases." Similarly, in distributing these items by the "immediate research goals," we see that most are concerned with etiology. A redistribution of these research support activities according to the primary research interests of the principal investigators, shows a relatively flat distribution of efforts amongst the various aspects of the neuromuscular system. There is currently a wave of interest in synaptology and properties of membranes. Nineteen projects consist of classic electrophysiology of individual units and systems of motor units. This work adds to our knowledge of how muscles work in concert.

Muscular Dystrophy

Muscular dystrophy refers to myopathy with two characteristics: a genetic basis and progressive weakness. The exact causes of the degenerative changes in the muscles are unknown. However, there appear to be disturbances in the enzyme systems concerned with muscle metabolism. Significant pathological findings tend to be confined to the muscles although the ventral horn cells have been shown to be degenerated or reduced in number.

There is no treatment which has proved to be effective in arresting the course of the disease.

Grant support for research in this area is comprised of 18 research grants, a portion of one clinical research center and one program project amounting to about $1.3 million.

Duchenne muscular dystrophy (DMD) is the best-defined of the muscular dystrophies. It is inherited as an X-linked recessive characteristic with complete penetrance. Female carriers are usually asymptomatic but evidence of the carrier state may show up under laboratory testing. Progression of the disease is rapid, usually being diagnosed at 3-6 years of age. Patients usually do not walk beyond 10-12 years of age. In fact, inactivity by the patient usually results in an inability to walk ever again. Cardiac muscle involvement occurs in almost all patients and there is a high frequency of mental retardation.

Theories of etiology emphasize the importance of one or another organ system; for instance, defective neurons or vasculature. However, both neurogenic and vascular theories have not been well-supported. One possible explanation in DMD, as in other genetic disorders, is a widespread independent expression of a metabolic defect in many tissues. Muscle may be the principal target because the biochemical defect most affects the function and integrity of this tissue.

Recently, there was reported abnormally impaired responses of adenyl cyclase in homogenates of muscle from patients with Duchenne dystrophy. Investigators have found the same abnormality in muscle cells growing in culture, reinforcing the concept that a fundamental fault in this disease affects the muscle surface membrane and is one that is not dependent upon neural influences. Several lines of evidence suggest that membranes of erythrocytes are also abnormal in Duchenne dystrophy. Investigators have found that epinephrine reverses the abnormal sensitivity of these cells to ouabain, but membrane adenyl cyclase is not stimulated by epinephrine as it is in normal cells.

Work using animal models continues. In dystrophic mouse muscle, muscle putrescine content was increased 5-10 times and spermacine content was twice normal, but spermine did not change. Even greater increases were found in experimentally denervated rabbit muscle with concomitant increase of a related enzyme. Acetycholine esterase (AChE) activity did not increase in dystrophic mouse muscle and the cytochemical distribution of this enzyme in dystrophic and denervated mouse muscle differed.

Another group is assessing the role of connective tissue in the development and progression of muscular dystrophy. They identified the cellular origin of the extracellular matrices in muscle tissue. Secondary cultures derived from embryonic avian musculature were employed to analyze both structural and biochemical associations that exist between muscle fibers and the extracellular matrix. In addition, human muscle biopsies were examined to identify structural relations between connective tissue and myofibers, and normal and disease tissue. To date, no observable differences have been noted in the development of cells and cultures derived from either normal or dystrophy biopsies. Fine structural studies of intact human muscle have revealed that regeneration is occurring in both young and old dystrophic patients, although it is more prominent in younger ones. In older patients, both degenerating and regenerating muscle cells appear to be functionally isolated from the blood supply as a result of the extensive fibrosis. This supports the possibility that vascular defects may influence the progression of human muscular dystrophy.

In the field of muscular dystrophy research, most investigators are divided as to whether the disease represents a primary myopathy or a neural defect. To date, no convincing evidence can be found to support either possibility as a mechanism active in human MD. Extensive fibrosis and apparent incapsulation of both myofibers and blood vessels seen in dystrophic human biopsies support this idea although one cannot rule out the possibility that the structural alterations only reflect a secondary phenomenon. The role of satellite cells in the regeneration response active in dystrophic muscle remains unclear. As indicated above, younger patients exhibit an active regeneration response while in older patients, regeneration is reduced. The question arises as to what has happened to the regenerating stem (satellite) cells in older patients. The reduction in regeneration activity may be due to either the loss by depletion of viable stem cells, or the inability of these cells to express their cytodifferentiation. Results from embryonic tissue culture studies support the view that an abnormal environment in dystrophic patients might alter stem cell expression. This is interesting in regard to the fact that determined myoblasts when modulated, can express themselves as either functional fibroblasts or adipose-like cells. If such a modulation were occurring in

dystrophic patients, not only would there be a loss of regeneration activity, but simultaneously, the modulated stem cells would contribute to both the fibrosis and fatty infiltration which are characteristic features of MD histopathology. Several investigators are studying at a very fundamental level the role of the satellite cells in regeneration.

Studies of chronic penicillamine treatment of chickens with inherited muscular dystrophy have indicated that this drug can prevent the development of muscular dystrophy in chickens and that the beneficial effect may outlast therapy. Improved righting reflex in the presence of the drug can be attributed to disappearance in the depression of transmitter release normally seen in untreated dystrophic birds, and possibly to a lowering of the threshold for activation of the direct elicited action potential in dystrophic skeletal muscle. Investigators suggest that penicillamine may alter Ca++ kinetics by some interaction with -SH groups on the presynaptic terminal, thus, preventing the deleterious effects of the disease on neuromuscular function.

Work continues on membrane development in normal versus dystrophic muscle. Much involves developing techniques for producing pure cultures. Investigators hope to obtain through the development of these cultures, an understanding of the role of the cell surface membrane in embryonic development generally, and in the development of striated muscle, specifically. They also hope to gain insight into the mechanism of membrane fusion. A natural outgrowth of this work would be identification of a putative primary membrane defect in genetically inherited dystrophies of muscle. Other research focuses on mechanisms that control the activity and localization of AChE during muscle development and maturation, and secondly, exploring the nature of the defect in AChE regulation in inherited muscular dystrophy in the chicken. Findings that AChE is released from cultured nerve reopens the question of whether nerve, muscle, or both, put AChE into motor endplates. The localization of AChE around the motor endplate supports the idea of a defect in motor endplate region in dystrophy. The difference in localization of AChE between a myogenic defect dystrophy and a neural lesion denervation together with the findings of AChE in human muscles, raises the possibility of a future clinical test to distinguish certain kinds of neuromuscular abnormalities.

Myasthenia Gravis

MG is a chronic neuromuscular disease characterized by progressive weakness and abnormally rapid fatigue of the voluntary muscles. The disease tends to be contracted by females at earlier ages and at later ages by males. There have been, in recent years, progressive improvements in the management of patients with this disease. These have been in hospital care, technical improvements in respiratory support devices, antibiotics and wide use of tracheostomy.

Impressive use of anticholinesterases, the preferred treatment for many years, has made it possible to relieve symptoms for many afflicted victims. The anticholinesterases facilitate transmission of nerve impulses across nerve-muscle junctions to activate muscles. In MG, transmission of the nerve-muscle impulse is defective.

ACTH and prednisone are believed to suppress the immunologic abnormality believed to be responsible for the transmission defect. Not too many years ago, MG patients died within the first few years of their illness. Today, under proper supervision, many can live virtually normal lives.

Drug therapy such as long-term, high-single-dose, alternate-day prednisone, perhaps coupled with thymectomy, thereby removing the immunologic tissue considered a major factor underlying impaired neuromuscular transmission, may make MG the most successfully managed neuromuscular disorder.

An immune factor has long been suspect in MG; the transmissable factor currently thought to be responsible is defective gamma-globulin. A number of investigators have demonstrated that rats and rabbits, immunized with acetylcholine receptor (AChR) present a similar autoimmune disease. At the neuromuscular junction, this muscle protein receives the transmitter acetylcholine released by the motor ending, and thus is critical to normal muscle excitation. Experiments have demonstrated that among a majority of MG patients studied, there is abnormal gamma-globulin which binds to and, presumably, blocks the AChR. The postulated abnormal gamma-globulin produced by B-cells of lymphoid tissue appears partially related to an abnormality of the thymus. It has been known for years that thymectomy sometimes improves the MG patient. Unfortunately, the picture is still unclear.

In the meantime, there is an array of studies dealing with the many aspects of the animal MG model, also known as EAMG or experimental autoimmune myasthenia gravis.

One group of investigators has demonstrated unequivocally that EAMG is a valid model for studying human myasthenia gravis by virtue of its clinical, electrophysiologic, pharmacologic, structural, and immunologic similarities in myasthenia gravis. They have also documented the truly autoimmune nature of EAMG by demonstrating in immunized rats, production of antibody to rat muscle acetylcholine receptor, and they have demonstrated the requirement for T-lymphocytes for the induction of both EAMG and antibody to acetylcholine receptor. They have demonstrated the detection of antibody to human muscle and AChR in patients with MG and have established its specificity as a useful diagnostic test for MG.

Other investigators have noted that the AChR content of muscle from MG patients is reduced and that many of the remaining AChR's had antibodies bound. Further, decrease in miniature endplate potential (mepp) amplitude was proportional to the amount of AChR remaining unbound by antibody in the muscle. These results show that decreased content of functional AChR is the most important factor contributing to impaired neuromuscular transmission in MG. This is consistent with other experimental findings that patients with MG have spontaneous mepp's that are 1/6 that of normal. This could reflect either a defect in the post-synaptic membrane or a decrease in the amount of transmitter.

Other work on the AChR reports that it is localized primarily on a specialized portion of the post-synaptic membrane which is at the crest of the junctional folds. Previous studies show that acetylcholine esterase (AChE), the enzyme that terminates the action of the ACh, is distributed throughout the primary

and secondary cleft of the neuromuscular junction. Thus, the receptor is present at the top of the junctional folds at a concentration which is ten times that of the esterases. At the bottom of the folds, the ratio is reversed. This molecular morphology has several physiological consequences in that it predicts the time course of miniature endplate currents. Detailed studies of the neural regulation of AChR metabolism requires a source of purified receptor and would benefit from an antiserum directed against a mammalian AChR.

The significance of much of this research falls in two areas. The understanding of AChR metabolism, and nerve and muscle, and, the development of procedures to investigate nerve muscle interaction in a controlled environment. Clearly, the understanding of normal cell function is requisite to understanding the basis of malfunctioning and diseased tissue. Knowledge of the mechanisms involved in nerve muscle interaction in tissue culture may well prove vital to the detection and correction of abnormal nerve muscle interaction in vivo. Recently, it has become possible to visualize the acetylcholine receptor on living cells. Fluorescence microscopy has been combined with electrophysiology to show that a fluorescent stain correlates with areas of high acetylcholine density. Cholinergic miniature endplate potentials were recorded in muscles that exhibited nerve induced localization of acetylcholine receptor. As the extent of acetylcholine receptor increases, the mepp amplitude and frequency increases. These results demonstrate that this system is a valid place to study functional synapse formation and the mechanism of AChR localization. It has also been demonstrated that antibody against the AChR induces an increase in the rate of receptor degradation. This results in an increase in receptor density and acetylcholine sensitivity. In addition, it has been shown that sera from patients with myasthenia gravis, increase the rate of AChR degradation and reduce human muscle sensitivity to acetylcholine. Studies of synaptogenesis may lead to an understanding of normal nervous system function and atrophic interactions between nerve and muscle. Many diseases may result from a breakdown of these trophic interactions. The work on the effect of antibody against the AChR has led to a greater understanding of the disease myasthenia gravis, and hopefully to a rational therapy.

Peripheral Neuropathies

There are approximately 3 1/2 million diabetics in the United States. About 10% of these have symptoms of painful burning, numbness, weakness or paralysis, or more serious symptoms. The causes of these symptoms are not known and treatment does not exist. In recent years, the Neurological Disorders Program has placed a special emphasis on research grants in this area. There are only a limited number of regular research grants focused on these problems; however, the NDP has recently awarded a specialized research center grant to the Mayo Clinic and Foundation in Rochester, Minnesota. The center will coordinate multidisciplinary research on human and experimental neuropathies, with the intent of elucidating their causes and hopefully, eventually develop treatments. Investigators are focusing on new approaches and techniques that combine morphologic, electrophysiologic, immunologic, biochemical, and physical research techniques. With an intimate intertwining of basic research and patient care, future prospects are bright. A system has been developed to quantitate the capabilities of sensory modalities in man, and others are to follow. There are

a number of morphometric studies applied to human and animal biopsy, and autopsy material. Electrophysiological studies of nerve function include the usual clinical methods as well as attempts at innovation, for instance, single fiber investigation in vitro. There are to be studies of abnormalities of axonal flow and endoneurial pressure. Immunologic methods are being used to assign cellular responsibility for segmental demyelination and to assess the roles of viruses in inflammatory neuropathy. Studies of lipid composition of nerve and the effect of modification of membrane lipids are to be undertaken by lipid chemists. The largest single responsibility of the center will deal with diabetic neuropathies, however, other neuropathies to be explored include a number of genetically determined diseases in man and animals, human and experimental heavy metal intoxication, and inflammatory diseases.

ANNUAL REPORT
For Period October 1, 1977 through September 30, 1978
Developmental Neurology Branch, Neurological Disorders Program
National Institute of Neurological and Communicative Disorders and Stroke
National Institutes of Health

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GENERAL SUMMARY

I. INTRODUCTION:

The completion of the Comprehensive Plan for Analysis and Interpretation of Collaborative Perinatal Project Data continued to be a major objective of the Developmental Neurology Branch (DNB) during this fiscal year. As this objective nears completion, the DNB is focusing increasing effort on developing and implementing a program of research on the neurological basis of the developmental disorders of children including cerebral palsy and motor disorders, autism and behavioral disorders, mental retardation and learning disorders, and central nervous system birth defects and genetic disorders. The DNB will continue to provide for the archival and retrieval system of NINCDS Collaborative Perinatal Project data and will promote the appropriate use of this national data resource commensurate with the goals and mission of the NINCDS.

The NINCDS Collaborative Perinatal Project (NCPP) is a longitudinal multidisciplinary research effort which seeks leads to the etiologies of cerebral palsy, mental retardation, learning disorders, congenital malformations, minimal brain dysfunction, convulsive disorders, visual abnormality and communicative disorders through studies which relate the events, conditions, and abnormalities of pregnancy, labor and delivery to the neurological and mental status of the children of these pregnancies as the child grows and develops. Data collection has been completed, and data analysis is nearing completion. The major emphasis of the project has therefore shifted to data interpretation and the writing of reports for publication of the NCPP research findings. A Comprehensive Plan for Analysis and Interpretation of Collaborative Perinatal Project Data provides the framework for this effort. The Comprehensive Plan includes 10 primary and 10 secondary areas of study. Significant publications stemming from the Comprehensive Plan are noted in the General Summary and in the individual contract narratives and project reports.

II. NCPP DATA COLLECTION:

The NINCDS Collaborative Perinatal Project was initiated in 1959 when the women began registering at each of the collaborating institutions during their pregnancies. Women continued to enroll in the study through December of 1965. The babies were delivered between 1959 and 1966 and received several examinations during their first year of life, at 3 years of age, and at 4 years of age. The 7-year extensive battery of examinations included a pediatric-neurological examination; a battery of psychological

tests, including an IQ determination; and a visual screening test. In June of 1974, an assessment of speech, language and hearing development completed follow-up of NCPP children. As of June, 1976 the last of these examination protocols was edited, coded and the data punched and transferred to computer tape thus completing the basic data file of the NCPP. Periodic updating of the file has occurred since that date, based on research findings. In addition, specialized study files exist for each of the primary areas and for a number of the secondary areas within the Comprehensive Plan. Creation of these files has been a part of the research process.

III. A COMPREHENSIVE PLAN FOR ANALYSIS AND INTERPRETATION OF COLLABORATIVE PERINATAL PROJECT DATA:

Broadly stated, the NINCDS Collaborative Perinatal Project is concerned with the identification of prenatal and postnatal factors that have sufficiently high association with adverse pregnancy outcome and subsequent neurological and mental development of the child to provide leads to the etiologies of the abnormalities and thus to the development of strategies for prevention and intervention. After a careful review of the objectives of the NINCDS Collaborative Perinatal Project, the data available for analysis and the work in progress, it was recommended that major efforts in analysis and interpretation were needed in ten primary areas in order to meet the basic objectives of the project. Reports in book or monograph form are in progress in each of the following primary areas:

Cerebral Palsy (See Individual Project Reports -
Project Nos. Z01 NS 02059-06 DNB,
Z01 NS 02172-04 DNB)

Mental Retardation (See Individual Project Reports -
Project Nos. Z01 NS 02106-05 DNB,
Z01 NS 02172-04 DNB)

Communicative Disorders (See Contract Narrative,
Contract N01 NS-4-2326)

Visual Abnormality (See Individual Project Report -
Project No. Z01 NS 02107-05 DNB)

Convulsive Disorders (See Individual Project Reports -
Project No. Z01 NS 02058-05 DNB,
Z01 NS 02234-03 DNB)

Learning Disorders (See Individual Project Report -
Project No. Z01 NS 02108-05 DNB)

Minimal Brain Dysfunction (See Individual Project Report -
Project No. Z01 NS 02062-06 DNB)

Congenital Malformations (See Individual Project Report -
Project No. Z01 NS 02109-05 DNB)

Birthweight-Gestational Age Relationships (Prematurity) (See Individual Project Report - Project No. ZO1 NS 02060-06 DNB)

Neuropathology, General Pathology and Placentology (See Contract Narratives, Contracts NO1-NS-3-2312 & NO1-NS-7-2376)

IV. IMPLEMENTATION OF THE COMPREHENSIVE PLAN

Implementation of the Comprehensive Plan for Analysis and Interpretation of Collaborative Perinatal Project Data is carried out through teams of researchers, headed in each of the ten primary areas by a member of the Professional Staff Consultants of the Developmental Neurology Branch. On each team, there is a member of the Office of Biometry and Epidemiology staff, who participates fully in the development of the analysis. In addition, members of the Production of Data Analysis Unit, Perinatal Research Section, and the software contractor (Computer Data Systems Inc.), are assigned to each primary area to facilitate data processing. The assignments are as follows:

PRIMARY DATA ANALYSIS AREAS	BRANCH	OFFICE OF BIOMETRY
Cerebral Palsy	Dr. K. B. Nelson	Dr. J. H. Ellenberg
Mental Retardation	Dr. S. H. Broman	Dr. P. W. Shaughnessy*
Communicative Disorders	Dr. P. J. LaBenz	No current assignment
Visual Abnormality	Dr. R. Feinberg	Miss E. C. Jackson**
Convulsive Disorders	Dr. K. B. Nelson	Dr. J. H. Ellenberg
Learning Disorders	Dr. S. H. Broman	Dr. P. W. Shaughnessy*
Minimal Brain Dysfunction	Dr. P. L. Nichols	Dr. T. C. Chen
Congenital Malformations	Dr. N.C. Myrianthopoulos	No current assignment
Birthweight-Gestational Age Relationships (Prematurity)	Dr. Bill H. Williams	Miss E. C. Jackson**
Neuropathology, General Pathology and Placentology	Dr. J. S. Drage	No current assignment

In each of the ten primary areas, a program plan has been developed and approved which expands in detail on the summary statements in the Comprehensive Plan and gives a detailed approach to the analysis and identifies

*No longer with the Office of Biometry, but continues to serve as a Consultant in this area.

**No longer with the Office of Biometry after December 31, 1977.

major components. Written monthly reports are prepared and meetings held to record progress and identify problem areas. In order to facilitate the primary data analyses and/or complete major efforts well underway, additional analyses are to be completed in the following secondary areas:

Toxemia (Completed in FY'77 - see Friedman, E.A., and Neff, R.K.: Pregnancy Hypertension. Littleton, Mass., PSG Publishing Co., Inc., 1977, 258 pp.)

Maternal Infection during Pregnancy (See report by Infectious Diseases Branch, NINCDS)

Neonatal Hyperbilirubinemia (See Individual Project Report - Project No. Z01-NS-02112-05 DNB) (Phase I completed in FY'77 - see Scheidt, P.C., Mellits, E.D., Hardy, J.B., Drage, J.S., and Boggs, T.R.: Toxicity to bilirubin in neonates: Infant development during first year in relation to maximum neonatal bilirubin concentration. J. Pediatr. 91: 292-297, 1977)

Maternal Anesthesia-analgesia during Labor and Delivery (See Contract Narrative N01-NS-8-2381, and Individual Project Report - Project No. Z01-NS-02169-04 DNB)

Four-Year IQ (Completed in FY'75 - see Broman, S.H., Nichols, P.L., and Kennedy, W.A.: Preschool I.Q.: Prenatal and Early Developmental Correlates. Hillsdale, N.J., Lawrence Erlbaum Associates (distributor, Halsted Press, John Wiley & Sons, New York) 1975, 360 pp.)

Physical Growth and Development (Birth to Seven Years) (See Contract Narrative, Contract N01-NS-5-2308) (Two publications during the year were: Garn, S.M., Shaw, H.A., and McCabe, K.D.: Effects of Socioeconomic Status (SES) and Race on Weight-defined and Gestational Prematurity in the U.S.A. In Reed, D.M. and Stanley, F.J. (Eds.): The Epidemiology of Prematurity. Baltimore, Urban and Swarzenberg, 1977; and Garn, S.M., Shaw, H.A., Wainright, R.L., and McCabe, K.D.: The effect of sample size on normative values. Ecol. Food & Nutr. 6:153-157, 1977.)

Twins (See Individual Project Report - Project No. Z01-NS-02332-01 DNB)

Genetic and Socio-economic Factors (See Individual Project Reports - Project Nos. Z01 NS 01514-12 DNB, Z01 NS 01857-09 DNB Z01 NS 01754-10 DNB, Z01 NS 01274-14 DNB)

Drugs taken during Pregnancy (Completed in FY'77 - see Heinonen, O.P., Slone, D., and Shapiro, S: Birth Defects and Drugs in Pregnancy. Littleton, Mass., Publishing Sciences Group, Inc., 1977, 516 pp.)

Labor and Delivery (See Contract Narrative N01-NS-8-2381)

Entire analyses or portions of an analysis are conducted by utilizing the contract mechanism, within both the primary and secondary areas of the Comprehensive Plan. A member of the Developmental Neurology Branch Staff serves as Project Officer on each contract. Other analyses are conducted directly by Developmental Neurology Branch and Office of Biometry staff with data processing support provided by the software contractor (Computer Data Systems, Inc.).

V. SUMMARY OF WORK IN PROGRESS:

In the cerebral palsy area, a univariate screen has been run, evaluating those maternal and pediatric conditions most strongly associated with cerebral palsy. This screen is the basis for multivariate analysis now beginning. Some of the special tape files to be used in the execution of this analysis have been created, some are being generated. A substudy of the double disability of cerebral palsy and severe mental retardation has been completed and was published. Nelson, K.B. and Broman, S.H.: Perinatal risk factors in children with serious motor and mental handicaps. Ann. Neurol. 2: 371-377, 1977.

In the mental retardation area, the incidence of severe retardation was found not to differ by ethnic group, but mild retardation was more frequent among blacks than whites. The incidence of mild retardation, and to a lesser extent, severe retardation, decreased as social class increased. Major neurological problems were more frequent among whites than blacks in both retarded groups. Within ethnic group, the proportion of neurologically involved retarded children increased with social class. Risk factors for mental retardation include urinary tract infections during pregnancy, teen-age pregnancy, clinical signs of perinatal anoxia, and poor psychomotor performance in infancy. Multivariate analyses of these data are being completed, and a monograph is in preparation.

Studies in the area of communicative disorders have been completed under contract. A book-length manuscript has been written and was reviewed by the DNB staff and by an ad hoc panel of experts from outside the DNB. Final editing has been completed and the manuscript has been submitted for publication in book form.

In the area of visual abnormality additional computer-generated outputs were produced and are undergoing analysis and interpretation. An outline of the monograph has been prepared and assignments to specific chapters from the investigative team were made. Writing of chapters is underway and several have been completed. Work continues on the bibliography to be used in conjunction with the monograph. Both the monograph and bibliography are to be completed in 1978. Two additions to the study this year were a consideration of handedness and eye dominance, and an inspection of the consequences of retinal hemorrhages in the neonate.

In the convulsive disorders area, data on the prevalence of specific seizure disorders in early childhood are now available and are being prepared for publication. Demographic data analysis and the univariate

screen of antecedent maternal and pediatric characteristics are complete. Special tape files to be used in multivariate analysis are partially completed, and initial regressions are beginning.

A study of febrile seizures was a major focus of the convulsive disorders area. Three publications have dealt with the likelihood of development of chronic epilepsy and other adverse outcomes in children who have experienced febrile seizures. These findings are an important element in the development of a rational approach to clinical management. The principal investigators received PHS Special Recognition Awards, primarily for work in this area. Ellenberg, J.H. and Nelson, K.G.: Febrile seizures and later intellectual performance. Arch. Neurol. 35: 17-21, 1978. Nelson, K.B. and Ellenberg, J.H.: Prognosis in children with febrile seizures. Pediatrics 61: 720-727, 1978. Nelson, K.B.: Febrile seizures. In Gellis and Kagan (Eds.): Current Pediatric Therapy. Philadelphia, W.B. Saunders, Co., 1978, Vol. 8, pp. 85-87.

In the learning disorders area, low achievers, or children with average IQ scores and below-average achievement test scores in reading or spelling, were found to have been born into large families or relatively low socio-economic status. They were primarily male. As preschoolers, they had difficulties with language and relatively low IQ scores. At age 7, signs of deviant behavior, verbal and non-verbal cognitive deficits, and neurological soft signs were present. Hyperactive low achievers had an increased frequency of obstetrical complications. The major statistical analyses are completed. A monograph reporting on these data is in preparation.

In the area of minimal brain dysfunction, significant associations have been found between major symptoms (low achievement, hyperkinesis, and minor neurological signs) and socioeconomic, perinatal, developmental, and familial variables; many antecedents were common to all three symptom groups (for example, small size at birth and later ages, family configuration, motor performance in infancy, behavior and motor performance at age four, and affected siblings).

A sixth and a seventh part of the eleven-part congenital malformations analysis have been completed. These deal with the association of ABO and Rh blood types with, and the effects of maternal factors on, congenital malformations. Reports have been written. Special studies of external ear malformations, maternal exposure to radiation in childhood tumors, and hyperthermia as a possible teratogenic agent in man have been written. In-depth studies of neural tube defects, microcephaly, and the role of placental type on abnormal outcome of twins have been initiated and partially completed.

In the birthweight- gestational age area, the analyses, including graphs and tables of Phases I and II, have been completed. The examination of a Birthweight Index to determine its predictive value for birthweight-gestational age outcomes is in progress. Writing of the text for Phases I and II of the study is underway.

Studies in the area of pathology continued under two separate contracts, one for neuropathology and one for general and placental pathology. In the neuropathology segment work on the manuscript of the monograph report continued during the Fiscal Year, and publication is expected in the spring of 1979. In the general pathology segment work continued under a newly awarded contract to follow-up leads generated during the previous contract, specifically in the areas of teratogenic effects of cigarette smoking and the relationships between placental abnormalities, gestational infections, and noxious antenatal events as these affect mental and motor status of the children. To date, three papers have been published from this new effort. Naeye, R.L.: Relationship of cigarette smoking to congenital anomalies and perinatal death: a prospective study. Am. J. Path. 90:2: 289-293, 1978. Naeye, R.L.: Causes and consequences of placental growth retardation. JAMA 239:12: 1145-1147, 1978. Naeye, R.L., Harkness, W.L., and Utts, J.: Abruptio placentae and perinatal death: a prospective study. Am. J. Obstet. Gynecol. 128: 740-746, 1977.

The contracted study of toxemia of pregnancy is complete and a book published. Friedman, E.A., and Neff, R.K.: Pregnancy Hypertension. Littleton, Mass., PSG Publishing Company, Inc., 1977, 258 pp.

"The First Year of Life," a book-length manuscript describing the characteristics of NCPP children during the first year, is in the hands of a publisher. Publication is expected early in 1979.

The neonatal Hyperbilirubinemia study is entering Phases II and III which will include analysis of data obtained when children were 7 years old. Phase I is complete and a paper published. Scheidt, P.C., Mellits, E.D., Hardy, J.B., Drage, J.S., and Boggs, T.R.: Toxicity to bilirubin in neonates: infant development during first year in relation to maximum neonatal serum bilirubin concentration. J. Pediatr. 91: 292-297, 1977. This paper suggests that neurological impairment may occur with serum bilirubin levels below 20 mg%.

A comprehensive analysis of labor and delivery factors, incorporating effects of maternal anesthesia on offspring was begun this fiscal year under a new contract. Work also continued in-house in the area of maternal anesthesia-analgesia and infant and child development. Analysis of a cohort of normal births indicates that there were marked effects throughout the infants' first year of pain-relieving agents administered during labor and delivery. The effects of anesthetic were particularly noticeable for motor development. Relationships between obstetrical medication and cognitive functioning at 4 and 7 years, and physical and neurological status at 7 years are currently being analysed.

Work in the area of physical growth continued during the third year of the contract. Socioeconomic and smoking effects on growth were explored in depth and several papers are currently in press. Work on the odontometric data from the University of Wisconsin is nearing completion. Analysis of incremental growth was completed. Planning for and writing of the monograph on physical growth has begun. Publications during the

year include Garn, S.M., Shaw, H.A., and McCabe, K.D.: Effect of socio-economic status in early growth as measured by three different indicators, accepted by Ecology, Food and Nutrition; and Garn S.M., Shaw, H.A., Wainright, R.L., and McCabe, K.D.: The effect of sample size on normative values. Ecology, Food and Nutrition, 6: 153-157, 1977.

Studies in the area of twins became a formal project this Fiscal Year. The objective of this project is to assess the influence of maternal, socioeconomic, neonatal, medical and other environmental factors on survival, growth, and development of twins and on abnormal outcomes. Twins have been classified into monozygotic (MZ) and dizygotic (DZ), and MZ twins into monochorionic (MC) and dichorionic (DC). Comparisons between and among these groups, including singletons, are made. Studies of IQ scores, physical measurements, and blood pressures have been conducted and three papers are now in press.

The contracted screening study of maternal drug ingestion is complete and a book published. Heinonen, O.P., Slone, D., and Shapiro, S.: Birth Defects and Drugs in Pregnancy. Littleton, Mass., Publishing Sciences Group, Inc., 1977, 516 pp.

VI. CONTRACT DEVELOPMENT:

Three new contracts awarded are as follows: (1) A Prospective Cohort Epidemiologic Study of Learning Handicaps in Children Attending School, (2) A Comprehensive Study of Labor and Delivery Effects on Offspring and (3) Analysis of General and Placental Pathology Data.

VII. SUPPORT FUNCTIONS:

The Unit for Data Collection is responsible for maintaining the NINCDS Collaborative Project files and the microfilming of the records in accordance with a system designed to facilitate data retrieval. During the Fiscal Year the major efforts were concentrated on preparation of records for microfilming, editing microfilm, and supplying records to the professional staff, outside investigators and consultants.

The Unit for Production of Data Analysis has as its basic mission the processing and storage by digital computer of the medical research data collected by the NCPP. The unit provides data processing support to the researchers in their analysis of the data. Most of the major research files have been completed, and in-depth statistical analysis is now being performed. By the end of the fiscal year, approximately 45 requests for analysis will have been completed. The following automated systems are in operation: (1) A financial system that accounts for all computer funds spent by the unit or programming contractor. (2) A follow-up job system that monitors all active jobs. The following systems are under study: (1) An automated bibliography of all publications emanating from the NCPP, (2) An index of all variables found in the Master-File, Variable File and peripheral files, (3) A tape documentation system that will describe and define all tapes created and stored by DNB.

VIII. OTHER MAJOR ACTIVITIES:

It is the policy of the DNB to encourage the appropriate use of the NCPP data by providing advice, data, and assistance to qualified biomedical and behavioral researchers who wish to utilize these data in their research. During the fiscal year a policy statement was developed which specifies the requirements to be met by a researcher who wishes to utilize NCPP data supplied by the DNB. The policy statement was formally approved by the Director, NINCDS and is available on request.

Requests for NCPP data to initiate new studies continue to be received from researchers who receive their support from agencies and institutions other than the NINCDS. These new initiatives include Child Study Center, Brown University, on maternal diabetes; Department of Mental Hygiene, New York State Psychiatric Institute, on the children of schizophrenic parents, and on the relationship between neurological soft signs and learning difficulties; and the Division of Special Education, The Pennsylvania State University, on early identification of handicapping conditions in children. In addition, a printout of alimentary tract conditions in NCPP children was supplied to the National Digestive Diseases Commission as a possible source of data for research. A study on occupational hazards to NCPP women during their pregnancies has been completed under a grant 1 R01 OH 00645-01 to Dr. Vilma R. Hunt, The Pennsylvania State University, from the National Institute of Occupational Safety and Health (NIOSH). A report on the findings of this study has been submitted to NIOSH and is being readied for publication.

Proceedings of the Workshop on the Neurological Basis of Autism are being edited by teams of workshop participants and DNB staff. A publisher is being sought. The volume will report on the presentations and discussions of the following topics: definition, neuropsychology, language, neurophysiology (vestibular and evoked potential research) and neurochemistry. A recruitment effort for the best qualified professional person available to head a new section within the DNB on Autism and Behavioral Disorders continues.

IX. ADDITIONAL ACTIVITIES:

The Office of the Chief, DNB, continues to be involved in Privacy Act matters for the NINCDS and the NIH. The Chief, DNB, continues to serve as NINCDS Privacy Act Coordinator. Activities for this fiscal year include the following: (1) The NIH pamphlet PRIVACY was distributed to all NINCDS staff (this publication has also been used to inform individuals outside the NIH of basic Privacy Act requirements); (2) determinations were made of the applicability of the Privacy Act to each new NINCDS contract involving human subjects; (3) quarterly reports, the annual report, and annual system notices were prepared and submitted to the NIH Privacy Act Coordinator; (4) requests for access to or ammendment of grant records are now at approximately the 800/year level (replies to

these requests are reviewed by Office of Chief, DNB staff); (5) impact of the Privacy Act on peer review continues to be assessed and recommendations made to the NIH Privacy Act Coordinator.

The Office of the Chief, DNB, continues to administer the NINCDS Clinical Research Panel for extramural contracts and the Chief, DNB, serves as Chairman. This panel has the responsibility for reviewing NINCDS contracts for adherence to DHEW and NIH rules and regulations regarding the protection of human subjects in research and recommending approval or disapproval to the Director, NINCDS. During the fiscal year to date, eighteen new contract proposals and twelve renewals have been reviewed by the Panel.

The Chief, DNB, and the Staff Assistant to the Chief, DNB are members of the Contract Compliance Committee for Project Officers, chaired by the Contract Compliance Coordinator, NIH. This committee has as its charge the development of a questionnaire to be administered by project officers to principal investigators/project directors on NIH contracts for the purpose of increasing awareness of and monitoring compliance with Federal requirements for Equal Employment Opportunity.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
October 1, 1977 through September 30, 1978

CHILDREN'S HOSPITAL MEDICAL CENTER, BOSTON, MASSACHUSETTS (NO1-NS-3-2312)

Title: Combined Neuropathologic and Epidemiologic Study

Contractor's Project Director: Floyd H. Gilles, M.D.

Current Annual Level: $ 0.00

Objectives: The contract will analyze the neuropathology collection of the NINCDS Collaborative Perinatal Project (NCPP). An estimate of the quality of the material and a catalogue of gross brain abnormalities will be prepared. Plots of fetal brain weight of grossly normal brains against estimated gestational age, utilizing a Gompertz function, will be made and an analysis will be made relating events of pregnancy, labor, and delivery. A comparison will be made of rate of brain weight acquisition in utero to rate of brain weight acquisition after birth as a function of total (gestational plus survival) age. A study will be made of intracranial hemorrhage including topography of hemorrhage. A study will be done on the risk factors associated with perinatal telencephalic leucoencephalopathy. A study of cerebral necrosis is to be completed which would include criteria of necrosis in the perinatal brain, and an evaluation of selected risk factors in relation to subclassification of neuronal and white matter necrosis.

Major Findings: Review and classification of pathology material are complete. Data analysis is complete and a monograph report is nearing completion.

Course of Contract: June 1, 1973 through December 31, 1976. The contract is terminated; extra time is being allowed to complete and publish the monograph report.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
October 1, 1977 through September 30, 1978

UNIVERSITY OF MINNESOTA (NO1-NS-4-2326)

Title: Analysis of Speech, Language and Hearing Deficits to Facilitate Prevention, Diagnosis and Treatment

Contractor's Project Directors: Frank M. Lassman, Ph.D., and Robert O. Fisch, M.D.

Current Annual Level: None

Objectives: Speech, language and hearing (SLH) data were collected as part of the Collaborative Perinatal Project (NCPP) of the National Institute of Neurological and Communicative Disorders and Stroke. Clinical populations and a group of private patients were sampled at twelve medical institutions located largely in eastern and southern states. Data from SLH examinations administered at 3YR and 8YR were analyzed for interrelationships and for associations with findings in other areas of the NCPP study. These include variables relating to pregnancy, labor and delivery, family characteristics and the physical, mental and behavioral characteristics of the children. Relationships among the variables were studied to provide clues to the etiology of communicative disorders, and to uncover findings which might be clinically applicable as well as predictive of outcome.

The cohort chosen for analytic study included all White and Black children who were given valid SLH examinations within age limits specified in the study protocols, and for whom there were sufficient prenatal, socioeconomic and other non-SLH data. The selection criteria yielded a cohort of 27,558 children consisting of three subsamples: 1) those tested at 3YR (19,885); 2) those tested at 8YR (20,137); and 3) those tested at both ages (12,464). These subsamples were compared with the children who took neither test (11,225) on selected SLH and NCPP variables and were described statistically by race, sex and Socioeconomic Index (SEI) for each medical institution and in total. They appeared to be similar in all respects one to another and, except for SEI and race, to the group not given either of the SLH examinations.

The speech, language and hearing examination administered at 3YR covered Language Comprehension and Language Expression, Hearing, Speech Mechanism, Speech Production and Memory for Digits and Syllables. The battery was devised especially for use in the NCPP study, and its component tests were largely unstandardized. At the 8YR level, the same general SLH areas were examined but in greater detail with more standardized tests. Reading and writing were assessed at 8YR but not at 3YR.

The SLH data were examined for quality as it pertains to availability of the data, reasonableness of values and stability of findings. Examiner variabil was evaluated on the basis of test-retest results obtained from the NCPP quality control program. Institutional variability was studied, with particular

emphasis on two data-gathering institutions which used atypical sampling methods at the 3YR level. Intercorrelations were calculated for the 3YR SLH variables and the 8YR SLH variables, using data on the population examined at both ages (3YR/8YR). The quality of most SLH variables appeared satisfactory in general. The NCPP variables were scrutinized by consultants and the NCPP staff for validity, reliability, redundancy and missing data. This resulted in the selection of a list of 676 NCPP variables for study.

In order to facilitate study of the relationships with NCPP variables, key SLH variables were identified and a number of composite indexes were constructed to serve as summary descriptors. The SLH variables were evaluated by consultants and staff for acceptability as potential components of indexes and were combined according to criteria recommended on the basis of expert judgment. Frequency distributions and descriptive statistics were generated for each index, and inter-correlations were obtained among indexes and between indexes and individual SLH variables. Additionally, the method of Principal Components was used to obtain weightings for construction of another set of indexes. These were correlated with indexes constructed by the consultant panel. Three other comprehensive indexes proposed by another task force also were subjected to the same evaluative process. A total of 27 SLH indexes and key variables emerged, and these were used to construct a correlation screen with the 676 NCPP variables selected earlier. Multiple regression analyses, using early variables which survived the correlation screen, yielded equations with varying predictive abilities. Correlations of residuals yielded no potential variables as additions to the multiple regression analyses. In addition, conditional probabilities were calculated to assess the risk of SLH failure given success or failure on certain other antecedent variables.

Major Findings: Two multiple regression analyses were performed using the 3YR SLH indexes as outcomes. One used data at birth, and the other used at-birth plus 8-month mental and motor scores. In general, these predictors explained less than 8% of the variance. The better predictions were for 3YR Articulation, Intelligibility, Language Comprehension and Sentence Complexity. Addition of the 8-month data did not materially improve prediction.

Five multiple regression analyses were done using the 8YR indexes as outcomes. In addition to the two sets of predictor variables used for 3YR outcome, the 4YR IQ and 3YR indexes were used in various combinations. Best predictions occurred for 8YR Word Identification (.75), Concept Development (.69), Written Communication (.61), Language Production (.54), Language Comprehension (.51), Auditory Memory (.49) and Articulation (.45).

The 4YR IQ demonstrated (by means of large beta coefficients) pronounced effects in almost every multiple regression in which it was entered. The 8-month variables contributed very little to any of the predictions, and 3YR indexes alone were not successful predictors of 8YR indexes. A combination of at-birth variables and 3YR indexes performed slightly better in predicting more than half the 8YR outcomes than did a combination of at-birth variables with 8-month scores and 4YR IQ.

Measures of lip, tongue and palate movement revealed little about communication skills such as Articulation, or about speech intelligibility, at 3YR or at 8YR for most children. They were useful to help identify childr with neuromotor impairments and anatomic deficiencies of the speech apparatus Higher than average incidence of speech mechanism dysfunction occurred in identified syndromes, deviant eye and skeletal conditions, and suspected neurologic abnormalities.

Social variables, such as SEI and mother's education, were more strongly related to articulation development than were physical variables other than clearly organic deficiencies. Premature children showed a slight performance deficit in articulation. No specific variables or sets of variables could be identified as predictive of poor fluency of speech. Failure on Articulati and Intelligibility at 3YR was predicted best by behavior on sound orientatio items in the Bayley Test at eight months. Speech production at 8YR could not be predicted reliably by performance at 3YR.

In accord with reports in the literature, sex differences in language performance favoring females were not consistently found at 8 years. Race differences were confounded by SEI and other effects tending to favor performance by Whites. Race, sex, SEI and IQ contributed most to predicting the 8YR language indexes. Failure on 8-month Bayley vocalization items increased by 2 to 3 times the risk of later poor language performance. Po language performance was associated with lower IQ, low birthweight, prematur low Apgar scores and hearing loss. Children with dysfluent speech or cleft palates were not inferior in language. Single children performed better than those with siblings, and adopted children did better than those with biological parents or children in foster homes.

Written communication (reading, writing, spelling) likewise showed systematic improvement with increasing IQ, SEI and education of the parents. Performanc was better also for Whites, females, singletons and adopted children. Scores were poorer for Blacks, males, stutterers, siblings, twins and those with adverse birth conditions or organic problems. Highest correlations were found to occur with SEI, parental education, IQ at 4YR and 7YR, and performar on the Bender Gestalt and the Auditory-Vocal Association Test (ITPA). Low correlations were found with early physiologic and behavioral observations. Handedness showed no relationship to performance. Single adverse perinatal events were ineffective predictors of written communication ability; environmental and later developmental multiple factors, including SEI, were better predictors.

Digit and syllable memory span tests were found to be relatively free of cultural bias. Race, sex and SEI differences were small. Performance was related to reading and writing and other language tests and to speech articulation. Performance was not predicted well by other variables except for IQ, attentional variables and neurological abnormality.

Hearing tests at 3YR revealed that 4.3% (N = 16,000) failed one or more frequencies in either or both ears. Spondee words were failed by 7.7%. No

systematic relationships were found between hearing at 3YR and other 3YR SLH variables or with NCPP variables. However, failure of the 3YR Pure Tone Hearing Screen was predictive of loss in hearing sensitivity at 8YR.

Mean air conduction thresholds at 8YR were comparable to those reported in other studies. Discrimination scores were higher for Whites and for higher SEI ratings. Abnormal conditions of the ears observed during the first year of life were not highly indicative of hearing loss at 8YR. Of 1186 children judged otoscopically abnormal at 7YR, about 100 appeared in the monaural or binaural conductive categories at 8YR, and less than half as many in the sensorineural loss group. Only about 1% of children falling outside normal range in the conductive categories were judged "deaf" in the 12 month pediatric examination, as compared with about 20% in the sensorineural group.

Relationships between 8YR hearing and antecedent medical and non-medical factors were generally weak; the strongest was between the binaural sensorineural category and infectious diseases during pregnancy ($r = .20$). Relationships between 8YR hearing and other SLH performance were stronger, particularly with Articulation (-.66), Intelligibility (-.66) and Word Identification (-.52). Only short attention span appeared to be related to monaural loss indexes. Weak relationships were found for conductive binaural loss and the Direct Coombs' Test (-.23), bilirubin level (.21) and Observable Ear Condition (.25-.33). Early responses to sound at eight months showed only weak association with the binaural indexes at 8YR.

Hearing was found to be worse in the poorer ear during colder months but no geographic effects were noted. Average hearing levels improved with higher birthweight. There was greater incidence of hearing loss among foster children than among adopted children. A small difference (1.5dB) in hearing sensitivity was found in favor of singletons compared with twins and children with siblings. Greater than average loss in the poorer ear occurred for children diagnosed as Cleft Palate (14.7dB, $n = 24$), Mentally Retarded (14.3dB, $n = 53$), Cerebral Palsy (11.3dB, $n = 83$) and dysfluent (11.4dB, $n = 35$). Premature children with true distress (5-minute Apgar) were similar to the total sample in poorer ear sensitivity.

Higher relative risks for sensorineural hearing loss in children was suggested for certain drugs administered to mothers during pregnancy. These include: a) several Sulfa Drugs (sulfathiazole, sulfacetamide, sulfabenzamide, sulfamethoxypridazine); b) Phenothiazines (promazine or buclizine); c) Streptomycin; d) Methamphetamine; and e) 17 tranquilizers. The analyses were controlled variously for SEI, Hyperemesis, Hematuria, bacterial infection, prior stillbirth or neonatal death, short gestation, no weight gain in pregnancy, smoking, 2nd trimester bleeding and Hypertension, although most characteristics of pregnancy were weakly or not at all associated with hearing loss. High relative risks were indicated for some other drugs as well (antidepressants, general anesthetics, non-barbiturate anticonvulsants and antihypertension agents), but too-few cases precluded analysis.

In brief, perinatal and physical factors associated with favorable SLH outcome included the following: low parity, maternal age 18YR, maternal height 61 inches, weight gain in pregnancy 15-25lb, full term infant, no forceps used, birthweight 2500 gm, larger head circumference, obesity, Femal normal neurologic findings. Factors associated with less favorable SLH outco included the following: high parity, maternal age 18YR, maternal height 6 inches, weight gain in pregnancy 15 or 25lb, prematurity, breech delivery, forceps used, birthweight 2500 gm, microcephaly, leanness, maternal mental retardation, Male, abnormal neurologic findings.

Psychosocial factors associated with SLH outcome at 8YR included SEI, race, sex, mental and motor scores at eight months, IQ at four and seven years and performance on the Bender Gestalt test. Other useful indicators were perform ance on vocalization items of the Bayley Scales, the Auditory-Vocal Association Test of the ITPA and some 3YR SLH indexes. Variables indicative of socioeconomic level have the strongest relationship to SLH outcome at 8YR. Correlational methods appeared to be less useful for predictive purposes than calculated conditional probabilities regarding outcome.

Course of Contract: June 29, 1974 through June 28, 1976. Extension of time was required for completion, but with no additional funding.

Publication: Forthcoming.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
October 1, 1977 through September 30, 1978

UNIVERSITY OF MICHIGAN (NO1-NS-5-2308)

Title: Physical Growth Analysis

Contractor's Project Director: Stanley M. Garn, Ph.D.

Money Allocated: $161,185.00 (Estimate for entire course of contract)

Objectives: To develop the physical growth measurement data on the 50,000 children examined within the framework of the NINCDS Collaborative Perinatal Project (NCPP). Specifically:

1. Develop for body weight, length, chest circumference and head circumference, a set of tabular, percentile, normative tables of (a) size-for-age, (b) increments of size for age-interval, (c) size-for-size for age and size for gestation length for whites, blacks and Puerto Ricans separately and for boys and girls separately. This set of tables is largely intended as a reference document for the NINCDS Collaborative Perinatal Project.

2. Develop a set of summary tabulations and reports, directed to the major pediatric and growth-related users, complete with narrative and graphs, with the purpose of providing in the professional literature both an account of major substantive findings, and an in-the-literature account of the major data based along lines described in 1, but simplified as necessary.

3. To correlate the incidence and prevalence of dental and facial abnormalities with neurological defects, congenital abnormalities and other disorders of childhood.

Major findings: (1) Effect of maternal socioeconomic status and smoking habits on birthweight and hemoglobin levels; (2) the effect of gestation length on subsequent growth patterns, and; (3) effect of birthweight and gestation length on tooth eruption sequences.

Significance to the Program: The above findings support previous study findings and are important to the pediatric community as well as to physical anthropologists in that they represent results from the largest longitudinal data base yet studied in the U.S.

Proposed Course: Prepare detailed analyses of the effects on physical growth data of socio-economic variables, birthweight variables, family size and parity, gestation variables, including various restrictions for gestation length, effects of race, family-line effects, channelwise progression (canalization), and the effects of exclusions for normality in final publication form and to be submitted for publication as results allow.

In addition to investigative research, chapter headings have been developed for the growth monograph required under this contract and are as follows:

Monograph Title: Determinants of Size and Growth in Infancy and Childhood

Chapter headings:

1. Introduction
2. Maternal size as a factor in growth
3. Placental size and growth
4. Maternal maturational timing as a factor in child growth
5. Maternal smoking and effects on the offspring
6. Gestation length and subsequent growth
7. Birth size as a determinant of size and subsequent growth
8. The birth weight factor and subsequent growth
9. Head circumference and its implications
10. Bivariate growth (size for size)
11. Race, ethnic group and prenatal growth
12. Race and ethnic group in postnatal growth and development
13. Incremental growth
14. Familial aspects of growth and development
15. Growth and teeth

Course of Contract: May 1, 1975 through April 30, 1979

Publications:

Garn, S.M., Shaw, H.A., and McCabe, K.D.: Apportioning black-white hemoglobin and hematocrit differences during pregnancy. Am. J. Clin. Nutr. 30:461-462, 1977.

Garn, S.M., Shaw, H.A., and McCabe, K.D.: Effects of socioeconomic status (SE and race on weight-defined and gestational prematurity in the U.S.A. In Reed, D.M. and Stanley, F.J. (Eds.): The Epidemiology of Prematurity. Baltimore, Urban and Schwarzenberg, 1977.

Garn, S.M., Shaw, H.A., Wainright, R.L., and McCabe, K.D.: The effect of sample size on normative values. Ecol. Food & Nutr. 6:153-157, 1977.

Garn, S.M., Shaw, H.A., and McCabe, K.D.: Relative effects of smoking and other variables on size of newborn (letter). Lancet 2:667, 1977.

Garn, S.M., Shaw, H.A., and McCabe, K.D.: Reply to Hoobler and Hunscher (letter). Am. J. Clin. Nutr. 30:1935-1937, 1977.

Garn, S.M., Shaw, H.A., and McCabe, K.D.: No Ifs, Ands or Butts. In Schemmel, R. (Ed.): Essays in Honor of Olaf Mickelsen. Michigan, Agricultura Experiment Station, in press.

Garn, S.M., Shaw, H.A., and McCabe, K.D.: Effect of maternal smoking on hemoglobins and hematocrits of the newborn. Am. J. Clin. Nutr., in press.

Garn, S.M., Shaw, H.A., and McCabe, K.D.: Dose response effect of maternal smoking (letter). Pediatrics, in press.

Garn, S.M., Shaw, H.A., and McCabe, K.D.: Effect of smoking during pregnancy in hemoglobin and hematocrit levels. Am. J. Obstet. Gynecol., in press.

Garn, S.M., Shaw, H.A., and McCabe, K.D.: Effect of socioeconomic status in early growth as measured by three different indicators. Ecol. Food & Nutr., in press.

Garn, S.M., Shaw, H.A., and McCabe, K.D.: The differential socioeconomic effect on the male. Am. J. Phys. Anthropol., in press.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
October 1, 1977 to September 30, 1978

UNIVERSITY OF COLORADO (NO1-NS-5-2326)

Title: Congenital anomalies and chromosome variation

Contractor's Project Director: Herbert A. Lubs, M.D.

Objectives: To determine if there is a significant association between congenital anomalies and minor chromosomal variants, particularly Q, C and length heteromorphisms.

Major findings: About 40 "independent" significant associations were found but considering the large number of analyses performed, these are not more than would be expected by chance. The most significant associations were between strawberry/portwine hemangioma and increasing amounts of qh material; and pale Q banding heteromorphisms and skin anomalies. These results indicate that the total amount of qh or p11 and p13 material may be more important in the determination of developmental anomalies than any particular heteromorphis per se.

Current status: The contract has expired and the work has been completed. A final report has been submitted.

Publications: Patil, S.R., Lubs, H.A., Brown, J., Cohen, M., Gerald, P., Hecht, F., Kimberling, W., Myrianthopoulos, N. and Summitt, R.L. Incidence of major chromosomal abnormalities in children. Cytogenet. Cell Genet. 18:302-306, 1977.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
October 1, 1977 through September 30, 1978

THE PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PA. (NO1-NS-7-2376)

Title: Analysis of General and Placental Pathology Data

Contractor's Project Director: Richard L. Naeye, M.D.

Current Annual Level: $ 42,360.00

Objectives: The objectives are (1) determine the teratogenic effects of cigarette smoking, (2) correlate gestational infections with placental abnormalities, and (3) search for the leads to the etiologies of sensory, mental and motor defects by making correlations between placental abnormalities and noxious antenatal events.

Major Findings: Two papers have been published and two submitted for publication. Work on completion of reports for publication from the previous contract (NO1-NS-3-2311) continues.

Course of Contract: September 30, 1977 through January 31, 1979.

Publications:

Naeye, R.L.: Relationship of cigarette smoking to congenital anomalies and perinatal death: a prospective study. Am. J. Path. 90:2:289-293, 1978.

Naeye, R.L.: Causes and consequences of placental growth retardation. JAMA 239:12:1145-1147, 1978.

Naeye, R.L., Harkness, W.L., and Utts, J.: Abruptio placentae and perinatal death: a prospective study. Am. J. Obstet. Gynecol. 128:740-746, 1977.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
October 1, 1977 through September 30, 1978

CHILDREN'S HOSPITAL MEDICAL CENTER, BOSTON, MASSACHUSETTS: (NO1-NS-7-2377)

TITLE: A Prospective Cohort Epidemiologic Study of Learning Handicaps in Children Attending School

Contractor's Project Director: Alan Leviton, M.D.

Current Annual Level: $83,000.00

Objectives: Conduct analyses of antecedents of school behavior and school achievement at age 9 in an identified sample of children in the Boston component of the NINCDS Collaborative Perinatal Project (NCPP) for the purpose of identifying risk factors for learning disorders.

Major Findings: Review and merger of tape files containing (1) teacher questionnaire data at age 9; (2) reading test scores between ages 7 and 9; and (3) the DNB variable file is complete. Cluster analysis of items on the teacher's questionnaire have revealed two syndromes related to learning handicaps: behavioral and cognitive-perceptual. Analyses of these data are continuing in the areas of syndrome definition and identification of antecedent factors.

Course of Contract: September 30, 1977 through September 29, 1979.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
October 1, 1977 through September 30, 1978

BETH ISRAEL HOSPITAL, BOSTON, MASSACHUSETTS (NO1-NS-8-2381)

Title: Comprehensive Study of Labor and Delivery Effects on Offspring

Contractor's Project Director: Emanuel A. Friedman, M.D.

Current Annual Level: $127,000.00

Objectives: The objectives are (1) to determine the effects on the fetus and the surviving infant of clinically defineable labor factors, labor disorders and the spectrum of delivery procedures, and thus to identify and quantitate the specific risk factors in labor and delivery that contribute to perinatal mortality and to the development of long term neurological and developmental disorders in children, and (2) to determine relationships between the various types of maternal anesthesia-analgesia and development of the child; specifically, to examine in detail the time-dose relationships and drugs used in combination during the course of labor and delivery, in relation to long-term neurological outcome in the child.

Major Findings: As this contract was awarded March 13, 1978, the first progress report has not been received at this writing. Data tapes have been requested and delivered to the contractor.

Course of Contract: March 13, 1978 through March 12, 1982. A fourth year was requested in the original proposal.

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 01163-16-DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Selected Maternal Risk Factors and Congenital Cardiovascular Anomalies

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

PI: L. Bajda Medical Consultant · DNB, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	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)

This investigation into significant maternal factors related to pregnancy outcome at high "risk" for congenital heart disease uses data from records of the longitudinal epidemiologic NINCDS Collaborative Perinatal Project. Observations on some 50,000 pregnancy records provide case and control data for analysis of selected maternal variables suggesting significant risk for cardiovascular anomalies. From over 500 suspect cardiacs specific diagnoses were available for some 460 cases. These include cardiac conditions as part of known syndromes. Analysis separates the various anomalies in order to clarify possible etiologies but also considers the group as a whole in the presentation of significance of the problem to society and to providers of care for handicapped children.

Project Description

The Study has as its primary objective an epidemiologic investigation of relationships between maternal conditions and congenital cardiovascular anomalies. Identification of conditions putting the child "at risk" are sought.

Additional objectives include relating early signs of cardiac abnormality to cardiac diagnosis, growth, and mental status at 7-8 years of age as well as at intermediate levels. Emphasis on clinical attributes of the congenital heart case at various ages as well as the maternal history involved may provide a ready guide to the optimal care of the child.

Methodology

Study records from the NINCDS Collaborative Perinatal Project (approximately 50,000 population) provide the data. Case number print-outs for children diagnosed as suspect or definite cardiacs on the one-year and seven-year summaries are used as indicators for the records searched for preselected maternal variables. After tabulation, analysis and comparison with computer provided control data, the use of statistical techniques should present a maternal "profile" for the infant "at risk" for congenital cardiovascular disease.

Major Findings

Presentations of preliminary findings on an initial sample of 82 and then 112 definite congenital cardiac cases emphasized the need for a larger study group in order that the maternal factors could relate to a specific diagnosis. General findings included a definite preponderance of mothers over 30 years age in both races after eliminating known chromosomal aberrations. There were also a greater than expected number of gravida with systemic disease complications and prior pregnancy loss. Underway is a larger analysis considering 457 cases with specific congenital cardiac anomalies.

Significance to Biomedical Research

Use of epidemiological techniques to reveal source of disease is well proven. It is anticipated that the resultant guides to prevention of that once very crippling condition called "congenital heart" are at hand. The extent to which the clinician and basic scientist desires, and is able, in today's sociological setting, to apply these findings will determine the significance of this study.

Proposed Course

Continuation of the above methodology and 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 01184-16 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Population Dynamics of Tay-Sachs Disease and Other Sphingolipidoses

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

PI: N.C. Myrianthopoulos Research Geneticist DNB NINCDS

COOPERATING UNITS (if any)

Dr. D. Gröschel, University of Texas

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05	.05	.00

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 objective is to confirm experimentally the epidemiologic finding that the selective advantage of the TSD heterozygote is due to possible protection of the heterozygote from tuberculosis. Current experiments measure the phagocytic activity of mouse macrophages for mycobacteria in the presence and absence of GM_2 ganglioside.

Project Description:

Objectives: To confirm experimentally the epidemiologic finding that the selective advantage of the Jewish TSD heterozygote is due to possible protection of the heterozygote from tuberculosis.

Methodology: The experimental design is to measure the rate of growth of the mycobacterium tuberculosis in media with and without hexosominidase A, and the rate of infection by the mycobacterium of tissues with and without lipid accumulation. Experiments have been designed in which mouse macrophages are fed GM_2 ganglioside *in vivo* and *in vitro*, and the phagocytic activity for mycobacteria measured. Due to technical difficulties, no progress has been made with these experiments.

Major findings: None

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 01274-14 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Genetic Bases of Neonatal Reflexes

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

PI:	A.F. Naylor	Research Geneticist	DNB NINCDS
OTHER:	N.C. Myrianthopoulos	Research Geneticist	DNB NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.10	.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)

Neonatal reflexes (suck, rooting, palmar grasp, plantar grasp, Moro, etc.) are usually tested as signs of neurological and general well-being. A study has long been planned as to whether genetic variables may be sometimes responsible for absence of specific reflexes. A data file which contains genetic and background information has been enlarged to include data on neonatal reflexes so that the investigation can finally be carried out.

Project Description:

Objectives: To investigate the validity of regarding the suck, rooting and other neonatal reflexes as genetic entities.

Major findings: A review of cases has led to criteria for accepting children as normal but lacking specific reflexes and rejecting those cases which are clearly abnormal. Appropriate codes, in the same format as malformations, have been included in the now completed Family Analysis File, which includes items on most possible abnormalities (physical or mental), background variables and relationships to other NCPP children.

Proposed Course: These variables will be analyzed familiarly by methods under development for all variables in the Family Analysis File. Special supplementary analyses will be carried out.

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 01276-14 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Sequential Aspects of Occurrence of Spontaneous Abortion in Family Histories

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

PI: A.F. Naylor Research Geneticist DNB NINCDS

COOPERATING UNITS (if any)

D. Warburton, College of Physicians and Surgeons at Columbia University

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.20	.00

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 objective of this study has been to relate the risk of spontaneous abortion to maternal age and prior reproductive experience. Careful reconsideration of long available tabulations has shown convincingly that gravidity is a major correlate of spontaneous abortion and maternal age is of no importance. A manuscript, subject to revision, has been accepted by Fertility and Sterility.

Project Description:

Objectives: To relate the risk of spontaneous abortion to maternal age and prior reproductive experience. A special point under investigation is whether apparent age effects are explicable by a tendency for intrinsic habitual aborters to remain in the reproductive population longer in attempts to compensate for unsuccessful pregnancies. Also conditional risks have been estimated.

Proposed course: A manuscript arguing that age effects are real but laying emphasis on the decided importance of parity effects has been published. Naylor, A.F.: Sequential aspects of spontaneous abortion: Maternal age, parity and pregnancy compensation artifact. Soc. Biol. 21:195-204, 1974. Reconsideration of data tabulated from OB-2 forms has shown the following: (1) the anamnestic reproductive histories are statistically reliable; (2) maternal age does not affect abortion risk once prior reproductive experience is taken into account; (3) gravidity effects are responsible for upwards of 30% of spontaneous abortions.

A manuscript, subject to revision, making these points and setting out a conditional table has been accepted by Fertility and Sterility.

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 ZO1 NS 01514-12 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Record Linkage of Relatives Registered in the Collaborative Study

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

PI:	A.F. Naylor	Research Geneticist	DNB NINCDS
OTHER:	N.C. Myrianthopoulos	Research Geneticist	DNB NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.40	.05

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 file has been created which can link NINCDS Collaborative Perinatal Project data for women with relatives also in the Project. This has been imbedded in a file which classifies registrants by the number of Project pregnancies if they have no relatives registered. This larger file, has in turn, been merged with a file containing other genetic information, such as twin zygosity, and medical and psychological data for familial studies of malformations and other conditions. The record linkage file, as such, will not be changed unless experience in its use shows it to be erroneous or cumbersome.

Project Description:

The objective of this study is to identify all relatives of gravidae registered in the NINCDS Collaborative Perinatal Project and link their records to facilitate genetic studies of obstetric, pediatric, psychological and sensory data.

The main record linkage file and its auxiliaries have been completed. Although it is being preserved as a separate file, on one set of magnetic tapes, it has also been merged with medical and psychological data preliminary to actual use in genetic and family studies.

Tabulations have been produced indicating that enough familial information is present to make analyses of not very rare abnormalities quite feasible. The first of three major computer programs to perform such analyses has been written and the second is well along.

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 01515-12 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Rh Hemolytic Disease in Negro and White Infants

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

PI: A.F. Naylor Research Geneticist DNB NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.00	.00	.00

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)

To carry out an investigation of a report in the literature that high Rh (but not ABO) antibody levels have smaller morbid effects in black than white babies, a data file under development, which is rich in information on both control and outcome variables, will be augmented with the needed laboratory test information.

Project Description:

Objectives: To confirm a report that high Rh antibody levels have smaller morbid effects in Negro than in white babies, although this is not true for ABO antibodies.

Major findings: Preliminary and indirect confirmation has been obtained, from a small data sample under study, for reports in the literature that high Rh antibody titers are not as highly associated with serious morbidity in Negroes as in whites.

Proposed course: An intermediate data file being mainly created for use in familial studies of malformations and other conditions will be augmented with variables needed for this particular study.

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 01754-10 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Growth and Intellectual Development of Children from Interracial Matings

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

PI:	L. Willerman	University of Texas at Austin	
OTHER:	A.F. Naylor	Research Geneticist	DNB NINCDS
	N.C. Myrianthopoulos	Research Geneticist	DNB NINCDS

COOPERATING UNITS (if any)

Department of Psychology, University of Texas at Austin

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.20	.00

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 small subpopulation within NINCDS Collaborative Perinatal Project children has been identified as being of mixed black and white parentage. Within the interracial group of matings there is no evidence that genetic or socio-economic differences are related to race of mother (or father). Thus socio-psychological influences, presumably operating through mother-child interactions, can be examined indirectly. Two papers have been published which indicate that, although early childhood differences are wholly negligible, children of white mothers eventually develop positive intellectual differentials. A careful reanalysis suggested by a journal referee of the comparative rates of growth of interracial and control children has removed the appearance of important differences. It is probable that no revised manuscript will be prepared.

Project Description:

Results of analyses of growth and intellectual development have been prepared for separate publication.

The conclusions reached in an initial publication in Science, that analyses of NCPP interracial for IQ data indicate that race of mother as a postnatal environmental indicator accounts for much of the black-white IQ differences, have been strengthened in a paper published last year. Analyses of Bayley Mental and Motor Scores, taken at 8 months, when maternal social influences will have had little effect show no differences between children of black/white and white/black matings. Also a more convincing demonstration has been made of lack of bias in genetic or socio-economic factors affecting four year IQ on the paternal side.

The physical development data have been re-analyzed to compare all properly selected NCPP interracial and monoracial matings. Hospital variation and other background factors were corrected for by multivariate regression. At birth children born to white mothers, whether by white or black fathers, are very similar in weight and length. Monoracial blacks are definitely smaller and interracials with black mothers may be intermediate in size (small numbers cloud the issue). At four months interracials with white mothers fall behind in weight (but catch up after one year), perhaps because of social stresses in the household. Re-analysis of data to meet criticisms of a referee has led to the probable conclusion that physical growth does not differ in important ways between interracial and control children. Consequently termination of the project is being considered.

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 01857 - 09 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
The Genetics of Intellectual and Motor Performance

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

PI:	Sarah H. Broman	Research Psychologist	DNB NINCDS
Other:	Paul L. Nichols	Research Psychologist	DNB NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.04	.04	.00

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)

Familial influences on tests of mental and motor performance at ages four and seven were examined by comparing correlation coefficients among monozygotic twins, dizygotic twins, full siblings, and half siblings, within race, sex, and social class groups. The correlations between scores of twin and sibling pairs on the Stanford-Binet (age 4) and Wechsler (age 7) intelligence tests suggested a greater genetic influence than was found for infant test scores. The monozygotic-dizygotic twin differences and full sibling-half sibling differences in correlations indicated more of a genetic influence for height and weight at ages four and seven than for the cognitive measures.

Project Description:

This study assessed the contribution of genetics to the variance in intellectual and motor performance at eight months, four years, seven years by correlating scores and measurements of twin, sibling, and half sibling pairs. Four year measurements included the Stanford-Binet IQ, Graham Block Sort Test, average fine and gross motor scores, height, weight, and head circumference. Seven year correlations were calculated for WISC verbal, performance, and total IQ, seven WISC subtests, Bender-Gestalt, Draw-A-Person Test, Auditory-Vocal Association Test (ITPA), Wide Range Achievement Tests, height, weight, and head circumference. An initial report has been published on genetics of infant mental test performance, in which scores of twins and singletons were compared. The estimated relative genetic influence on the variability of the four and seven year measurements ranged from very high (e.g., weight at seven years) to moderate (IQ at four and seven years) to low (some WISC subtests). The genetic influence on all the four and seven year outcomes appeared to be greater than that found and reported earlier for behavior in infancy.

Publications:

Nichols, P. L. and Anderson, V. E.: Intellectual performance, race and socioeconomic status. Soc. Biol., 20: 367-374, 1973.

Nichols, P. L. and Broman, S. H.: Familial resemblance in infant mental development. Dev. Psychol., 10: 442-446, 1974.

Nichols, P. L. and Broman, S. H.: Infant mental development. Beh. Genet., 7: 347-348, 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 ZO1 NS 02052-06 DNB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

The First Year of Life

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

PI.:	J. S. Drage	Chief	DNB, NINCDS
Other:	E. C. Jackson	Biostatistician	OBE, NINCDS

COOPERATING UNITS (if any)

J. B. Hardy, The Johns Hopkins University

LAB/BRANCH

Developmental Neurology Branch

SECTION

Perinatal Research Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) MINORS ☐ (a2) INTERVIEWS

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

"The First Year of Life" is planned as a volume to report on the frequency distribution of a number of findings reported on NINCDS Collaborative Perinatal Project children during the first year of their lives. It will include information on birthweight-gestation distribution, bilirubin levels, age at hospital discharge, and distributions of various pathological findings detected during the nursery stay and during the first year of life. Of particular interest will be information regarding brain abnormality as detected during the nursery period. This volume is intended to serve as a general description of the Collaborative Project children during their first year of life and as a reference document for further in-depth studies. The analyses for this work have been completed. The document is ready for publication.

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 ZO1 NS 02058-06 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Convulsive Disorders Data Analysis Group

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

PI: K.B. Nelson	Pediatric Neurologist	DNB NINCDS
PI: J.H. Ellenberg	Mathematical Statistician	OBE NINCDS

COOPERATING UNITS (if any)

Dr. J. Freeman, Johns Hopkins
Dr. K. Holden, Johns Hopkins

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.6	0.3

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 study examines the relationship between perinatal factors and the occurrence of seizure disorders in childhood in a large, prospectively studied population. In addition to the central question of etiology, it investigates frequency, prognosis, demographic characteristics, and a number of other aspects of these disorders. Extensive hand review and classification of cases has been completed, and files created. Univariate screen of maternal, obstetric, and pediatric risk factors, and demographic analysis, have been completed. File creation for multivariate analysis is partially complete, and regression analyses have begun. Selected topics of particular clinical relevance are under examination.

Project Description:

Objectives: To examine maternal characteristics, conditions of pregnancy, labor, delivery and the neonatal period, and illness and injuries of early childhood for their association with seizure disorders. To seek clinically useful indices for prediction, to evaluate clustering of other handicaps with convulsive disorders, and to examine the frequency of seizure disorders in the population of the NINCDS Collaborative Perinatal Project.

Methodology: The preliminary program to screen antecedent obstetric variables and early clinical manifestations with regard to their association with seizure disorder diagnoses has been completed. The analysis of demographic factors (e.g. institution, race, socioeconomic status, etc.) and their impact on the incidence and risk of seizure disorders is now available, and is in the process of assessment. A study into the natural history of seizure disorders from one to seven years of life is underway, taking full advantage of the prospective nature of the NINCDS Collaborative Perinatal Project.

Drs. Freeman and Holden are participating in the study of the prognosis of neonatal seizures.

Major Findings: Approximately one in twenty children (57/1000) followed to the age of seven years had at least one seizure. About one-tenth that number (4.8/1000) had active epilepsy by the age of seven. As studied in the NINCDS Collaborative Perinatal Project, active epilepsy in childhood is slightly more common in girls than boys, and approximately equal in rate in whites and blacks.

A substudy on febrile seizures (ZO1 NS 02234-03 DNB) is described separately.

Data on the prevalence of specific seizure disorders in early childhood are now available, and a manuscript is in preparation on this subject.

Approximately a quarter of children with epilepsy in early childhood have another major neurological handicap: mental retardation or cerebral palsy, or both. The other three-quarters of epileptic children did not have these additional disabilities. Analysis of the antecedents and clinical course in these two major groups is in progress.

Seizures occurring in the first month of life were associated with a relatively high rate of death or subsequent disability, including cerebral palsy. Neonata seizures are a major marker of risk for subsequent neurologic morbidity.

A major substudy concerning febrile seizures has been completed. Three reports have been published.

A manuscript is nearing completion on birthweight and gestational age as risk factors for seizure disorders.

Maternal, obstetrical, and early childhood characteristics which are associated

with seizure disorders are now under study.

Future Course: Demographic data on convulsive disorders in Study children, and the univariate screen of antecedent maternal and pediatric characteristics are now complete. Multivariate analysis has begun. Selected topics of particular medical importance will be examined in sub-studies. A monograph on convulsive disorders in childhood will be prepared; target date is July, 1978.

Significance: The convulsive disorders are a common and socially costly medical problem. It is estimated that about 4 million Americans have some form of epilepsy. The cost of the epilepsies in direct payments and medical expenses has been calculated at more than $4 billion per year. The information generated in the NINCDS Collaborative Perinatal Project may contribute useful information in this important problem area.

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 02059-06 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cerebral Palsy Data Analysis Group

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

PI: K.B. Nelson	Pediatric Neurologist	DNB NINCDS
PI: J.H. Ellenberg	Mathematical Statistician	OBE NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.8	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)

This large prospective study attempts to add to available knowledge of the perinatal factors associated with motor handicaps in childhood, the primary goal being to identify areas for possible preventive efforts.

Data on demographic analysis and a univariate screen of maternal and pediatric factors associated with cerebral palsy are available. Multivariate analysis is underway.

Project Description:

Objectives: To examine the etiology of motor disabilities in children, to improve clinical prediction, and to examine the relative frequencies of motor and associated disabilities in the population of the NINCDS Collaborative Perinatal Project.

Methodology: The univariate screen relating antecedent obstetric variables and early clinical manifestations with regard to their association with cerebral palsy diagnoses has been completed and multivariate analyses have begun. The analysis of demographic factors (e.g. institution, race, socioeconomic status, etc.) and their impact on the incidence and risk of cerebral palsy is available. Study of the natural history of cerebral palsy from one to seven years of life has been performed, taking full advantage of the prospective nature of the NINCDS Collaborative Perinatal Project.

Future Course: The multivariate analysis of the obstetric, early clinical and demographic factors is under way. Extensive use is being made of the NEUROMED statistical package developed in the Office of Biometry and Epidemiology to facilitate the rapid execution of this complicated last phase of analysis.

Major Findings: Cerebral palsy at seven years is somewhat more frequent in boys than girls, and among whites than blacks. Ten per cent of cerebral palsy is apparently caused by events occurring after the first month of life, most often infection or trauma.

Clearly handicapping cerebral palsy was present at age seven in 22-32/10,000 children, the range being related to race and sex. Within each birthweight and gestational age group examined, white males were at highest risk of cerebral palsy.

A listing of maternal and pediatric conditions most strongly associated with cerebral palsy outcomes has been made, and is the basis for multivariate analysis.

Studies have been completed, and manuscripts are in preparation, concerning:

1. The relationship of birthweight and gestational age to cerebral palsy (CP). Although low birthweight and immaturity are risk factors for CP, 59% of CP and 69% of CP other than spastic diplegia, occurred in infants of term weight and full 37 or more weeks gestational age. The apparent relationship of CP to smallness for dates noted by Scandinavian investigators may be due to the weighting of their series with twins, who are regularly smaller for dates than singleton births.

2. Children who "outgrew" CP. Subjects who were free of CP at seven years were more likely to be mentally retarded, to have recurrent convulsions, speech articulation problems, and other disabilities if they had shown abnormal motor signs on examination at one year of age. Children with documented early motor

abnormalities, especially those of mild degree, may be full of motor handicaps by early school age but at risk for other disabilities.

3. Associated handicap in children with CP. In addition to motor disability, children with CP have an increased frequency of intellectual, sensory and behavioral disorders, and seizure disorders. This study quantifies the degree of the association of CP with other handicaps, according to severity of CP and to specific CP type.

4. Signs of neonatal neurologic dysfunction as predictors of CP. A paper on this subject was delivered to the American Academy of Neurology (April, 1978). Certain signs on neonatal neurological examinations, and observations in the newborn nursery, are strongly associated with the likelihood of later CP. The predictive value of combinations of signs is under evaluation.

5. Early recognition of the infant at high risk for CP. Findings on examination at four months of age, especially among babies who were of term gestational age at birth, serve as predictors of later CP and, in a study parallel to that of newborn signs, is being evaluated for utility in the early recognition of infants at risk for motor handicap. Preliminary findings indicate that, for instance, a term infant who was considered to be neurologically abnormal at age four months, was at 200 times the risk of CP at age seven years as a baby who was considered neurologically normal when four months old.

Significance: Approximately 750,000 persons in the United States are victims of cerebral palsy; milder forms of cerebral palsy are more frequent still. The loss in social and economic terms is immense, and individuals with CP are lifelong dependents of their families or the state. Our aims are to identify areas in which preventive efforts may be effectively directed, to improve clinical prognostication, to examine clustering of handicaps, and to estimate relative frequency of cerebral palsy conditions.

Publications: Nelson, K.B. and Ellenberg, J.H.: Epidemiology of cerebral palsy. In Schoenberg, B.S. (Ed.): Advances in Neurology. New York, Raven Press, 1978, Vol. 19.

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 02060-06 DNB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Birthweight-Gestational Age

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

PI:	B. H. Williams	Assistant Head	PRS, DNB, NINCDS
Other:	J. S. Drage	Chief	DNB, NINCDS
	K. D. McCabe	Consultant	DNB, NINCDS
	E. C. Jackson	Biostatistician	OBE, NINCDS

COOPERATING UNITS (if any)
J. B. Hardy, The Johns Hopkins University
E. D. Mellits, The Johns Hopkins University

LAB/BRANCH
Developmental Neurology Branch

SECTION
Perinatal Research Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.50	0.25	0.25

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)

During the past year Phase II, a multivariant analysis to determine the relationship of birthweight as the dependent variable to a large number of antecedent (prenatal) independent variables, has been completed. The examination of a Birthweight Index derived from Phases I and II to determine its predictive value for birthweight-gestational age outcomes is in progress.

The final draft of the birthweight-gestational study will be submitted this year.

This work is being completed under personal services contracts with J. B. Hardy and E. D. Mellits.

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 02062 - 06 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Minimal Brain Dysfunction

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

PI:	P. L. Nichols	Research Psychologist	DNB NINCDS
Other:	Ta-chaun Chen	Sr. Math. Statistician	OBE NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.70	.60	.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)

Antecedents of minimal brain dysfunction were investigated by examining the association of three major symptoms -- school achievement, or learning disabilities; hyperkinetic impulse disorders; and minor neurological problems, or neurological soft signs -- with socioeconomic, perinatal, developmental, and familial variables. A major report describing the project is in preparation. Variables significantly associated with low achievement included socioeconomic status, number of family moves, family size, affected siblings, and behavior and motor performance at age 4. Hyperactivity was associated with cigarette smoking during pregnancy, size at birth and in infancy and childhood, father absent from the home, affected siblings, and behavior and motor performance at age 4. Associations with neurological signs included motor performance in infancy and at 4 years of age, cigarette smoking during pregnancy, size at birth and later, affected siblings, and neonatal seizures.

Project Description:

Specific diagnoses of minimal brain dysfunction (MBD) have not been made for children in the longitudinal NINCDS Collaborative Perinatal Project, but there is information available related to the most frequently cited symptoms (e.g., hyperactivity, learning difficulties, and equivocal neurological signs). This study has developed MBD criteria from the available NCPP data and characterized children with these symptoms in terms of demographic, psychological, and physical variables. Univariate analyses of the associations between MBD symptoms and hundreds of antecedent conditions have been performed. Many significant associations were found; some conditions related to poor school achievement were low socioeconomic status (including low levels of parental education, occupational status, and income; public assistance, and high housing density), frequent family moves, large family size, affected siblings, small size at birth and during infancy and later childhood, and abnormal behavior and motor performance at age 4. Hyperactivity was associated with cigarette smoking during pregnancy, absence of fathers from the home, small size at birth and later ages, being an "only child," affected siblings, abnormal motor performance and behavior at 8 months, and abnormal behavior and motor performance at 4 years. Neurological soft signs were related to poor motor performance at 8 months, neonatal seizures, cigarette smoking during pregnancy, small size at birth and later, affected siblings, and abnormal behavior and motor performance at age 4. Multivariate analyses have shown that most of the above associations remained significant when examined in combination with other variables. Familial associations were especially important. Of all the early predictors simultaneously examined, including socioeconomic status and perinatal complications, mean achievement score of siblings was the best discriminator between children with and without poor academic performance. The presence of a hyperactive sibling was the best discriminator between children with and without hyperactivity. And, presence of a sibling with neurological soft signs was one of the best discriminators between children with and without these conditions. A book length report describing the entire project 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 02106 - 05 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Developmental Factors Associated with Mental Retardation

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

PI:	S. H. Broman	Research Psychologist	DNB NINCDS
Other:	P. L. Nichols	Research Psychologist	DNB NINCDS
	J. P. Pomeroy	Systems Analyst	DNB NINCDS

COOPERATING UNITS (if any)
Dr. Peter Shaughnessy, University of Colorado Medical Center
Dr. Wallace Kennedy, Florida State University

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.7	.6	.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)
Data collected in the NCPP are being analysed to determine primary and contributing roles of biological and environmental factors in mental retardation in a population of 37,000 children followed from the prenatal period to age 7. The incidence of severe retardation did not differ by ethnic group, but mild retardation was more frequent among blacks than among whites. Retardation was negatively related to social class. Major neurological problems were more frequent among severely retarded than mildly retarded children, and were more frequent among whites than blacks in both retarded groups. Within ethnic group, the proportion of neurologically involved retarded children increased with social class. Risk factors for mental retardation include urinary tract infections during pregnancy, teen-age pregnancy, clinical signs of perinatal anoxia, and poor psychomotor performance in infancy.

Project Description:

Objectives: Data collected in the NCPP are being analysed to determine the primary and contributing roles of biological and environmental factors in mental retardation in a population of 37,000 children followed from the prenatal period to age 7. The identification of early signs of mental retardation will facilitate prevention, diagnosis and treatment, and will add substantially to knowledge in this area that has been largely derived from small retrospective studies of institutionalized retardates.

Method: Mental retardation was defined as an IQ of 70 or less on the Weschler Intelligence Scale for Children, or, for the relatively few children who could not be tested according to study protocol, equivalent IQs from other tests or reliable clinical judgements of retardation. Since children with IQs under 70 form a heterogeneous group, they were subdivided into four major categories consisting of those with severe retardation (IQ under 50) with and without signs of central nervous system damage, and those with mild retardation (IQ between 50 and 69) with and without such signs. Specific neurological diagnoses were obtained from the neurological examination given at age seven. The four groups were further subdivided by ethnic group and sex. Comparison groups are composed of children with IQs in the borderline, average and superior ranges.

Major Findings:

The incidence of severe retardation (0.5%) did not differ by ethnic group, but mild retardation was more frequent among blacks (5%) than whites (1%). The incidence of mild retardation, and to a lesser extent severe retardation, decreased as social class increased. Among severely retarded children, three-fourths of the whites but only one-half of the blacks had major neurological problems. Among mildly retarded children, 14% of the whites and 6% of the blacks had major neurological problems. In general, the proportion of retarded children with major neurological involvement increased as social class increased.

Urinary tract infection during pregnancy has a strong independent association with severe mental retardation without major neurological involvement.

Adolescent childbearing is associated with low IQ scores (under 70) at age four, and among whites, at age 7.

Clinical signs of perinatal anoxia are associated with below-average cognitive development. The risk for mental retardation is greatest when signs of central nervous system impairment are also present.

Infant psychomotor test scores at 8 months are good predictors of severe mental retardation at age 7.

IQ scores at age 4 are good predictors of all levels of mental development at age 7.

Proposed Course: Multivariate analyses of these data are being completed, and a monograph is in preparation.

Publications: Broman, S. H.: Perinatal Anoxia and Cognitive Development in Early Childhood. In Field, T., Sostek, A. M., Goldberg, S. and Shuman, H. H. (Eds): Infants Born at Risk. New York: Spectrum, 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 02107-05 DNB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
The Study of Visual Abnormalities in the NINCDS Collaborative Perinatal Project

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

PI: R. Feinberg — Research Psychologist — DNB, NINCDS

COOPERATING UNITS (if any) W.R. Baldwin, New England College of Optometry; R.E. Hoover, Baltimore, Md.; R.P. Kling, Georgetown Univ. Hosp.; M.A. Whitcomb, Nat. Acad. of Sc.; S.Z. Wood, Washington, D.C.; F.A. Young, Wash. State Univ.

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) MINORS ☐ (a2) INTERVIEWS

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

This project includes the analysis between visual abnormalities and outcome variables; anecdotal treatment based on case histories of unusual visual abnormalities; special studies of high-incidence disorders; and an overview of handedness and eye dominance as ascertained in our data; case studies of the blind children; and, preparation of a bibliography (monograph) encompassing these subjects.

Project Description:

The objectives of this project are to determine the extent to which genetic, maternal, obstetric, pediatric and environmental factors produce eye and visual abnormalities in the Study children; to assess the relative frequency of such anomalies; and, to study the concomitance of visual abnormalities with other sensory and motor neurological and systemic disorders.

A reference cohort of visual defects has been defined for which the specified outcomes were tabulated. Outcome variables have been reviewed. Computer print-outs have been obtained and are in process of being analyzed.

Both the monograph and the bibliographical supplement are expected to be completed in 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 02108 - 05 DNB

PERIOD COVERED
October 1, 1977 to Sepember 30, 1978

TITLE OF PROJECT (80 characters or less)
Developmental Factors Associated with Learning Disorders

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

PI:	S. H. Broman	Research Psychologist	DNB NINCDS
Other:	J. D. Pomeroy	Systems Analyst	DNB NINCDS

COOPERATING UNITS (if any)
Dr. Peter Shaughnessy, University of Colorado Medical Center

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.7	.5	.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)

The purpose of this study was to identify early behavioral, physical and familial characteristics of children with a significant discrepancy between intellectual ability and school achievement in the first and second grades. Low achievers, followed from the prenatal period to age 7, were compared with their IQ-matched academically successful controls on prospectively ascertained indices of cognitive and physical development and family characteristics. Cognitive deficits and behavioral deviations were found in the preschool perioc Indices of socioeconomic status (SES) and family structure were more strongly related to low achievement than were indices of physical development and medical status.

Low achievers were born into low SES, large families and were primarily male. As preschoolers, they had difficulties with language and relatively low IQ scores. At age 7, signs of deviant behavior, verbal and non-verbal cognitive deficits, and neurological soft signs were present. Hyperactive low achievers had an increased frequency of obstetrical complications.

Project Description:

Objectives: Children with normal intelligence and poor school performance, particularly in reading, present significant problems in etiology and the development of effective remedial techniques. Longitudinal data collected in the NCPP on a population of 37,000 children permit a study to be made of early events, beginning in the prenatal period, which differentiate between children with learning disorders in the first and second grades and those without a significant discrepancy between intellectual ability and school performance. The accurate identification of precursors of behavior patterns identified as learning disorders will facilitate prevention, early diagnosis and treatment. The objectives of this study are to determine the degree of association between learning disorders at age seven and the following classes of variables:

1) Biological factors

   a) Complications of pregnancy and delivery
   b) Adverse neonatal conditions
   c) Neurological and general medical status at one and seven years
   d) Childhood diseases and accidents
   e) Physical growth rates

2) Socio-environmental factors

   a) Socioeconomic status and social mobility of family
   b) Parental education
   c) Maternal intelligence level
   d) Family size and composition

3) Familial factors: occurrence of learning disorders in siblings

4) Cognitive and behavioral factors

   a) Mental and motor development and behavior ratings at eight months
   b) IQ scores, fine and gross motor development, concept formation and behavior ratings at age four
   c) Verbal and performance IQ scores, conceptual and visual-motor development and behavior ratings at age seven

Method: A child was considered to have a learning disorder if he or she had an IQ of 90 or above on the Weschler Intelligence Scale for Children and was more than one year below grade placement in reading or spelling on the Wide Range Achievement Test. Children with learning disorders,

referred to as low achievers, were compared with IQ-matched academically successful controls in order to isolate early developmental patterns and concurrent characteristics. Comparisons were made within ethnic group. Variables were first screened individually. The significant ones were then entered into discriminant function analyses.

Major Findings: (1) children with IQs of 90 or above and below average achievement test scores in reading or spelling were found to make up approximately 3% of the large unselected population of the Collaborative Perinatal Project, a proportion that agrees with the lower limit of most estimates of prevalence of "learning disabilities"; (2) low achievers were born into low SES, large families and were primarily male. In the preschool period, they exhibited language difficulties and relatively low IQ or aptitude scores. At age 7, their school problems were accompanied by signs of deviant behavior, non-verbal as well as verbal cognitive deficits, presence of neurological soft signs, and other signs of physical impairment also, particularly among blacks; (3) hyperactivity among low achievers appears to be related primarily to obstetrical complications, and, possibly, past reproductive problems. SES factors appear minimal, although less maternal education was a characteristic of the white subgroup. At age 7, hyperactive children were judged to be highly impulsive, and to show generalized behavioral deficits, and to a lesser extent, abnormalities of reflexes and gait.

The social status, size, and stability of the family all appear to be closely related to school performance of the child, particularly the male child. The characteristics of other subgroups of low achievers are being studied. They, like the hyperactives, may also have a distinct etiology.

Proposed Course: The major statistical analyses are completed. A monograph reporting on these data 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 ZO1 NS 02109-05 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Comprehensive Analysis of the NCPP Data on Congenital Malformations

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

PI:	N.C. Myrianthopoulos	Research Geneticist	DNB NINCDS
OTHER:	A.F. Naylor	Research Geneticist	DNB NINCDS

COOPERATING UNITS (if any)

C.S. Chung, Univ. of Hawaii; H. Lubs and M.L. Lubs, Univ. of Colorado; D. Smith, Univ. of Washington; J. Frias, Univ. of Florida; M. Melnick, Univ. of Indiana

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.50	3.70	0.80

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 is a long-term project to study the epidemiologic characteristics of congenital malformations in singletons and twins; to assess and interpret the influence of maternal, socioeconomic, neonatal, medical and other environmental factors on the occurrence of congenital malformations; to determine the risk of familial occurrence and to elucidate the role of genetic factors and the mode of inheritance of certain malformations; to determine the severity and clinical significance of congenital malformations and their associations with neurological, psychological and sensory handicaps; and to assess the long-range effects of malformations on survival, growth and development.

Project Description:

Objectives: This is a long-term project to study the epidemiologic characteristics of congenital malformations in singletons and twins; to assess and interpret the influence of maternal, socioeconomic, neonatal, medical and other environmental factors on the occurrence of congenital malformations; to determine the risk of familial occurrence and to elucidate the role of genetic factors and the mode of inheritance of certain malformations; to determine the severity and clinical significance of congenital malformations and their associations with neurological, psychological and sensory handicaps; and to assess the long-range effects of malformations on survival, growth and development.

Methodology: A congenital malformation is defined as a gross physical or anatomical developmental anomaly which was present at birth or was detected during the first year of life. Malformations have been classified into major and minor categories on the basis of their severity, threat to life and cosmetic significance.

The analysis is divided in 11 parts, and for each appropriate epidemiologic and statistical methods are being employed. In addition, in-depth studies of specific malformations or group of malformations are performed.

Current status and major findings:

I. Epidemiology of congenital malformations in singletons.

This part has been completed. About 15 percent of single-born children had one or more malformations. Half of the malformations were major. Only about a third of malformations observed during the first year of life were diagnosed at birth. Except for three minor malformations which were more frequent among blacks, there were no significant differences in malformation incidences between blacks and whites.

A report of this part has been published.

II. Epidemiology of congenital malformations in twins.

This part has been completed. Twins have significantly more major and minor malformations than singletons but the difference is wholly contributed by monozygotic (MZ) twins. The incidence of malformations in dizygotic (DZ) twins is the same as that in singletons. Monoamniotic twins have more malformations than diamniotic twins. Concordance rates are significantly higher among MZ than among DZ twins for all malformation categories but among specific malformations only those of the musculoskeletal system show significant differences.

A report of this part has been published.

III. A study of the effects of medical, genetic and socioeconomic factors in the occurrence of congenital malformations.

This part has been completed. Multiple birth, pregnancy complications (mostly through hydramnios) and male birth were positively correlated with increased risk in major malformations, whereas maternal weight gain was negatively correlated with major malformations. Maternal diabetes during pregnancy was significantly correlated with single or multiple major malformations.

Over one-third of children of chronic alcoholic mothers show a constellation of developmental deficits and clinical symptoms known as the "fetal alcohol syndrome". An analysis of malformations in children of mothers who took dilantin during pregnancy shows that 11% of these children show a constellation of developmental deficits and clinical symptoms consistent with the "fetal hydantoin syndrome".

Reports of this part have been published.

IV. Special analysis of the effects of diabetes in the mother on the occurrence of congenital malformations in the offspring.

This part has been completed. The risk of having a malformed child in mothers with continuous diabetes is doubled compared to that of non-diabetic mothers, with regard to major malformations and increased significantly with regard to minor malformations. The effect seems to be associated with the severity of the disease and not with the intake of insulin.

A report for this part has been published.

V. Genetic studies of congenital malformations.

This part is in progress. The studies have been designed to derive empiric risk figures of repeating a malformation when it has once appeared in a family; to identify familial aggregations of specific malformations; and to clarify the mode of inheritance in identified familial aggregations.

The basic file to be used in the analysis has been considerably enlarged and is now being documented. This "Family Linkage File" contains a record for each NCPP child including all information on its relationships to other NCPP children. It also combines genetic information such as family linkages, twin zygosity, etc., with important maternal and outcome variables such as malformations, minimal brain dysfunction, mental retardation, speech and hearing disorders, seizures, cerebral palsy, and visual disorders.

VI. Study of associations of ABO and Rh blood types with congenital malformations.

This study has been designed to confirm earlier suggestions of association of blood groups with congenital malformations, based on small samples.

The study has been completed. Several associations have been detected, some of which are fortuitous. The most interesting finding is a strong negative association between mother's blood group O and anencephaly. Another strong and interesting association is between pyloric stenosis and Rh incompatibility in boys. The nature of these associations should be explored in in-depth studies.

A report is being written.

VII. Study of the clinical significance of minor malformations.

VIII. Longitudinal study of development, morbidity and survival of children with malformations.

These two parts have been combined for purposes of analysis. The objective of Part VII is to establish which minor malformations are worthwhile detecting and why, and which ones can be ignored or considered as normal variants. Part VIII is a continuation of Part VII and deals with the effects of single and multiple malformations on growth and development.

All cases with multiple malformations have been carefully reviewed. Of 1,477 cases with multiple malformations, 531 were considered to have significant multiple malformations. The remaining 946 were found to have multiple minor anomalies or single malformations. Analysis of 531 cases showed that in 234 (44%) the condition was a localized error in morphogenesis (anomalad); in 154 (29%) a specific syndrome could be identified; and in 143 (26.9%) the pattern of malformation could not be identified.

A study to assess the load and achieve a classification of these malformations in terms of mortality and morbidity has been designed and will be performed on contract with the most qualified investigator or laboratory.

IX. Study of the effects of maternal factors in the production of congenital malformations.

This part has been completed. Families containing half siblings have been used in the analysis to determine maternal influences, genetic or environmental. Among informative malformations, clubfoot, congenital heart defects, umbilical and inguinal hernias, polydactyly, and café au lait spots occurred with significantly higher frequencies in half siblings than in the NCPP population. The recurrence risks of these malformations were the same in full and half siblings. While this approach cannot differentiate between genetic and environmental maternal factors, it provides clues for formulating and testing biological hypotheses.

A report for this part has been published.

X. Study of 7-year malformations.

This part will study the epidemiologic characteristics of congenital malformations in NCPP children surviving to age 7 years, make comparisons with

the 1-year malformations, and provide a basis for studies of the effects of these malformations on neurological outcome, psychological tests performance, and speech, language and hearing performance.

The study is now in progress. The 7-year malformations have been defined, identified, and classified into major and minor. New codes have been assigned for comparison with the 1-year malformations and a new file containing the 1-year and 7-year malformations has been created. Frequency distributions of the 7-year malformations have been produced by race, sex and institution and analyses are under way.

XI. Correlation of minor chromosomal variants with congenital malformations.

This part has been carried out as a contract operation with Dr. Herbert Lubs, University of Colorado, Denver, as principal investigator. The study utilizes data which have been developed by five NINCDS Collaborating Perinatal Project institutions which are currently participating in the Denver-based chromosomal study of minor variants. See Contract Narrative NO1-NS-5-2326.

The study has been completed and a report has been written.

In-depth studies of congenital malformations

These studies have been planned as a logical extension of the original Program Plan for Comprehensive Analysis of the NCPP Data on Congenital Malformations. The specific objective is to study the epidemiologic and genetic characteristics, and natural history of specific malformations or groups of related malformations.

1. External ear malformations.

This study has been completed. The frequency of external ear malformations was 1.72% in blacks and 0.42% in whites. The frequency of familial cases was 6% in both races. Segregation analysis showed that there was a substantial number of chance isolated cases. In hereditary cases, no distinction could be made between a common recessive trait with 40% penetrance and a rare dominant trait with 20% penetrance, or genetic heterogeneity. A most interesting finding was a significantly increased risk for hearing loss and speech abnormalities among white sporadic cases and a lesser increase among black sporadic cases.

A report has been written.

2. Maternal exposure to radiation and childhood tumors.

The relative risk of childhood malignancies was found to be 2.61 that of controls when mothers were exposed to radiation prior to pregnancy, and 1.5 when mothers were exposed during pregnancy. The risk was approximately the same for malignant and benign tumors. High dose examinations consistently

had a higher risk than medium dose examinations.

A report has been written.

3. Hyperthermia as a possible teratogenic agent in man.

The offspring of pregnancies exposed to high fever during the first trimester did not show significant differences from controls with regard to growth deficiencies, neurologic abnormalities or dysmorphic features. Incremental increase in fever height was negatively correlated with Binet scores but other variables such as socioeconomic status and etiologic agent could not be eliminated as alternative explanations for this finding.

A report has been written.

Publications:

Myrianthopoulos, N.C.: Congenital malformations in twins. Acta Genet. Med. Gemellol. 25:331-335, 1976.

Myrianthopoulos, N.C. and Burdé, B.: A case of conjoined twins. Acta Genet. Med. Gemellol. 25:59-61, 1976.

Myrianthopoulos, N.C.: Concepts, definitions and classification of congenital and developmental malformations of the central nervous system and related structures. In Handbook of Clinical Neurology. Amsterdam, North Holland, 1977, Vol. 30, pp. 1-13.

Myrianthopoulos, N.C.: Epidemiology of central nervous system malformations. In Handbook of Clinical Neurology. Amsterdam, North Holland, 1977, Vol. 30, pp. 139-171.

Myrianthopoulos, N.C.: Other skull defects: craniolacunia, foramina parietalia permagna, aplasia of the sphenoid wings. In Handbook of Clinical Neurology. Amsterdam, North Holland, 1977, Vol. 30, pp. 269-284.

Myrianthopoulos, N.C.: An approach to the investigation of maternal factors in congenital malformations. In Chung, C.S. and Morton N.E. (Eds.): Genetic Epidemiology. New York, Academic 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 02112-05 DNB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Neonatal Hyperbilirubinemia

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

PI:	J. S. Drage	Chief	DNB, NINCDS
Other:	E. C. Jackson	Biostatistician	OBE, NINCDS

COOPERATING UNITS (if any)

J. B. Hardy, The Johns Hopkins University

LAB/BRANCH
Developmental Neurology Branch

SECTION
Perinatal Research Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.10	0.05	0.05

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) MINORS ☐ (a2) INTERVIEWS

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

The neonatal hyperbilirubinemia study has been designed to assess the relationship of intermediate levels of serum bilirubin on the subsequent neurological and mental development of NINCDS Collaborative Perinatal Project children. There has been increasing concern that neonatal serum bilirubin levels between 10-20 mg% may be damaging to the central nervous system, not in the classical sense of 'kernicterus' associated with levels above 20 mg%, but rather damaging in more subtle yet clinically significant ways. Neonates have been studied in five birthweight-gestational age categories, by three socioeconomic classes, for a variety of outcome measures, including mental and motor assessments at age 8 months, and spectrum of neurological findings at age one year which will include motor performance, reflexes, tone, abnormal movements, eye findings and the over-all neurological classification of normal, suspect or abnormal. The analysis of Phase I of this study has been published. The analysis of Phase II and III which include data obtained at age seven years is in progress.

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 02169 - 04 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Long Term, Differential Effects of Obstetrical Medication on Infants

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

PI:	S. H. Broman	Research Psychologist	DNB NINCDS
PI:	Y. Brackbill	University of Florida	

COOPERATING UNITS (if any)
University of Florida

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.08	.07	.01

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 two-fold purpose of this study was to determine how long-lasting are the effects of obstetrical medication on infant psychophysiology, and to determine how long they continue to affect functions differentially. It has been reported that both anesthetics and analgesics depress the quality of infant test performance and that the drug produced dysfunction is not short-lived. Items from examinations at birth and at 4, 8, and 12 months of age that reflect CNS integrity were analysed in a cohort of normal births. Overall, there were marked effects throughout the infant's first year of pain-relieving agents administered during labor and delivery. In some cases these effects decreased (e.g., oxytocin) or even reversed (e.g., promethazine) with age but in others, principally inhalant anesthetics, they did neither. The effects of anesthetic were particularly noticeable for motor development.

Project Description:

Objectives: The two-fold purpose of this study was to determine how long-lasting are the effects of obstetrical medication on infant psychophysiology, and to determine how long they continue to affect functions differentially. It has been reported that both anesthetics and analgesics depress the quality of infant test performance and that the drug produced dysfunction is not short-lived. Items from examinations at birth and at 4, 8, and 12 months of age that reflect CNS integrity were analysed in a cohort of normal births. Overall, there were marked effects throughout the infant's first year of pain-relieving agents administered during labor and delivery. In some cases these effects decreased (e.g., oxytocin) or even reversed (e.g., promethazine) with age but in others, principally inhalant anesthetics, they did neither. The effects of anesthetic were particularly noticeable for motor development.

Relationships between obstetrical medication and (1) cognitive functioning at 4 and 7 years and (2) physical and neurological status at 7 years are currently being analysed.

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 02170 - 04 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Offspring of Schizophrenics: Developmental Factors Related to Intelligence

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

PI:	R. O. Rieder	Psychiatrist	LPP NIMH
PI:	S. H. Broman	Research Psychologist	DNB NINCDS

COOPERATING UNITS (if any)
National Institute of Mental Health

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.00	.00	.00

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 objective of this study was to assess the hypothesis that offspring of schizophrenics are more susceptible to perinatal complications than are offspring of normals. Correlations between perinatal and early childhood events and IQ at age 7 in the offspring of a schizophrenic group were compared with those in a matched control group and in a large unselected population. Male offspring of schizophrenics had a slightly lower mean IQ than their matched controls. Lower correlations between IQ and socio-economic indices, and higher negative correlations between IQ and certain perinatal events were found in the offspring of "continuous" schizophrenics. If these perinatal events are more negatively correlated with IQ because the children of continuous schizophrenics are specifically susceptible to them, it is possible that these factors are also influential in the later development of schizophrenia.

Project Description:

Within a sample of 60 children of schizophrenic parents, IQ and the correlates of IQ were examined. The Wechsler Intelligence Scale for Children was administered at age 7. The offspring of parents with schizophrenia were found to have a slightly lower IQ than their matched controls. This deficit could be attributed entirely to the male offspring.

Using a second comparison group numbering several thousand, we computed correlations for various perinatal and socioeconomic factors with seven-year IQ. These correlations were also computed for the children of schizophrenics, and the difference in correlations was examined. IQs for the offspring of "continuous schizophrenics" (chronic, borderline, and chronic schizo-affective schizophrenics) were found to have lower correlations with socioeconomic indices and higher correlations, in a negative direction, with certain perinatal events. The findings were found to a lesser, non-significant degree among the small sample of offspring of acute schizophrenics. If these perinatal events are more negatively correlated with IQ because the children of continuous schizophrenics are specifically susceptible to them, it is possible that these factors are also influential in the later development of schizophrenia. This study has been completed.

Publications: Rieder, R.O., Broman, S.H., & Rosenthal D. The offspring of schizophrenics. II: Perinatal factors and IQ. Arch. Gen. Psychiatry. 34: 789-799, 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 02171-04 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Compendium of Heritable Disorders of the Nervous System

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

PI: N.C. Myrianthopoulos Research Geneticist DNB NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.10	0.05	0.05

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) MINORS ☐ (a2) INTERVIEWS

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

The purpose is to prepare a comprehensive list of all known heritable disorders of the nervous system, including disorders and malformation syndromes which, though not primarily neurological, have neurological involvement.

Project Description:

Objectives: To prepare a comprehensive list of all known heritable disorders of the nervous system, including disorders and malformation syndromes which, though not primarily neurological, have neurological involvement.

Methods employed: Sources for the compendium are published reports in the past and current literature containing convincing evidence of familial occurrence.

Current status: Recent additions to the list have brought the number of neurological disorders to about 950. These have been classified into 23 nosological categories, including malformations, spinal atrophies, ataxias, demyelinating disorders, epilepsies, metabolic disorders, neuromuscular disorders, neoplasms and vascular disorders, mental retardation, syndromes, and others. A revised listing has been produced and additions and revisions are constantly being made.

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 02172-04 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Children with Moderate or Severe Cerebral Palsy and Severe Mental Retardation

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

PI: K.B. Nelson	Pediatric Neurologist	DNB NINCDS
PI: S.H. Broman	Research Psychologist	DNB NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) MINORS ☐ (a2) INTERVIEWS

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

Perinatal risk factors were examined in a group of children with moderate or severe cerebral palsy and severe mental retardation. Of 179 characteristics considered, 31 distinguished the severely handicapped group from controls. Only one of these significant risk factors, low birthweight of prior liveborn child, could be recognized at the time gravida registered for prenatal care. Three factors of labor and delivery (arrested progress of labor, lowest fetal heart rate in second stage of labor, and use of mid forceps) distinguished affected children from controls. Twenty-five characteristics of the neonate were different, and most were markedly different, in children who were later severely handicapped as compared with controls. The greatest differences had to do with the occurrence of neonatal seizures, intracranial hemorrhage, neonatal neurological abnormality, respiratory difficulty, small size at birth, and low hemoglobin and hematocrit.

Project Description:

Objectives: The gravity of disability of children with both cerebral palsy and severe mental retardation is so great that none is likely to become an independent citizen. Many are institutionalized, at a cost of approximately $7000 per year per child. Each child so handicapped represents both a human tragedy and a very substantial societal burden.

Methodology: From a population of 45,300 children, 90 children were found who had moderate or severe degrees of cerebral palsy and an IQ below 50 at seven years, or met these criteria at one year and died before seven. Comparisons were made with a control population of more than 34,000 children unselected for motor or mental characteristics, with respect to 179 variables relating to maternal and family characteristics, obstetrical factors, and neonatal findings.

Major Findings: The double disability of cerebral palsy and severe mental retardation was present in 1/590 liveborn children in the Collaborative Project. Of the ninety children with both CP and severe mental retardation, half were institutionalized by seven years. Seventy per cent survived at least to their seventh birthdays. Thirteen (14%) acquired their grave disabilities after the first month of life. Of factors significantly associated with serious mental and motor handicaps among the 50 children whose deficits were not "accounted for" by structural, metabolic, or other known factors, the most striking relationships were found among neonatal characteristics. In particular, four groups of factors appeared especially important: 1) small size at birth (low weight, length, head circumference), 2) difficulty initiating and maintaining independent respiration, 3) low hematocrit and hemoglobin, and 4) neonatal seizures. A discriminant function analysis indicates that the occurrence of neonatal seizures, intracranial hemorrhage, an overall impression of abnormal neurological status in the newborn nursery, 5 minute Apgar score, and the variables describing need for respiratory support, were the most effective independent discriminators between the severely handicapped group and controls.

Significance to Biomedical Research: It is a major concern of the NINCDS Collaborative Perinatal Project to identify antecedents to childhood neurological disability, to suggest where preventive efforts may be directed with best effect. The present study suggests that, for the extremely disabled, neonatal abnormalities and certain observations during labor are much more strongly related to bad outcome than earlier predictors involving maternal and family characteristics, and that interventions focussing upon labor and neonatal periods may be most productive in attempting to prevent such very unfavorable outcomes.

Proposed Course: A report describing the results of this study has been published. With its publication, this study has terminated.

Publications: Nelson, K.B. and Broman, S.H.: Perinatal risk factors in children with serious motor and mental handicaps. Annals of Neurology 2:371-377, 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 02234-03 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Febrile seizures study

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

PI:	K.B. Nelson	Pediatric Neurologist	DNB NINCDS
PI:	J.H. Ellenberg	Mathematical Statistician	OBE NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) MINORS ☐ (a2) INTERVIEWS

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

The aim of this study is to evaluate the prevalence and prognosis of febrile seizures, which is the most common convulsive disorder in any age group. Because it has an established base population, the NINCDS Collaborative Perinatal Project has the denominator data for a quantitative statement of risks. Because these were prospectively documented early examinations, this study could include information on neurological status of children before any seizure.

An initial report dealt with the likelihood of development of chronic epilepsy in children who have experienced febrile seizures. A subsequent study indicates that intellectual deficit and early learning disorder are not more frequent in children with febrile seizures only. Work now completed has delineated the remaining risks with febrile seizures. The quantification of these risks is an important element in development of a rational approach to clinical management.

Project Description: Children in the population of the NINCDS Collaborative Project who experienced febrile seizures were identified, and their seizure histories and subsequent histories examined.

Objectives: To evaluate the prevalence, associated conditions, and prognosis of this extremely common convulsive disorder. Quantification of the risks associated with febrile seizures is necessary for rational decision-making as to therapy.

Methodology: Information abstracted from the records of children with febrile seizures was used to create a tape for data analysis on a time-share system in the Office of Biometry and Epidemiology. Clinical characteristics of children with febrile seizures, and other seizure experience by the age of seven years, as well as results of intelligence testing at seven years, were examined. Sibling contols of children with febrile seizures were evaluated.

Results: Of 1821 children with febrile seizures in the Collaborative Project population, 1706 were followed to the age of seven years. Two per cent had become epileptic by the age of seven, and another one per cent had had at least one afebrile seizure not meeting the definition of epilepsy employed. Race, sex, birthweight and Apgar score were not significant predictors of epilepsy, but clinical features of the seizures, abnormal neurological status prior to the first seizure, and afebrile seizure disorders in the immediate family increase the risk of epilepsy in a child who has had a febrile seizure.

Comparison of 431 children who have had febrile seizures only with their seizure-free siblings indicates that febrile seizures do not "cost" the child a loss in IQ, or an increased vulnerability to early learning disorder. There were no deaths and no acquired motor defects associated with febrile seizures in this series.

Predictors of recurrence, and further refinement of the relationships of characteristics of the child and types of febrile seizures to the risk of subsequent convulsive disorders have been completed.

Significance: Three to four per cent of children experience at least one febrile seizure. This is the most common seizure disorder in any age group, and one of the most common acute problems in child neurology. Considerable disagreement as to optimal medical management exists, and it has been our objective to supply clinically useful information as to the spectrum of risks associated with febrile seizures, as a component in therapeutic decision making.

Honors and Awards: Public Health Service Special Recognition Award to Dr. Nelson, 1977
Public Health Service Special Recognition Award to Dr. Ellenberg, 1978

Publications:

Ellenberg, J.H. and Nelson, K.B.: Febrile seizures and later intellectual performance. Arch. Neurol. 35:17-21, 1978.

Nelson, K.B. and Ellenberg, J.H.: Prognosis in children with febrile seizures. Pediatrics 61:720-727, 1978.

Nelson, K.B.: Febrile seizures. In Gellis and Kagan (Eds.): Current Pediatric Therapy. Philadelphia, W.B. Saunders, Co., 1978, Vol. 8, pp. 85-87.

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 02332-01 DNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Analysis of NCPP Twin Data

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

PI: N.C. Myrianthopoulos Research Geneticist DNB NINCDS

COOPERATING UNITS (if any)

NHLBI; M. Melnick, University of Indiana

LAB/BRANCH
Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.00	0.80	0.20

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 is a secondary area within the program plan for analysis of NCPP data. The objectives of the project is to assess and interpret the influence of maternal, socioeconomic, neonatal, medical and other environmental factors on survival, growth and development of twins and on abnormal outcome exclusive of congenital malformations.

Project Description:

Objectives: The objective of the project is to assess and interpret the influence of maternal, socioeconomic, neonatal, medical and other environmental factors on survival, growth and development of twins and on abnormal outcome exclusive of congenital malformations.

Methodology: Twins have been classified into monozygotic (MZ) and dizygotic (DZ); and MZ twins into monochorionic (MC) and dichorionic (DC). Comparisons between and among these groups, including singletons will be made using concordance, correlation and heritability statistics.

Current status and major findings: Studies of the effects of chorion type on normal and abnormal variation showed that chorion type has no effect on and cannot explain the higher frequency of congenital malformations in MZ twins. Likewise, chorion type has no effect on head circumference, height, and right-left asymmetry. Total ridge count, however, showed a significantly greater within pair variation for DC-MZ twins than for MC-MZ twins.

Studies involving 7-year IQ scores showed that in white MZ twins but not in blacks there is greater within pair mean square for DC than MC twins, suggesting that in white twins DC placentas are of greater influence than the similarity or dissimilarity of genomes with regard to intrapair IQ development.

Studies involving blood pressure have shown significant genetic variability for diastolic blood pressure in twins with a heritability estimate of 0.53. Systolic blood pressure results tended in the same direction but were not significantly significant. The trends were comparable for both sexes, in blacks and whites.

Publications:

Myrianthopoulos, N.C., Nichols, P.L. and Broman, S.H.: Intellectual development of twins. Comparison with singletons. Acta Genet. Med. Gemellol. 25:376-380, 1976.

Melnick, M. and Myrianthopoulos, N.C.: The effects of chorion type on normal and abnormal developmental variation in monozygous twins. Am. J. Clin. Genet., in press.

Melnick, M., Myrianthopoulos, N.C. and Christian, J.: The effects of chorion type on variation in IQ in the NCPP twin population. Am. J. Hum. Genet., in press.

Havlik, R.J., Garrison, R.J., Katz, S.H., Ellison, R.C., Feinleib, M. and Myrianthopoulos, N.C.: Detection of genetic variance in blood pressure of seven year old twins. Am. J. Epidemiol., in press.

ANNUAL REPORT
October 1, 1977--September 30, 1978

Epilepsy Branch
Neurological Disorders Program
National Institute of Neurological and Communicative Disorders and Stroke
National Institutes of Health

The programs of the Epilepsy Branch are conducted from three locations of the National Institutes of Health: The Federal Building, which serves as the principal location; Building 36, housing the pharmacology laboratory; and Building 10, with facilities for the clinical epilepsy research program, conducted in cooperation with the Experimental Therapeutics Branch, Intramural Research Program. The previously noted difficulties concerning environmental conditions of the Federal Building have been rectified to a large extent. Heat and humidity controls have been installed by the General Services Administration within the limits required for video and audio tape stability. Occasional problems in maintenance still occur however.

An event of extreme importance to persons with epilepsy occurred February 28, 1978, when the Food and Drug Administration approved valproic acid for the treatment of absence seizures. The Epilepsy Branch has long considered this drug a significant advance in therapy, and it appeared that it would require several years to reach the marketplace. However, a combination of several events served to accelerate development of the drug, resulting in its marketing this year. An international conference on antiepileptic drug development with specific reference to valproic acid which served to show the existing problems was held by the Commission on Epilepsy and Its Consequences last fiscal year. In January, 1978, a patient was seen in the NIH Clinical Center who would have benefitted from valproate, but who was not admissible for treatment because Abbott Laboratories was not investigating her type of seizure disorder. Her parents took her to Birmingham, England, for treatment, and subsequently began a "campaign" to seek early FDA approval of valproate. During the summer months, NBC News provided national coverage on the plight of patients with seizures and describing the need for valproate. As a result of this public interest, the Food and Drug Administration requested Abbott Laboratories to provide the New Drug Application information available at that time. An NDA was filed by Abbott Laboratories on September 22, 1977. On October 12, 1977, the FDA's Advisory Committee on Neurological Drug Products met and unanimously recommended that the FDA approve marketing valproic acid. However, on December 14, 1977, the FDA Bureau of Drugs wrote to Abbott Laboratories and said data from an additional controlled study was needed. This was provided to the FDA on December 31, 1977, and the drug was subsequently approved on February 28, 1978. The indications approved at this time are absence seizures and mixed seizure types including absence seizures with dosage not to exceed 30 mg/kg. The drug was available for prescription use in March, 1978. The Epilepsy Branch did not participate in the publicity and public outcry for valproate, but did provide scientific information and other facts about the drug to the FDA, patients, physicians, the news media, and the Congress. Data from two controlled studies supported by the Institute

were contained in the New Drug Application. The marketing of valproic acid is a significant milestone in the treatment of epilepsy because of its anticipated broad spectrum of activity and relative lack of serious side-effects. Further studies are planned to determine its effectiveness in other seizure types, and at higher doses for greater effectiveness.

A clinical epilepsy research program is conducted in the NIH Clinical Center with partial support from the Experimental Therapeutics Branch, IRP, NINCDS. Support from the latter organization is limited to one full time secretary, $17,000, and one clinical center module. The Epilepsy Branch provides all the remaining support: professional neurologic services for in-patients, including a full-time neurologist, and the weekly epilepsy clinic; an EEG technician; video and telemetry consultation, and pharmacology laboratory support for routine serum concentrations, (including a full-time technician) necessary for research protocols. The Clinical Epilepsy Section conducts studies on the diagnosis and therapeutic evaluation of patients with intractable seizures, studies on the clinical pharmacology of antiepileptic drugs, and trains neurologists desiring special competence in treatment of seizure disorders. This year, one neurologist spent 15 months in the section and 2 others, 3 months each in this informal training program. In addition, the staff has participated in training of neurologists in epilepsy in connection with the course in epilepsy conducted by the American Academy of Neurology. Further details of this program are contained in the annual report of the Experimental Therapeutics Branch. Three publications arising this year from studies conducted by this section are likewise referenced therein.

The Branch's drug development program continued its support of clinical trials of investigational antiepileptic drugs, and preclinical development of potential new agents. The screening program for new anticonvulsant activity of new chemicals was expanded at the University of Utah to include Screen 3, an evaluation of anticonvulsant potential, for those relatively few compounds which show promise. A Source Sought announcement was issued to determine contractors capable of performing general pharmacology and/or intermediate toxicity. A Request for Proposals will be issued early in the new fiscal year. Funding of this aspect of the program will provide additional capability for drug development. Also, in response to a demonstrated need, a request for grant applications was made to solicit research in the synthesis of new chemicals with anticonvulsant activity. These applications will be subjected to a special review and funded next fiscal year.

Having served as Secretary General of the International League Against Epilepsy, J. Kiffin Penry, M.D. was elected to the office of President at the organization's meeting in Amsterdam in September, 1977. The international epilepsy movement was furthered with the establishment of Epilepsy International with an office in Geneva, Switzerland. Richard H. Gibbs, formerly Project Coordinator of the Comprehensive Epilepsy Program at the University of Virginia, assumed the office of Executive Director in March, 1978. An International Epilepsy Symposium was held in Vancouver, British Columbia, Canada, September 10-14, 1978. The meeting was organized by the International League Against Epilepsy and

the International Bureau for Epilepsy. Other organizations are sponsoring plenary themes: Intensive Monitoring by the American Epilepsy Society; Natural History and Prognosis of Epilepsy by the Japanese Epilepsy Society; New Surgical Treatment Through Experimental Models by the Canadian League Against Epilepsy; New Drug Testing, Marketing and Availability by the International League Against Epilepsy; and Comprehensive Care of Epilepsy by the Canadian Epilepsy Association, the Epilepsy Foundation of America, International Bureau for Epilepsy and the Western Institute on Epilepsy. A publication, Epilepsy: The Eighth International Symposium, Raven Press, 1977, contained papers from the meeting held in Dublin in September, 1976. It became available during the year and received wide distribution.

Epilepsy Branch staff was saddened and epilepsy drug research deterred when the principal investigator of the research contract at New Castle State Hospital, New Castle, Indiana, died suddenly in February, 1978. Studies comparing the efficacy of phenytoin-valproate and phenytoin-phenobarbital combinations in patients with refractory seizures have been completed. Further studies are pending recruitment of a physician to collaborate with the Epilepsy Branch staff in conducting controlled clinical trials in this institutionalized population.

More than 40 visitors interested in epilepsy from the United States and foreign countries visited the Epilepsy Branch. Each visitor received a description of the program at the National Institutes of Health, and had the opportunity to discuss their interest in detail with the staff. Many of the visitors were especially interested in the intensive monitoring of patients using telemetered electroencephalograms and video techniques in the Clinical Center. Others were particularly interested in the pharmacology and metabolic aspects of the Branch's work. These visits enable others to observe and adapt our techniques to their situation and provides the opportunity for the transfer of technology to persons practicing medicine and to others conducting research in seizure disorders.

The Commission on the Control of Epilepsy and Its Consequences completed its work by submitting its recommendations to the Congress in August, 1977. The Branch was fortunate to obtain the services of the Commission's former Deputy Director to aid in the awesome task of implementation of the Commission's recommendations. Of more than 400 recommendations, over 60 are the responsibility of this Institute. Many require legislative action and all require funding. Detailed plans are being made for the Institute's role in implementation of the recommendations in an orderly manner. The Institute has provided copies of the report to hundreds of interested individuals upon request.

The Institute supports 5 Comprehensive Epilepsy Programs. The first 3 to be established were subjected to technical merit review by ad hoc experts at the end of their third year. Two years additional support, as planned, was recommended for each of the three: The University of Minnesota, the Good Samaritan Hospital and Medical Center, Portland, Oregon, and the University of Virginia. The remaining 2 programs,

Medical College of Georgia and University of Washington, will be similarly reviewed in the coming fiscal year.

The monthly publication Epilepsy Abstracts continues from the Excerpta Medica Foundation with support from this Branch. A computer tape from this contract is provided to the National Library of Medicine so that a data retrieval system, EPILEPSYLINE may be made available to the users of MEDLINE or TOXLINE. With an interagency personnel agreement, a medical librarian from Emory University has worked with the Branch and the National Library of Medicine to enhance and evaluate the effectiveness of EPILEPSYLINE The central component and foundation of the epilepsy information system is a collection of more than 30,000 references. These comprise most of the current scientific literature as well as much of the pertinent past literature. Much effort has been devoted to the computer assisted production of three Epilepsy Indexes, each containing 10,000 citations: 1-10,000; 10,001-20,000; and 20,001-30,000. Availability of these citations will greatly facilitate reference to the epilepsy literature, and makes Branch resources available worldwide. These citations are also available on microfiche.

The Epilepsy Branch Pharmacology Laboratory has continued its research into the metabolism of anticonvulsant drugs. It also provides training for individuals establishing laboratories for serum concentration determinations of drugs. Many individuals have visited the laboratory for instruction. Others are aided through publications such as "Determination of Phenobarbital" by HJ Kupferberg which appeared in Test of the Month, Volume 4, 1978. Consultation was also provided to the Clinical Pathology Department, NIH Clinical Center, in developing automated analysis for routine antiepileptic drug analysis (McClean SW, Young DS, Yonekawa W. Anticonvulsants in Serum Determined with a Fully Mechanized Enzyme Analyzer. Clin Chem 23:116-118, 1977).

The metabolism of methsuximide and phensuximide was determined and results published: Kupferberg HJ, Yonekawa W. Lacy JR, Porter RJ and Penry JK. Comparison of Methsuximide and Phensuximide Metabolism in Epileptic Patients. In: Gardner-Thorpe C, Janz D, Meinardi H and Pippenger CE (Eds), Antiepileptic Drug Monitoring, Tunbridge Wells, (England), Pittman Press, 1977, pp 173-180.

A symposium attended by 250 persons was held in St. Louis, Missouri, on the topic Mechanisms of Action of Antiepileptic Drugs. Noted experts from the field discussed the known and proposed mechanisms of action for each of the principal therapeutic agents. Examples of their research and gaps in knowledge were given. A book devoted to this subject will be published in late 1978 by Raven Press.

The Branch was fortunate to have Dixon M. Woodbury, Ph.D., as a visiting scientist for a six-month period during the year. Dr. Woodbury is a noted neuropharmacologist and Chairman of the Pharmacology Department, University of Utah. Dr. Woodbury spent considerable time in scholarly writing, attending seminars, providing consultation, and laboratory research.

Another instance of aiding patients with seizures through education of physicians was provided through the publication of an article, "Getting to Know the Epilepsies", which appeared in Patient Care, Volume 11, 1977, pp 102-136. HJ Baird, F Carter, C Lombroso, HR McFarland, JK Penry and CE Pippenger cooperated in providing useful information on the diagnosis and treatment to the practicing physician

Although support ended from a grant from the Developmental Disabilities Office of the Department of Health, Education and Welfare, this quality control program has been vigorously continued by participating laboratories making token payments. A blind survey taken in 1976 showed few proficient laboratories. A second blind survey was undertaken, and it was gratifying to find that although some laboratories are still not proficient, a very great improvement had occurred in the number of laboratories adequately performing phenytoin levels. Two publications describe this program: Pippenger CE, Paris-Kutt H, and Penry JK: Proficiency Testing in Determination of Antiepileptic Drugs. J Anal Toxicol 1:118-122, 1977, and Pippenger CE, Penry JK, Daly DD and Paris-Kutt H: The North American Drug Quality Control Program. In: Gardner-Thorpe C, Janz D, Meinardi H and Pippenger CE (Eds), Antiepileptic Drug Monitoring, Tunbridge Wells (England), Pittman Press, 1977 pp 42-46. A book was published in January, 1978, which will greatly aid physicians and laboratory workers who analyze body fluids for antiepileptic drugs: Antiepileptic Drugs: Quantitative Analysis and Interpretation, edited by CE Pippenger, JK Penry, and H Kutt, Raven Press. This work followed an NINCDS sponsored symposium held in Kansas City in November, 1976.

A long standing collaboration between Branch staff and scientists at the University of Vriginia, Department of Neurology, produced numerous studies in absence drug evaluation. Recently, a new automated system of video and telemetry recording was installed to replace individual components. Recordings are greatly enhanced through elimination of variables and the possibility of operator error. This system will provide continued high quality data collection. Improvements were made in the in-house video laboratory in the Federal Building with the installation of 2 stations to monitor video tapes from collaborating centers. Storage facilities were increased, and recording capability extended.

The senior professional staff contributed to the education of physicians and other professionals through numerous lectures to groups throughtout the country. There is a high level of interest in seizure disorders, their treatment and improved care, making this activity highly appropriate in transferring findings to a practical level. Talks of this kind are very well received and gratifying to the program.

An 8-channel electroencephalograph telemetry device was installed in the Clinical Center for intensive monitoring in the clinical epilepsy research program. Eight channels of EEG are displayed on the video screen in connection with this installation. Field studies of the wearable EEG recording system developed by Stanford Research Institute under contract were conducted at the Clinical Center, and the Naval Hospital of Bethesda; at the University of Virginia, Department of Neurology, Charlottesville; and at the University of

Washington, Seattle. It is anticipated that these studies will result in refinements and data to allow marketing of this device to further research in epilepsy.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
October 1, 1977--September 30, 1978

NEW CASTLE STATE HOSPITAL (NO1-NS-8-1310)

Title: Development of a Model for Assessment of New Anticonvulsant Agents

Contractor's Project Director: Joseph T. Brock, M.D.

Current Annual Level: $60,000.

Objectives: To further develop models to study the efficacy, safety and bioavailability of new antiepileptic drugs in humans. During the past year, investigation included study of the antiepileptic property of sodium valproate when substituted for phenobarbital in the therapy of patients with generalized seizures refractory to treatment, the evaluation of possible side effects of the drug, and evaluation of drug serum concentrations.

Course of Contract: The study of sodium valproate was completed in the fall of 1977. Clinical data was sent to the Epilepsy Branch, NINCDS, Bethesda, for review and preparation for computer aided analysis. Final verification and analysis of the data is underway.

Major Findings: Data collected during previous fiscal years evaluating mexiletine as an adjunct antiepileptic drug has indicated that mexiletine is not effective as an antiepileptic adjunct in this population. Analysis of data from the pilot study of sodium valproate correlating dosage with serum concentration has been completed and submitted for publication.

Significance to NINCDS Program and Biomedical Research: The pharmaceutical industry has demonstrated little interest in developing new antiepiletic agents. Aside from the economic factors involved, one of the industry's major problems is to obtain satisfactory clinical studies of antiepileptic drugs. Through this contract and others, NINCDS has supported clinical studies of antiepileptic drugs. Well controlled studies--as conducted at New Castle State Hospital--will be significant indicators of therapeutic merit of new antiepileptic drugs and may encourage the pharmaceutical industry to develop promising agents for clinical trial . It is anticipated NINCDS-sponsored studies will enable drugs to reach the market more readily, and thus be available to physicians who treat patients with seizures.

Proposed Course of Contract; Evaluations of other investigational antiepleptic drugs and further development of the model for drug evaluation are pending.

Publications: None

C.T. No. 0700761

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
October 1, 1977--September 30, 1978

University of Virginia School of Medicine (NO1-NS-9-2196)

Title: Absence Seizure Drug Studies

Contractor's Project Director: Fritz E. Dreifuss, M.D.

Current Annual Level: $130,000

Objectives: To evaluate the effectiveness of valproic acid and of ethosuximide on the frequency and intensity of absence (petit mal) seizures in patients previously untreated for this disease, and in patients who have failed to be controlled by ethosuximide; to evaluate drug effects on physiologic and other functions.

Course of Contract: A pilot study preceded the present controlled study; 47 patients have entered the study as of July, 1978. Twenty-five of these were in Phase I where maximum valproic acid dose was 30 mg/kg, and 22 are in Phase II where the maximum is 60 mg/kg.

Major Findings: Dose-serum concentration relationships were determined and appropriate dose schedules devised. A protocol was developed for a double-blind controlled study of valproate sodium. Children with absence seizures are treated with ethosuximide or valproate according to a proven data collection procedure developed by NINCDS--University of Virginia. Long-term EEG telemetry and video recording are used as an important measure of evaluating absence seizure frequency and duration.

Proposed Course: When 50 patients complete the study protocol, the data will be analyzed to answer the hypotheses proposed.

Publications: None

C.T. No. 0700759

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
October 1, 1977--September 30, 1978

UNIVERSITY OF WASHINGTON (NO1-NS-0-2281)

Title: Complex Partial Seizure Drug Studies

Contractor's Project Director: Alan J. Wilensky, M.D., Ph.D.

Current Annual Level: $240,000

Objectives: To compare the relative antiepileptic efficacy and safety of clorazepate dipotassium and phenobarbital in patients receiving phenytoin for partial seizures; to measure drug concentrations in patients' blood; and to assess patients' psychological competence.

Course of Contract: Sixty patients have entered the double-blind controlled study so that 50 will complete the protocol.

Major Findings: This study design is similar to previously conducted controlled drug trials with other drugs. Preliminary results indicate that clorazepate is as effective as phenobarbital as an adjunct to phenytoin. Some of the neuropsychological tests appear to favor clorazepate.

Proposed Course: The double-blind evaluation with crossover will continue comparing clorazepate and phenobarbital as adjuncts to phenytoin treatment. Neuropsychological comparisons of the two drugs will be made. Drug serum concentrations will be correlated with drug dosage. The study will be completed in the coming year.

Publications:

Dodrill CB and Troupin AS: Psychotropic effects of carbamazepine in epilepsy: A double-blind comparison with phenytoin. Neurol 27:1023-1028, 1977.

Dodrill CB and Dikmen SS: The seashore tonal memory test as a neuropsychological measure. Consul Clin Psychol 46:192-193, 1978.

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Troupin AS, Friel P, Wilensky AJ, Moretti-Ojemann L, Levy RH and Fiegl P: Evaluation of clorazepate (Tranxene) as an anticonvulsant--A pilot study. Neurol 27:376, 1977.

Wilkus RJ, Dodrill CB and Troupin AS: Carbamazepine and the electroencephalogram of epileptics: A double-blind study in comparison to phenytoin. Epilepsia, 1913:283, 1978.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
October 1, 1977--September 30, 1978

UNIVERSITY OF WASHINGTON (NO1-NS-1-2282)

Title: Study of Experimental Antiepileptic Drugs in Animals

Contractor's Project Director: Joan S. Lockard, Ph.D.

Current Annual Level: $479,000

Objectives: To compare the antiepileptic efficacy of drugs in spontaneous motor seizures of primates. Seizure frequency and behavioral toxicity are compared with drug dosage and drug blood concentration. Metabolic and pharmacokinetic studies are conducted.

Course of Contract: Since this anticonvulsant drug research contract in the primate was awarded competitively in June, 1971, it has been highly effective and productive. The first 18 months were devoted to the development of validation of the primate model of focal seizures. A series of quantitative drug evaluations were then performed. These studies were efficacy of anticonvulsants, documentation of the role of social factors in drug therapy, single dose pharmacokinetic experiments, long-term studies, and mass-spectrometry of metabolites of anticonvulsant drugs.

Major Findings: An investigational anticonvulsant, cinromide, was evaluated in 6 epileptic monkeys. Preliminary findings show the drug effective in reducing the number of seizures and in shortening the duration of seizures which remain. Clonazepam was evaluated and found effective in eliminating tonic-clonic seizures and appreciably reducing the number of focal seizures. Some tolerance and withdrawal effects were found.

Polyethyleneglycol (PEG) 400 is a frequently employed solvent. It had been observed that in higher concentrations, PEG 400 itself may have an influence on seizures. Studies were performed which indicated that a 60% solution of PEG 400 significantly reduced seizure frequency and also produced severe side-effects. Thus, the solvent should be cautiously employed. Carbamazepine was found to induce its metabolism and that of its 10-11 epoxide metabolite measured by mass-spectrometry. Diurnal oscillations in carbamazepine blood concentrations were found.

Cerebellar stimulation in man has been suggested as a possible therapeutic measure. Its efficacy in the chronic lesion primate was evaluated under the conditions studied. Seizure frequency was increased and EEG bursting during stimulation decreased; no animal became seizure free. A rebound of seizure occurrence was obvious after stimulation was terminated. Thus, the therapeutic usefulness of this technique must be questioned. When valproic acid was evaluated in this model, a 2-step decrease in seizure frequency was found. Further, a delayed return of the seizure frequency to pre-drug levels for 2 weeks was observed with no valproic acid in the animal's blood. The

efficacy of valproic acid and ethosuximide were compared in a crossover design. Valproic acid was found effective in the plasma range of 50-150 ug/ml with focal seizures attenuated only at the higher plasma concentrations. Ethosuximide on the other hand exacerbated seizure frequency to some extent. These findings provided the opportunity to quantify correlative processes in efficacy evaluation leaving to a greater understanding of mechanisms of action of antiepileptic drugs.

The interactions of phenytoin and phenobarbital were studied. Phenytoin was found capable of autoinduction; phenobarbital lowered the levels of phenytoin, and phenytoin affected the levels of phenobarbital.

Proposed Course: Studies are contemplated to continue the pharmacokinetic, metabolic, and efficacy studies, as they are the only chronic model of seizures for focal motor and secondarily generalized tonic-clonic seizures. It has been proved valid on a genetic basis, and emperically evaluated and found that antiepiletic drugs efficacious in man in gross motor seizures are also efficacious in this model. Drugs specific to other seizure types are not efficacious. An ingenious constant rate infusion system for administration of drugs via a femoral catheter has been developed along with chronic sampling for drug concentrations via a jugular catheter. Drugs of long biologic half-life but insoluble may be administered orally on a chronic basis using a nasogastric tube. Drugs of short half-life may be administered using a gastric duodenal cannula for constant infusion. Using an implanted EEG plug, continuous frequent electroencephalographic sampling possible without disturbing the monkeys. Quantification of epileptiform activity as sleep staging is routinely ascertained using an intraventricular catheter and reservoir system. This model will soon be capable of performing chronic sampling of cerebrospinal fluid.

This model also provides the ability to perform extensive behavioral assessment of drug effects, such as reaction time, judgment, toxicity, psychotropic or sedative effects in restrained animals and in the free roaming room. Computerized techniques provide data from these assessments. Also, this model is suitable for drug interaction and drug tolerance and withdrawal studies. Precision pharmacokinetic and metabolic studies are also possible. Likewise, the proximity of this research within the Department of Neurological Surgery provides short-term spectral analysis of EEG, single neuron, and histologic studies associated with the anticonvulsant drug studies.

Publications:

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Levy RH, Lockard JS, Patel IH, and Lai AA. Efficacy testing of valproic acid compared to ethosuximide in monkey model: I. Dosage regimen design in the present of diurnal oscillations. Epilepsia, 18:191-203, 1977.

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Lai AA and Levy RH. Pharmacokinetic Profile of Clonazepam in Rhesus Monkeys. J. Pharm. Sci., 67:295-299, 1978.

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Trager, WF. Levy, RH, Patel, IH and Neal JM. Simultaneous analysis of carbamazepine and carbamazepine-10, 11-epoxide by GC/CI/MS, Stable Isotope Methodology. Anal Let J, B11(2), 119, 1978.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
October 1, 1977--September 30, 1978

UNIVERSITY OF KANSAS MEDICAL CENTER (NO1-NS-2-2313)

Title: Investigation of Pharmacologic Posttraumatic Epilepsy Prophylaxis

Contractor's Project Director: Charles Brackett, M.D.

Current Annual Level: $130,000

Objectives: A pilot study to determine the effectiveness of prophylactic treatment with phenytoin and phenobarbital in persons who suffer head injury and are thus liable to posttraumatic epilepsy.

Course of Contract: One hundred and twenty-five patients have been accessioned in the study and all have completed the required 18-month treatment period; they continue to be followed with neurological exams, plasma levels, and EEGs. As of 7/7/78 all of the still eligible patients will have completed both the 18-month drug treatment period followed by another 18-month period of no drug treatment. Eleven patients experienced seizures while on the study, and four have had seizures after completion of drug therapy. Data has been coded and analysis is underway.

Proposed Course of Contract: Analyses to answer the proposed hypotheses will be done, and further investigation will be made into the relationship between the study drug dosage and plasma levels which are somewhat lower than expected. Based upon the pilot study, the role of pharmacologic prophylaxis in posttraumatic epilepsy is being investigated using therapeutic serum concentrations of drugs rather than a fixed dose combination treatment.

C.T. No. 0700757

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
October 1, 1977--September 30, 1978

EXCERPTA MEDICA FOUNDATION (NO1-NS-3-2303)

Title: Publication of Epilepsy Abstracts, Volume 10.

Current Annual Level: $42,000

Objectives: To scan serial publications and periodicals from approximately 3500 world biomedical journals and select appropriate articles to be included in Epilepsy Abstracts in accordance with the guidance of the Project Officer and his editorial advisors; prepare abstracts with appropriate translations into English from foreign languages; classify, index, and store the abstracts in a computer retrievable form; and produce a 9-tract, 1600 bpi computer tape for use at NIH. The text is automatically set by computer-operated photocomposition. The Excerpta Medica Foundation produces camera-ready copy for each monthly issue of Epilepsy Abstracts, which includes an index of subjects and authors, prints and distributes the journal monthly with a cumulative index at the end of the volume. In order to pay for the production of the camera-ready copy, printing, and distribution, the Excerpta Medica Foundation sells subscriptions to recover these costs.

Course of Contract: Subscriptions to Epilepsy Abstracts, each at annual cost of $69.00, have been acquired from interested persons by Excerpta Medica at a satisfactory rate. Interest in the publication continues at a high level throughout the world.

Proposed Course of Contract: Continue distribution of monthly issues as scheduled and computer tapes delivered to NIH bimonthly in accordance with the contract. These tapes comprise the Epilepsyline database retrievable throughout the country via MEDLINE or TOXLINE terminals.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
October 1, 1977--September 30, 1978

Stanford Research Institute (NO1-NS-3-2322)

Title: Continuous Development of a Wearable Eight-Channel EEG Cassette Recording System

Contractor's Project Director: Charles S. Weaver, Ph.D.

Current Annual Level of Funding: $135,000

Objectives: The objective of this project is to fabricate, maintain and evaluate a wearable EEG recording systems; to design and fabricate an economical playback system without computer tape format; and, to design a data compression scheme. This development includes a hybridized amplifier system to provide for eight-channels of differential recording connected to a wearable tape recorder with appropriate signal processing electronics. Digital encoding circuitry provides for a full dynamic range and suitable bandwidth to allow for automatic processing of EEG data by computer. In addition to the design and development of the recording technique and implementation in hardware, the overall system is to be clinically evaluated with a selected group of patients under environmental conditions similar to those in which it will ultimately be used. The playback system is to transcribe the encoded data from initial tape cassettes and reform the original analog EEG on a strip chart through micro processor, as well as to interface to a computer.

Major Findings: The first eight-channel recorder and playback system was delivered on December 3, 1976. Now, two on-body systems with hybridized amplifiers are in use. The system weighs approximately five pounds and records eight-leads of EEG for twelve hours on a standard C-120 audio cassette with a bandwidth for each lead of 40Hz. The system successfully underwent engineering evaluation and was accepted in March 1977. It is now undergoing extensive clinical evaluation at the Clinical Center, NIH, and elsewhere.

Planning is well underway for transfer of this new technology to allow commercial production of the recording system.

Significance to Biomedical Research and the Program of the Institute: The availability of a lightweight high-fidelity portable EEG tape recorder will allow significant improvement in both diagnosis and therapy of some types of epilepsy, and provide numerous research opportunities, e.g., evaluation of drug treatment of seizures.

Proposed Course of the Contract: Additional systems will be produced and more extensive field evaluation in different settings will be conducted.

Collaborating Units: This project is being carried out as a collaborative effort of the Neurological Disorders Program and the Fundamental Neurosciences Program. The Neurological Disoders Program is providing the

clinical direction and the funding, while the Fundamental Neurosciences Program is providing the technical direction and project management.

Publications:

Sato S, Penry JK and Dreifuss FE: Electroencephalographic Monitoring of Generalized Spike-Wave Paroxyms in the Hospital and at Home. Quantitative Analytic Studies in Epilepsy. Edited by Peter Kellaway and Ingemar Petersen. Raven Press, New York 1976.

Sato S, Penry JK and Burch JD: Eight-channel Digital Recorder for Monitoring Partial and Generalized Seizures. Proceeding of International Symposium of Ambulatory Monitoring, September 1977, Northwick Hospital, London, England. Academic Press, 1978.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
October 1, 1977--September 30, 1978

UNIVERSITY OF UTAH (NO1-NS-5-2302)

Title: Initial Pharmacologic Development of New Drugs

Contractor's Project Director: Ewart A. Swinyard, Ph.D.

Current Annual Level: $254,000

Objective: To determine the anticonvulsant properties of novel organic compounds in mice at various times following intraperitoneal administration.

Course of Contract: Compounds are received by NINCDS from academic and industrial medicinal chemists and sent to the University of Utah. Initially, the levels of anticonvulsant and neurotoxicity activities are determined on all compounds submitted. For those compounds which exhibit relative potency, the ED_{50} and TD_{50} are determined. The pharmacologic data is then provided to NINCDS where it is reviewed and analyzed. The submitters of compounds are then informed of the results for their use for further synthesis. Those compounds which are selected as the most promising are scheduled for further testing (Screen 3). Screen 3 testing, in part, uses the same format as that of determining the ED_{50} and TD_{50} previously with the exception that the candidate compounds are administered orally in mice and rats. The LD_{50} and HD_{50}s are determined in the mouse and the rat, along with a profile of drug-induced behavior between the TD_{50} and LD_{50}. Subacute studies are also performed on the most promising compounds being evaluated in Screen 3 to study sustained effect, tolerance levels, and withdrawal symptoms.

Proposed Course of Contract: This program began January 1, 1975 and during the period October 1, 1977 through September 30, 1978 screened 550 compounds for anticonvulsant activity. Currently, 25 candidate compounds are being evaluated in the Screen 3 phase of testing.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
October 1, 1977--September 30, 1978

Contractor		Project Director	Annual Level
Univ of Minnesota	(NO1-NS-5-2327)	R. Gumnit, M.D.	$1,396,446
Good Samaritan Hosp Portland	(NO1-NS-5-2328)	J. Schimschock, M.D.	971,917
Univ Va Med Center	(NO1-NS-5-2329)	F. Dreifuss, M.D.	545,853
Med Coll Georgia	(NO1-NS-6-2340)	J. Green, M.D.	500,000
Univ of Washington	(NO1-NS-6-2341)	A. Ward, Jr., M.D.	733,279

Title: Comprehensive Epilepsy Program

Objectives: The objective of the Comprehensive Epilepsy Program is to facilitate applied research and to coordinate research and teaching with health care services related to persons with all types of epileptic seizures within a defined geographic area.

Courses of Contract: Each contractor is conducting clinical and laboratory research in the diagnosis, treatment, prognosis and prevention of epilepsy. Each contractor is demonstrating to physicians and other professionals the newest advances in epilepsy research and treatment and is establishing a broad program for public education. In addition, each contractor is establishing the required procedures to assure, in a research setting, the availability to the person with epilepsy of complete and up-to-date preventive medical and rehabilitative psychological, vocational, educational, and social services.

Major Findings: During fiscal year 1975 three Comprehensive Epilepsy Programs were established. During fiscal year 1976 two additional programs were established. All of the contractors showed evidence for the feasibility of establishing a program in their geographic area by a detailed description of clinical research capability, health care delivery, rehabilitation resources, etc., for the person with epilepsy.

Proposed Course: During the coming year, the Comprehensive Epilepsy Programs will facilitate applied research and coordinate research and teaching with health care services related to persons with all types of epileptic seizures within their defined geographic area. Inpatient facilities will be made available to patients for periods up to 3-6 months to receive special diagnostic evaluation and intensive treatment of their seizure disorders and any other concurrent handicap or physical problem. Vocational training, psychological support, other ancillary services and continued schooling will be simultaneously provided. Existing services will be coordinated for maximum utilization. An integral part of the program will be demonstration at all levels in the management of patients with epilepsy for professionals, paraprofessionals, and the lay public. While it is recognized that care for persons with epilepsy is available from many sources at the present time, these are scattered and noninclusive so that a patient may or may not receive total care depending on the local resources and how well they are coordinated. This program is incrementally funded on an annual basis.

Publications:

Comprehensive Epilepsy Programs:

The Commission for the Contraol of Epilepsy and Its Consequences. Plan for Nationwide Action on Epilepsy, DHEW, Publication NO. (NIH) 78-312.

Comprehensive Epilepsy Service Network--Background Notes, Vol II, pt 1, pp 1065-1070.

Comprehensive Epilepsy Program of Georgia, Vol II, pt 1, pp 1071-1076.

Comprehensive Epilepsy Program of Minnesota, Vol II, pt 1, pp 1077-1096.

Comprehensive Epilepsy Program of Oregon, Vol II, pt 1, pp 1097-1103.

Comprehensive Epilepsy Program of the University of Virginia, Vol II, pt 1, pp 1104-1110.

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Medical College of Georgia:

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Smith DB and Obbens EAMT: Comments on epileptogenic properties of folic acid and N^5 methyltetrahydrofolate in cat. Epilepsia 17:35-36, 1976.

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University of Minnesota:

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Anderson VE, Chern MM and Schwanebeck EM: Computer simulated models for the study of heterogeneity. In Excerpta Medica International Congress Series No. 397, p 173, 1976. 5th International Congress of Human Genetics, Mexico City, Mexico, DF.

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Luederes H, Daube JR, Taylor WF, Klass DW: A computer system for statistical analysis of EEG transients. In: Quantitative Analytic Studies in Epilepsy, edited by Peter Kellaway and Ingemar Petersen, Raven Press, NY.

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CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
October 1, 1977--September 30, 1978

METHODIST HOSPITAL-HOUSTON (NO1-NS-6-2342)

Title: Quantification and Treatment of Infantile Spasms

Contractor's Project Director: Peter Kellaway, Ph.D.

Current Annual Level: $60,000

Objective: To develop a quantitative clinical methodology to describe infantile spasms; and, to conduct a pilot evaluation of their treatment with prednisone.

Course of Contract: Instrumentation was developed for quantification of infantile spasms employing video recordings, EEG and EMG. Twenty-five patients have been evaluated and the methodology employed in the treatment with prednisone of ten patients.

Proposed Course: Following this pilot study the methodology and results will be reviewed and analyzed. Six additional patients will be studied to complete the range of patients to be analyzed in the methodology study.

Publications:

Frost JD, Hrachovy RA, Kellaway P, Zion, T: Quantitative analysis and characterization of infantile spasms. Epilepsia 19:273-282, 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 01933 08 EB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Quantitation of Spike-wave Activity by a Reaction Time Method.

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

PI:	J. K. Penry	Chief, Epilepsy Branch	EB, NDP, NINCDS
Others:	S. Sato	Visiting Scientist	EB, NDP, NINCDS
	F. E. Dreifuss	Prof. of Neurology	University of Virginia

COOPERATING UNITS (if any)
Department of Neurology
University of Virginia

LAB/BRANCH
Epilepsy

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) MINORS ☐ (a2) INTERVIEWS

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

This study determines wheter reaction time in absence patients is or is not impaired in a gradual fashion from the point of spike-wave initiation. A reaction-time device is employed which gives instantaneous recognition by voltage criteria that a spike-wave burst has started. This burst is of much higher than normal background, and this factor alone is used to electronically trigger the reaction timer. On instantaneous recognition the reaction timer is triggered and a tone is delivered to the subject. The subject responds by turning off the high pitch tone with a telegraph key. Between paroxysms the patient is maintained in a state of alertness.

Project Description:

Objectives: To determine whether reaction time in ptients with absence (petit mal) seizures is or is not impaired in a gradual fashion from the point of spike-wave initiation as has been suggested by some authority but disputed by others. There is some evidence for a "trough-like" pattern decrease of consciousness. The onset of decreased clinical functions during apik-wave paroxysms is evaluated by the reaction time method.

Methodology: A device is employed which gives instantaneous recognition by voltage criteria that a spike-wave burst has started. This burst is of much higher than normal background, and this factor alone is used to electronically trigger the reaction timer. On instantaneous recognition the reaction timer is triggered and a tone is delivered to the subject. The subject responds by turning off the high pitch tone with a telegraph key. Between paroxysms the patient is maintained in a state of alertness by a program of approximately 10 random stimuli per minute. All the data is collected by television, including a portion of the screen reserved for the reaction time from the digital clock. There is no age limit in selecting patients, but they must all have spike-wave paroxysmal discharge. A second group of patients was studied with the apparatus altered slightly so that the auditory stimulus was delivered 0.5 seconds into the seizure in order to see if responsiveness becomes less as the seizure progresses. Oscillographic displays from magnetic tape recordings of spike-wave paroxysms revealed shifting asymmetries.

Major Findings: The first group of patients suggest that some ability to respond early during the paroxysmal burst is maintained; this responsiveness is frequently not seen 1-2 seconds after onset. Analysis of responsiveness during short bursts suggests that patients may retain a normal reaction time during such paroxysms.

Significance: This study has applied video recording techniques and sophisticated electronic methods to improve the quality of clinical research. Specifically, this study is an analysis of the relation of the patient's behavior to his EEG during paroxysmal electroencephalographic events. An understanding of this relationship is important--not only as a guidepost for further research in the mechanism of epilepsy, but also in determining the day-to-day therapeutics of the epileptic patient.

Future Course: This project will continue. Patients from an investigational drug study will be evaluated.

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 02097 07 EB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Diagnostic Value of Prolonged Telemetered EEG in Epilepsy

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

PI:	J. K. Penry	Chief, Epilepsy Branch	EB, NDP, NINCDS
Others:	S. Sato	Visiting Scientist	EB, NDP, NINCDS
	W. L. Brannon	Chief, Neurology Serv.	Naval Med. Center

COOPERATING UNITS (if any)

U.S. Naval Medical Center, Bethesda, MD. 20014

LAB/BRANCH
Epilepsy

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Telemetered EEGs are recorded for 6 hours in order to sample over a longer period of time than the usual 20-30 minutes, and during normal activity. The incidence of diagnostic paroxysmal abnormalities in the 6-hour telemetered EEG are compared with those from routine conventional EEGs. About 10% of patients have had diagnostic abnormalities on the 6-hour telemetered EEG which were not recorded in the routine EEG. The accessioning of patients will continue. The ability to detect and record diagnostic epilepsy format abnormalities in the EEG after a single seizure will aid in the early treatment and long-term prognosis of patients who suffer their initial seizure.

Project Descriptions:

Objectives: To develop a means of detecting and recording interictal paroxysmal abnormalities (epileptiform) in the EEG of patients who have suffered a clinical convulsion.

Methodology: Telemetered EEGs are recorded for 6 hours in order to sample over a period of time longer than the usual 20-30 minutes and during normal activity; in some patients, the latter may evoke interictal paroxysmal abnormalities. The study compares the incidence of diagnostic paroxysmal abnormalities in the 6-hour telemetered EEG with those from routine conventional EEGs. Seventy-five patients have been evaluated to date; one hundred patients are to be studied.

Major Findings: In the initial group of patients studied, about 10% have had diagnostic abnormalities on the 6-hour telemetered EEG which were not recorded in the routine EEG. The accessioning of patients will continue.

Significance: If patients could be detected before the occurrence of a seizure, they could be treated and the seizure prevented, without treating those patients who will not have a recurrence.

Publication: 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 02098 05 EB

PERIOD COVERED
October 1, 1977, to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Monaural-Auditory Evoked Potential to Measure Anticonvulsant Effect on Brain Function

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

PI:	J. R. Wolpaw	Research Associate	LNP, NIMH
Other:	J. K. Penry	Chief, Epilepsy Branch	EB, NDP, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Epilepsy Branch

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The monaural auditory evoked response is used as a measure of acute and chronic antiepileptic drug effect on central nervous system function. The origin and magnitude of the ipsilateral-contralateral peak latency difference were defined. Patients before and after initiation of antiepileptic drug therapy are being evaluated. In patients beginning phenobarbital treatment, and others beginning phenytoin, approximately half had significantly increased latency differences.

Project Description:

Objectives: To develop the ipsilateral-contralateral peak latency difference previously noted by others in the monaural auditory evoked response as a measure of acute and chronic antiepileptic drug effect on central nervous system function.

Major Findings: Through an extensive study in normal controls, the origin and magnitude of the previously reported ipsilateral-contralateral peak latency difference were defined. In pilot studies, moderate doses of ethanol and caffeine increased the ipsilateral-contralateral peak latency difference, often giving differences significantly higher than control values. Then data was collected from patients before and after initiation of antiepileptic drug therapy. Results show that phenobarbital and phenytoin give abnormally high ipsilateral-contralateral peak latency differences in about 50% of patients.

Significance: This technique provides a means of measurement of subtle, toxic effects of antiepileptic drugs on the central nervous system.

Proposed Course: This project has been completed.

Publications:

Wolpaw JR, Penry JK: Laboratory note. Hemispheric differences in the auditory evoked response. Electroencephalogr Clin Neurophysiol 43:99-102, 1977.

Wolpaw JR, Penry JK: Acute and Chronic Antiepileptic Drug Effect on the T Complex Interhemispheric Latency Difference. Epilepsia 19:99-107, 1978.

Wolpaw JR, Penry JK: Effects of ethanol, caffeine, and placebo on the auditory evoked response. Electroencephalogr Clin Neurophysiol 44:568-574, 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 02334 01 EB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
The relationship of Pentylenetetrazole (Metrazol) Brain Levels to Seizure Activity in Mouse Brain

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

PI:	H. J. Kupferberg	Pharmacologist	EB NDP NINCDS
Others:	W. Yonekawa	Pharmacologist	EB NDP NINCDS
	D. Woodbury	Professor	University of Utah

COOPERATING UNITS (if any)

LAB/BRANCH
Epilepsy

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Pentylenetetrazole (PTZ) when administered subcutaneously to mice causes various types of seizure activity. First observed is myoclonic jerks, followed by clonic seizures and finally by clonic running and generalized tonic extension seizures. With low doses the initial seizures only are observed. By increasing the dose, the entire seizure complex occurs. The convulsive dose$_{50}$ needed to induce clonic seizures in mice without inducing tonic hindlimb extension was found to be 60 mg/kg. The CD$_{50}$ of PTZ needed to induce the entire seizure complex was found to be 95 mg/kg.

Brain concentration of PTZ were measured by gas liquid chromatography following the administration of 60 mg/kg and 95 mg/kg at the time of clonic seizures and tonic hindlimb extension, and were found to be 53 ug/gm and 79 ug/gm respectively. There appears to be a relationship between the amount of PTZ in the brain and the severity of seizures induced by PTZ.

Project Description:

Objectives: To determine whether there exists a relationship between mouse brain levels of pentylenetetrazole and the severity of seizures.

Major Findings: The CD_{50} for pentylenetetrazole induced clonic seizures was 60 mg/kg and the brain level of PTZ at the time of clonic seizure was 53 ug/gm. The CD_{50} for PTZ induced maximal tonic hindlimb extension seizures was 95 mg/kg and the brain level at the time of tonic seizure was 79 ug/gm. There is a direct relationship between brain levels of PTZ and the seizure type.

Significance: This provides insight into the understanding of the mechanism of action of chemically induced seizures.

Proposed Course: This project will be continued to obtain additional data.

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 02335 01 EB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Metabolism of Mephenytoin in Epileptic Patients

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

PI:	H. J. Kupferberg	Pharmacologist	EB NDP NINCDS
Others:	W. Yonekawa	Pharmacologist	EB NDP NINCDS
	R. J. Porter	Sr. Staff Associate	EB NDP NINCDS
	M. E. Newmark	Staff Associate	EB NDP NINCDS
	B. Desai	Visiting Scientist	EB NDP NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Epilepsy

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The rate metabolism of mephenytoin to its metabolite, nirvanol, has not been determined although mephenytoin has been used for over 30 years in the treatment of seizures. Five epileptic patients were given single doses of 50 and 400 mg mephenytoin. The plasma levels of both drugs were measured by GC-MS-mass-fragmentography. Mephenytoin is rapidly absorbed and disappears from plasma with a plasma t1/2 range of 8 to 24 hours. Nirvanol appears in the plasma soon after ingestion of mephenytoin and reach peak plasma levels in 24 to 36 hours. Nirvanol then disappears from plasma with a plasma half-life of 84-144 hours. Chronic administration of mephenytoin (400 mg/day) produces steady-state levels of nirvanol much higher than those for mephenytoin. Steady-state levels of nirvanol are achieved in 14-21 days whereas mephenytoin steady-state levels are reached in 6 days following chronic administration.

Project Description:

Objectives: To determine some pharmacokinetic parameters of mephenytoin and its metabolite nirvanol in epileptic patients

Major Findings: The metabolic half-life of mephenytoin in epileptic patients following single oral doses of mephenytoin ranged between 8-24 hours for mephenytoin and 84-144 hours for nirvanol. Steady-state levels of nirvanol were greater than mephenytoin in epileptic patients receiving chronic mephenytoin therapy. The ratio of nirvanol to mephenytoin was 15 to 1.

Significance: Mephentyoin has been used in the treatment of seizures for 30 years. Nirvanol had been used prior to that time, but removed from the marketplace because of toxicity. The results indicate that nirvanol most likely accounts for the antiepileptic activity of mephenytoin because of the high plasma levels of nirvanol and much lower levels of mephenytoin with chronic therapy of mephenytoin.

Proposed Course: 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 02235 03 EB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Metabolism of Methsuximide and Phensuximide in Epileptic Patients

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

PI:	H. J. Kupferberg	Pharmacologist	EB NDP NINCDS
Others:	W. Yonekawa	Pharmacologist	EB NDP NINCDS
	R. J. Porter	Sr. Staff Associate	EB NDP NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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)

This project has been completed.

Publication: Kupferberg, H.J.; Yonekawa, W.; Lacy, J.R.; Porter, R.J.; Penry, J.K. Comparison of Methsuximide and Phensuximide Metabolism in Epileptic Patients. In: Gardner-Thorpe, C; Janz, D.; Meinardi, H.; and Pippenger, C.E. (Eds), Antiepileptic Drug Monitoring, Tunbridge Wells, (England), Pittman Press, 1977, pp 173-180.

ANNUAL REPORT
October 1, 1977--September 30, 1978

Stroke and Trauma Program
National Institute of Neurological and Communicative Disorders and Stroke

INTRODUCTION

The Stroke and Trauma Program of the NINCDS is responsible for research and related training and technical consensus activities relevant to the cerebrovascular disorders, head and spinal cord injury, neural plasticity and regeneration, and CNS neoplasms. It also serves as an Institute focal point for research on positron emission tomography (PET), psychosurgery, and for chronic pain including headache, backache and manipulative therapy. Fiscal Year 1978 was characterized by the launching of new initiatives in several of these areas (e.g., clinical research training, EC/IC controlled clinical trial, research on coma and on cerebral edema, comprehensive stroke centers); the planning of new initiatives to be considered for implementation in FY 1980 (e.g., comprehensive trauma centers, PET research initiative, research on chronic pain, establishment of a Joint Commission on Psychosurgery, establishment of an M.D.-M.S. research training award program, controlled clinical trial of steroids in spinal cord injury); the maintenance of previously initiated research and training activities of high program priority and scientific excellence; and the discontinuation of research awards of poor productivity.

Stroke and Trauma Program areas of responsibility include the clinical entities of highest incidence and prevalence, and of severest morbidity and greatest mortality of any of the neurological disorders. Because of this, high priority is given to clinical investigations of pathogenesis, prevention, diagnosis and therapy. In addition, usually by contract, the development and dissemination of research analyses and of technical consensus are integral parts of the program's research and information initiatives. As a foundation for these clinically oriented research and evaluative activities, the program provides support for basic research relevant to the clinical areas of its responsibility. The several administrative instruments of program support (research project grants, program-project grants, clinical research center grants, cooperative trials, research contracts and service contracts) are all used as necessary and appropriate to accomplish research objectives. Because of the known shortage of skilled clinical investigators in each of the areas of program responsibility, emphasis has been given to the recruitment and development of additional clinical investigators utilizing the institutional fellowship and teacher-investigator award mechanisms.

Thus, with the funds and other resources made available to it, the NINCDS Stroke and Trauma Program has served as the Institute focal point for the planning and operation of research and related activities in its

assigned areas of responsibility. Because of restricted resources, Fiscal Year 1978 was characterized by a reevaluation of program goals and objectives, the continuation of a broad-based but limited program in each of its areas of responsibility, and the initiation of a few new targeted efforts in research areas of the highest priority.

I. STROKE

The research project grants and the cerebrovascular clinical research center grants continue to be the focus for basic and clinical investigations related to the pathogenesis, prevention, diagnosis, and treatment of stroke. New programs which have been launched in the past year are the Comprehensive Stroke Centers with an emphasis on applied research and an international clinical trial of a microsurgical treatment for the prevention of stroke.

Current expenditures for 14 Cerebrovascular Research Center grants amount to approximately $7,000,000; for 50 research grants, $3,000,000; and for 4 contracts, $1,500,000.

The following selected topics illustrate the wide range of activities during the past year.

Comprehensive Stroke Center Program

Six feasibility studies for Pilot Comprehensive Stroke Centers completed during the past year demonstrated that the Program is scientifically viable and is responsive to the needs of the community. Awards were recently made to the following institutions for the establishment of Pilot Comprehensive Stroke Centers:

1. North Carolina Heart Association
   This program is based in the geographic area of the eastern coastal plains of North Carolina within the "stroke belt" of the southeastern United States. It involves the combined professional facilities of the Department of Neurology at Bowman Gray School of Medicine, the Departments of Epidemiology and Health Education at the University of North Carolina at Chapel Hill, the Research Triangle Institute, and the community hospitals and health providers. The program is characterized by a unique evaluation design for measuring its effectiveness.

2. University of Rochester
   The area served by this program is Monroe County, New York including the city of Rochester. The applied research projects in this center are focused on rehabilitation.

3. University of Oregon Health Sciences Center
   This is a state-wide program emphasizing applied research projects on stroke prevention and therapy as well as education and rehabilitation.

Extracranial/Intracranial By-Pass Study

A prospective randomized clinical trial on the efficacy of extracranial/intracranial arterial anastomosis for the treatment of cerebrovascular disease is underway. This is a fixed protocol, multi-institutional study for which the University of Western Ontario serves as the central office. During the past year the details of the study design have been refined and completed; operating procedures have been simplified and made more efficient. Major efforts are now centered on increasing patient entry. In order to increase more rapidly the number of cases admitted, institutions in Europe and Japan have been identified as possible participants.

The project is as yet only in its early phases, and not enough data have been accumulated to allow conclusions to be drawn regarding the defined objective of the study which is to determine whether the procedure will reduce by 50% or more, the incidence of first or recurrent strokes in patients with certain specific forms of cerebrovascular disease.

Diagnosis

An ultrasound B-scanner developed jointly by the Mayo Foundation and Stanford Research Institute has been under clinical trial since 1975. High resolution and real-time capacity of the instrument coupled with the safety, speed and reproducibility of the examination technique has made it possible to obtain good images of the carotid bifurcation in the majority of patients, some of whom have undergone subsequent angiography. Initial, favorable impressions prompted a prospective comparative study between carotid B-scanning and carotid angiography.

At the present stage of development and testing, real-time ultra-sound scanning of the carotid arteries represents a promising adjunct to clinical practice but so far there is no indication that it will substitute for the need for angiography in most patients being considered for surgical treatment of ischemic cerebrovascualr disease.

Metabolic Mapping Studies

Little information is available concerning the cerebral metabolic alterations that occur in man following acute stroke, and data which are available are only average values for the whole brain or one hemisphere. The availability of positron emmission tomography for measuring concentrations of radionuclides in vivo and for depicting their regional distribution now makes it possible to map local metabolic changes which would otherwise be undetected.

The following compounds labeled with positron-emitting radionuclides are being used in several of the Cerebrovascular Research Centers: $C^{15}O_2$, $^{13}NH_3$, ^{11}CO, ^{11}C-glucose, and ^{18}FDG. In one Center approximately 150 positron studies have been done on 100 patients, including 30 studies with transverse sectioning imaging. This number is made up of approximately 35 studies in volunteers and 65 in patients with ischemic disease,

20 with tumors, 20 with vascular malformations and 10 with miscellaneous problems (multiple sclerosis, dementia, migraine, etc.) Initial efforts were to define the normal positron scintigrams, to evaluate whether changes in the distributions could be identified in patients with obvious structural lesions, to assess whether alterations in the distributions could be found when physiology was disturbed without gross structural changes and, by correlating single or sequential scintigrams with other clinical data, to interpolate the physiologic meaning of changes in the positron images.

In another Center the rate of local glucose metabolism for different regions of the brain has been determined in human volunteers after the injection of 2-deoxy-2-fluoro-D-glucose (2FDG) into the brachial artery.

The effectiveness of positron techniques remains to be demonstrated but they have the potential of becoming powerful tools for defining mechanisms of stroke disease and for designing appropriate stroke therapy.

Computerized Information Systems

Considerable progress has been made in developing a computerized information system to study the relationship of the neurologic, arteriographic and systemic manifestations of transient cerebral ischemia (TIA) to the outcome of medical or surgical therapy of this illness. Thus far, more than 250 patients with TIA have been entered into this data bank. The data base designed for this study includes detailed information on the number, frequency and nature of the ischemic attacks, the precise location and extent of arteriographic lesions and the type of treatment. A new case of TIA has been entered into the data bank approximately each week in the past year and follow-up observations are being made at regular intervals by clinic visits, phone calls and letters to the patients and their physicians.

This computerized information system now provides the physician with an immediate printout of the risks and outcome of all TIA patients in the data bank with symptoms similar to those of the patient currently under his care. This "on-line" repository of information is often helpful to the physician in making a decision as to the need for arteriography and/or surgical therapy. In addition to providing this unique type of neurologic consultation, the computer bank represents a resource for clinical investigation of the various risk factors which may influence the natural history and therapeutic responses of this illness. It is believed that this data bank represents a potential chapter in a computerized textbook on stroke which will augment the present sources of medical information on this illness.

Conferences and Publications

Princeton Conference--The Eleventh Princeton Conference on Cerebrovascular Disease, supported jointly by NINCDS and NHLBI, was held at Nassau Inn, Princeton, New Jersey, March 5, 6, and 7, 1978. The program included a discussion of the following topics:

1. An Evaluation and Comparison of Ischemic Stroke Models
2. The In-vivo Mapping of Human Brain Biochemistry and Hemodynamics in Stroke
3. Edema and Brain Infarction
4. Transient Ischemic Attacks and Platelet Supression
5. Cerebral Vasospasm
6. Effect of Stroke on the Cardiovascular System.

Proceedings of this conference will be published.

Cerebrovascular Research Status Report

A request was approved and a grant awarded for the preparation, printing, and distribution of the Fifth Edition of the Cerebrovascular Research Status Report. The new edition will place increased emphasis on computerized tomography scans, ultrasound reconstruction of arteries, and physical therapy and rehabilitation.

Workshops on Monitoring the Acutely Brain-Injured Patient

An interdisciplinary conference held at Bowman Gray School of Medicine was designed to bring together clinicians, clinical researchers, and research engineers to discuss common problems in monitoring patients with acute brain injury, particularly acute cerebrovascular disorders and head trauma. The outgrowth of this workshop was the assessment of the reliability of the various monitoring methods and approaches and indications of the need for improvement of the technology which might allow broader application of monitoring systems.

II. SPINAL CORD INJURY

Spinal cord injury research continues to be directed primarily at an understanding of the secondary reactions of the spinal cord to injury, that is, to an accurate description and explanation of the anatomical, electrical and chemical changes that occur in the spinal cord following mechanical trauma. The impetus for this research is the possibility of reversing and preventing some of these secondary changes and so preventing or decreasing the neurologic deficit in the patient with spinal cord injury.

To stimulate and encourage research in this field, the NINCDS has funded five spinal cord injury clinical research centers. These centers are located at:

1. Medical University of South Carolina
2. New York University
3. Ohio State University
4. Yale University
5. University of Texas at San Antonio.

The thrust of investigation in these centers in the past year has been in the following fields:

A. Epidemiology

These centers have, as part of their clinical programs, recorded the number of patients admitted, level of injury, neurologic deficit, treatment and outcome. The Yale Spinal Cord Injury Clinical Research Center, studying all aspects of spinal cord trauma in Connecticut, has collected data which comprise the most complete U.S. study of spinal cord injury in a defined geographic area. In addition, this will be the most complete study in the world of causes, cost, treatment and results of treatment in the acute phase of spinal cord injury.

For a progress report on the important epidemiologic effort that was begun by contract in April, 1977, refer to the contract narrative for the Yale Spinal Cord Injury Registry.

B. Pathogenesis

Each center has a program of basic research encompassing the disciplines of anatomy, pathology, physiology, and neurochemistry. The following observations and findings are among those reported in the past year:

1. The methodology has been developed for analysis of injured CNS tissue by gas chromotography - mass spectroscopy to evaluate profiles of changes in phospholipid fatty acids, as well as membrane cholesterol.

2. Measurements of ascorbic acid levels in injured cat spinal cord reveal early decreases, suggesting that free radical reactions occur shortly after injury and may play an important pathogenic role in subsequent, irreversible structural changes.

3. Lipid-soluble barbiturates protect against the loss of membrane components, such as phospholipid fatty acids and cholesterol.

4. Preliminary results of studies of glucose metabolism and spinal cord blood flow in experimental spinal cord injury suggests that there are alterations in spinal cord metabolism as well as blood flow and that these factors may constitute a progressive injury force.

5. Cardiac output falls immediately after spinal cord injury suggesting autonomic paralysis and sequestration of circulating blood volume, possibly in large muscle masses.

6. Light microscopic analyses have revealed that the central grey matter of the spinal cord is less susceptible to damage by blunt trauma at high thoracic levels than at lower thoracic levels.

7. The source and course of descending monoaminergic pathways to the spinal cord have been investigated by ligating the monkey spinal cord at midthoracic levels. In this preparation, an increase is found in the fluorescent intensity of catacholamine neurons within the lateral reticular formation and the ventral portion of the locus ceruleus.

C. Diagnostic Aids

There has been continued study of somatosensory evoked potentials, both in the laboratory and clinically. The following observations have been reported:

1. Cortical evoked potentials, evoked by median or radial nerve stimulation, have been shown to be altered by low thoracic cord transection.

2. Studies have supported the hypothesis that a correlation exists between, on the one hand, activity and different fiber groups found in sensory nerves, their conduction pathways through the cord, and, on the other hand, the components of the cortical evoked potential.

3. The use of the median or radial nerve SEP as a monitor of the more caudal regions of the spinal cord is important because it may be possible to establish a method of determining changes in spinal cord function as they reflect the effects of time or therapy in cases where lower limb SEPs are absent.

D. Treatment

Data regarding the indications for various types of treatment, optimal use of existing modes of therapy, evaluation of new modes and assessment of outcome are being collected by the spinal cord injury centers. The cooperative effort of the centers in devising a data collection system is described in the contract narrative, "Yale Spinal Cord Injury Registry." A grant application has been received for the use of this system to study the effects of high dosage steroids in patients with spinal cord injury.

A new project of the Stroke and Trauma Program, designed to assess and improve community care of the CNS injured patient, is described under the separate contract narrative for "Feasibility Studies for Comprehensive Central Nervous System Trauma Centers." It is expected that the information collected through this effort will provide guidelines for improving the care of head and spinal cord injured patients.

In addition to the Spinal Cord Injury Clinical Research Centers there are twenty individual research grants dealing with the spinal cord. These include quite basic studies such as "Organization of the Primitive

Spinal Cord," and clinical investigations such as "Outcome vs. Emergency Care in Spinal Cord Injury."

III. HEAD INJURY

A better understanding of the pathophysiology of head injury and the consequent development of more effective methods of treatment of the head injured patient remain the goals of this research program. The bulk of this research is being done in the six Head Injury Clinical Research Centers in a variety of basic and clinical studies. The following observations and findings have been reported by the Head Injury Centers in the past year:

A. Epidemiology

Computerized data bases have been established. Information from patients' histories, from neurological examinations, and from diagnostic tests is being recorded and analyses undertaken of correlations of the neurologic status of the head-injured patient with diagnostic studies, prognosis, and long-term neurologic outcome and disability.

B. Basic Studies

1. In experimental brain injury, alterations can be produced in synaptic vesicles and a temporary passage of intravascular tracer can be caused through the vascular endothelium in the midline raphe of the brain stem. In the area of this leakage, neurons are in direct contact with vascular endothelium and not separated by astrocytic processes as elsewhere.

2. A study of sites of brain damage was made in 28 consecutive autopsies of patients dying after head injury. Lesions were concentrated in the frontal lobes and in the basilar medial portions of the temporal lobes, diencephalon and brain stem. A high incidence of infarctions was seen in the hypothalamus and pituitary. In 29% of the cases, subendocardial hemorrhages and myocardial necrosis were found in the left interventricular septum near the A-V node.

3. In head injury cases, GABA and LDH were found to be increased in ventricular fluid. K^+ levels were remarkably stable in ventricular CSF, even in severely injured patients.

4. Injection of Na^+ or Li^+ into cortical glia was found to evoke glial depolarization and discharge of adjacent neurons. The effects can be explained by the extrusion of K^+ from the glia after cation injection. The data suggest that a reduced rate of re-uptake of K^+ into the Na^+ loaded glia results in epileptiform firing of neurons and supports the hypothesis that glia function to buffer the environment of neurons.

5. Simultaneous placement of bilateral mesencephalic radio-frequency lesions in the rat caused a consistent reduction of glucose metabolism in the entire brain, both grey and white matter, as early as one hour after the lesions.

C. Diagnostic Aids

1. The direct cortical response (DCR) has been studied with in-dwelling electrodes in patients with severe brain injury. Cyclical changes in cortical excitability have been found in traumatized cortex. These changes bear a relationship to the development of periods of electrocortical seizure activity.

2. Somatosensory evoked potentials have become increasingly useful. Graded SEPs in brain injured patients appear to be a powerful prognostic tool in that they correlate with the patient's final outcome, even when recorded early after injury. Also, abnormal multi-modality evoked potentials have been found to define dysfunction of the visual, auditory and motor systems in comatose patients and have been more effective than the clinical neurological examination in diagnosing persisting deficits in these systems.

3. Regional cerebral blood flow also continues to be studied. In one study of acutely head injured patients, approximately one-half revealed transient hyperemias, either generalized or focal, which were true luxury perfusions, i.e., blood flow was in excess of metabolic demand.

4. A video-tape has been made for teaching physicians and medical students the neurologic examination of the comatose patient and for teaching the coma-grading system.

D. Treatment

One group has concluded from laboratory studies involving balloon compression of the brain, middle cerebral artery occlusion, spinal cord compression and gunshot wound of the head that DMSO behaved in a manner similar to Mannitol but was more effective. Dexamethazone, compared to DMSO in monkeys after middle cerebral artery clipping, clearly improved the swelling of the injured brain but did not improve the clinical function to the same degree and was inferior to DMSO both in affecting neurologic status and in reducing brain edema.

In addition to the research being done in the clinical research centers there are 14 individual research grants dealing with head injury. A major new effort of the trauma program has been initiated with the funding of feasibility studies for Comprehensive Central Nervous System Trauma Centers. (See separate contract narrative.)

IV. CNS NEOPLASMS

There are nine research grants dealing with CNS neoplasia. They are generally basic studies dealing with such problems as "Metabolic Regulation in Glioma Cells," "Studies of Glial Cell Membranes and Myelin," and "Fine Structure of Viral Induced Brain Tumors."

V. NEURAL REGENERATION AND PLASTICITY

The traditional belief that the injured central nervous system is incapable of recovery has been seriously challenged in recent years by an increasing number of effective neuroscientists. It has become well recognized that the neurons and axons of the transected or traumatized spinal cord can produce sprouts that initiate the process of re-growth toward target axons. The reasons for the typical inhibition or blockage of this re-growth process are being investigated through the use of enzymes, grafting, use of prosthetic guides or channels, chemical and electrical growth stimulators, and other approaches.

The eventual aim of these studies--recovery of function--has not yet been achieved. However, the quantity and especially the quality of specific knowledge of the biological phenomena taking place have increased markedly within the past few years.

Research activities in regeneration and plasticity of the injured nervous system are almost all at the basic science level, as required by the present state of knowledge. However, the nature, the breadth, and the ingenuity of such research has been changing rapidly. The increased use of nerve tissue culture techniques, of immunological and genetic principles in studies of nerve grafting, and computer-assisted three-dimensional reconstruction methods of visualizing dynamic nerve processes are all contributing to a rapidly progressing and promising research field.

Regeneration and plasticity research is supported by the Stroke and Trauma Program mainly through research grants to individuals or small teams of investigators in academic medical centers. There are 54 research project grants and 4 research program-project grants totalling $5.4 million. These cover a wide range of subjects. Progress is steady but, as in most basic fields, not spectacular; these studies are certainly extending and confirming the basic knowledge on which applied research, and eventually clinical application, will be based.

Research supported by Stroke and Trauma Program grants is in progress on:

- Development and regenerative properties of embryological and neonatal nerve tissues.

- Processes of regrowth of injured axons and reestablishment of synapses in severed nerves.

- The chemical nature and mode of biological activity of Nerve Growth Factor and related substances in promoting re-growth of injured nerves.

- Axonal transport including effects of axon-sheath relationships.

- Plastic phenomena as a means of restoration of partial function following nerve injury.

The Stroke and Trauma Program supports by contract a single study to replicate the claims by Matinian in the USSR that through the introduction of enzymes into the lesion following transection of the rat spinal cord, functional recovery can be achieved. Thus far the Matinian reports have not been verified.

Among recent publications in nerve regeneration and plasticity several reports are of special interest for future major advances in practical application. These include:

Establishment of the facts that peripheral nervous tissue is immunologically competant and that homografts of this tissue are governed by the same principles and limitations of histocompatibility that apply to other tissues such as the heart and kidney. Application of this knowledge is leading to increased success in peripheral nerve grafts of major defects.

Swedish investigators in 1977 published data showing that neurons grafted into the brain will regenerate and reinnervate damaged regions of the brain, thus proving that neurons can survive temporary loss of blood supply and that subsequent outgrowth of nerve processes can occur.

Also in 1977 it was reported by a leading investigator that there are many normally non-functional nerve connections in the mammalian spinal cord which become functional after injury to adjacent neurons. These redundant connections offer promise that some recovery of function following severe spinal cord injury does not require perfect anatomical restoration of all specific axonal connections.

Another very recent report deals with the effect of autografts of peripheral nerve in bridging the debrided gap in the transected spinal cord of the dog. Nerve fibers subsequently grew into the graft. If this promising lead results in proof of reestablished neural transmission across the transection the applicability to restoration of function may be very great.

The steady increase in publications in CNS regeneration and plasticity, in membership in scientific research societies concerned with this field, and in the reorientation of established neuroscience investigators to studies of CNS regeneration all point to the potential for advancement in the field of CNS regeneration.

VI. PSYCHOSURGERY

The indications, contraindications and efficacy of psychosurgery remain areas characterized by much rhetoric and relatively little fact. The joint research efforts of the NINCDS and NIMH addressing these clinical issues are awaiting the decision of the Secretary, DHEW, in response to the report and recommendations of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. The Commission's report has now been released and published in the Federal Register. As required by the National Research Act of 1974, the Secretary, DHEW, will make his determination in regard to these recommendations. In collaboration with ADAMHA (NIMH) and the OD-NIH, the NINCDS has served on a task force preparing the draft of a DHEW position. This includes the establishment of a Joint Commission on Psychosurgery under contract to the NINCDS and NIMH. The Joint Commission will: set clinical standards for the clinical practice of psychosurgery, request voluntary compliance with these standards through the participation of the relevant professional societies, operate a voluntary registry on clinical psychosurgery, and advise the NINCDS-NIMH on research areas requiring emphasis and initiation. In the interim, the NINCDS continued to support jointly with the NIMH two contracts for retrospective evaluations of the efficacy of these procedures. These contracts were initiated by the National Commission as background material for its report. These data indicated that psychosurgery is equal to and in some instances superior to other forms of therapy for selected affective disorders. Both contractors have been advised that future support for these studies will depend on competition in the research grant mechanisms of the NIH and NIMH.

VII. CHRONIC PAIN

Chronic pain has been identified as a special health initiative by the White House staff. An "Interagency Committee on New Therapies for Pain and Discomfort" has been established on which a representative of the NINCDS Stroke and Trauma Program and of the NINCDS Intramural Research Program serve. The Committee has reviewed the Department's present activities in this area and is formulating needed new activities. As part of this effort, an NINCDS new initiative on research on chronic pain was announced. An RFA describing the Institute's interest in research grant, program-project and clinical research center applications has been released. In addition, the staff has served as technical consultants to the HCF Administration on the development of Department policies in regard to the reimbursement of chiropractors under Medicare. NINCDS current research grant support on the pathogenesis and treatment of headache and backache is at a very small level. Research support includes a controlled clinical trial of chiropractic manipulative therapy initiated in previous years, and is still in progress. Also, the terminal year of a program-project on the fundamentals of chiropractic is in progress. In addition, an Institute supported research Workshop on the Neurobiologic Mechanisms in Manipulative Therapy was held in October 1977 and a publication of the proceedings is now available (Plenum Press). We anticipate increased investigator-initiated grant proposals in response to the chronic pain RFA.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
October 1, 1977 -- September 30, 1978

UNIVERSITY OF ROCHESTER (NO1-NS-8-2385)
NORTH CAROLINA HEART ASSOCIATION (NO1-NS-8-2386)
UNIVERSITY OF OREGON HEALTH SCIENCES CENTER (NO1-NS-8-2387)

Title: Comprehensive Stroke Center

Project Directors: John H. Feibel, M.D. (Univ. of Rochester)
Paul E. Hirschauer (North Carolina Heart)
Frank M. Yatsu, M.D. (Univ. of Oregon)

Current Level of Support: $450,470 (Rochester)
$356,917 (N.C. Heart)
$726,015 (Oregon)

Objectives: The objectives of these centers are to:

a. Develop integrated and coordinated community resources by means of which developments in cerebrovascular research (including diagnosis management) and prevention research can be evaluated on a community basis.

b. Evaluate on a community basis the efficacy of the results of research on the diagnosis, management, and prevention of the cerebrovascular disorders.

c. Conduct a program of applied clinical research in which fundamental advances are utilized in the development of specific approaches for the prevention, diagnosis and management of cerebrovascular disorders.

d. Demonstrate to physicians, other professionals and the public, by a broad public education program, the significant advances in cerebrovascular research and management.

Major Findings: These contracts were awarded in June 1978. The first progress report is not yet due, but the following preliminary activities are underway:

1. Identification of individuals with stroke risk factors

2. Organization of stroke teams to integrate and coordinate health care services for stroke patients

3. Preparation of educational materials for use in prevention programs.

Significance to the NINCDS Program and Biomedical Research: The NINCDS Commission on Stroke has recommended that the NIH give increased attention to the problem of stroke through the assignment of additional resources to the NINCDS for this activity. Better opportunities are needed for clinical research, clinical training, and community services to permit a level of accomplishment commensurate with the impact of stroke as the third leading cause of death and the major national cause of long-term disability. Information about cerebrovascular disease needs to be communicated to the medical and scientific community in a more effective manner and newly developed methods for stroke prevention and diagnosis evaluated on a community basis.

Proposed course:

CONTRACTOR	TERMINATION DATE
University of Rochester	6/28/81
North Carolina Heart Association	5/31/81
University of Oregon Health Sciences Ctr.	6/14/80*

*It is expected that a detailed proposal will be submitted for a third year of support.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
October 1, 1977 -- September 30, 1978

MAYO FOUNDATION (NO1-NS-0933)

Title: Bibliographic Service on Cerebrovascular Disease

Contractor's Project Director: Robert G. Siekert, M.D.

Current Annual Level of Support: $17,810

Objective: To provide an abstracting service relative to various aspects of cerebrovascular disease. Contents of 150 journals are scanned. The abstracts are published in Stroke, a Journal of Cerebrovascular Disease.

Significance to the NINCDS Program and Biomedical Research: The abstracts on cerebrovascular disease continue to be a valuable service to the health profession.

Proposed Course: This contract will terminate 5-31-80.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
October 1, 1977 -- September 30, 1978

UNIVERSITY OF MINNESOTA (NO1-NS-4-2306)

Title: Comparison of Isotope-Bolus Technique with Contrast and Isotope Angiography

Contractor's Project Director: Shelley N. Chou, M.D.

Current Annual Level of Support: 0

Objectives: To test a simple, portable, intravenous isotope-bolus technique for demonstrating the absence of cerebral perfusion by comparison with both isotope and contrast cerebral angiography.

Major Findings: Accession rate remained low during this study. However, Dr. Chou feels that there has been adequate study to reach the conclusions listed below:

1. The isotope-bolus study can be easily performed at the bedside in both adult and pediatric patients.

2. Most of the patients treated for neonatal asphyxia, subependymal hemorrhages, trauma, etc., received significant quantities of barbiturates to control seizures. Therefore, the bolus study in this group may have more validity than the EEG to determine brain viability.

3. There was perfect correlation between non-filling angiograms and no bolus effect on the isotope study.

4. There also was excellent correlation between biological activity on the EEG and the bolus study. All patients with iso-electric EEGs had no bolus effect. All patients with biologic activity on the EEG had a definite bolus effect, except for one patient. This patient had minimal low-voltage activity on an EEG and then several hours later had no bolus on the isotope study and the non-filling angiogram. This can best be explained by a gradual progression of his cerebral dysfunction.

5. There were two patients with barbiturate intoxications who had definite bolus effects, despite a deep coma. One of these was a neonate, the other a middle aged female. Both eventually died, despite aggressive therapy.

6. One patient with renal failure (endogenous intoxication) and considerable obtundation was studied and he also had a bolus effect present.

Significance to the NINCDS Program and Biomedical Research: Cerebral angiography has remained the most reliable method for demonstrating cerebral circulation. Four-vessel cerebral angiography has been used in Europe as one of the criteria for establishing the diagnosis of brain death. However, this procedure involves both the risk of transporting the moribund patient and his support equipment to the X-ray department and the risk of the technique itself. Therefore, if the isotope-bolus technique is as reliable as angiography in determining the absence of cerebral perfusion, a simple, portable and relatively safe procedure will be available for assessing brain death.

Dr. Chou states that he believes, "We will be able to recommend the use of this technique in all age groups to confirm the finding of ECS on the EEG and as a substitute for doing an angiogram, if its only purpose is to document cerebral death."

Proposed Course: This contract terminated June 24, 1978.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
October 1, 1977 -- September 30, 1978

YALE UNIVERSITY (NO1-NS-7-2361)

Title: Feasibility Study to Develop Data Collection Instruments and Protocols at the National Acute Spinal Cord Injury Centers

Project Director: William F. Collins, M.D.

Current Level of Support: $63,000

Objectives: To develop and test data collection forms to be used by all Spinal Cord Injury Clinical Research Centers. Also, to standardize, as much as possible, the methods of performing and recording the neurologic examination. Such a data collection system will permit pooling of cases and more rapid evaluation of therapy.

Major Findings: Representatives of the Spinal Cord Injury Clinical Research Centers have completed work on the data collection forms. These have been tested and found to function satisfactorily.

Significance to the NINCDS Program and Biomedical Research: This effort is of major importance to our clinical research program in spinal cord injury. The accession of spinal cord injury cases is too low in any one center to permit accumulation of data in a short enough time to assess any method of treatment. With this pooling of cases, such assessment should be possible.

Proposed Course: This contract is due to terminate September 30, 1978. Yale University, acting as representative and coordinator for the Spinal Cord Clinical Research Centers has submitted a grant application entitled, "Methylprednisolone and Spinal Cord Trauma." This is a proposal to study the effects of high dosage corticosteroids on patients with spinal cord injury. If approved, this will be the first project in which the new data collection instruments will be used.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
October 1, 1977 -- September 30, 1978

NATIONAL ACADEMY OF SCIENCES MEDICAL FOLLOW-UP AGENCY (NO1-NS-6-2346)

Title: Feasibility Study, Phase I, Follow-up Study of Vietnam Registry of Head and Spinal Cord Injuries

Contractor's Project Director: William Caveness, M.D.

Current Level of Support: $10,300

Objectives: To determine the alteration in central nervous system function 10 years after injury and so assess residua of penetrating wounds.

Major Findings: So far, 1200 records have been reviewed and coded. Analyses are in progress. A manuscript has been submitted to the Journal of Neurosurgery, entitled, "The Nature of Post Traumatic Epilepsy." Among the conclusions reported are: (1) The incidence of fits has remained the same from one war to another, in spite of marked improvement in patient transport, surgical techniques, medical management, and the prophylactic use of anticonvulsants in Vietnam; (2) In the population at risk, 65-75% never have a fit.

Significance to the NINCDS Program and Biomedical Research: An analysis of these war records may permit a better understanding of penetrating injuries, their natural history and treatment, in contrast to the more common blunt injuries of peacetime.

Proposed Course: This contract terminated on June 30, 1978. Some final work with the records is being done and analyses are being performed under professional services contracts to several individuals.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
October 1, 1977 - September 30, 1978

UNIVERSITY OF NEW MEXICO (N01-NS-5-2332)

Title: Quantitative Intracranial Pressure Measurement in Man

Project Director: A. Earl Walker, M.D.

Current Annual Level of Support: $72,232

Objective: To evaluate clinically a system for monitoring intracranal pressure.

Major Findings: Eighty intracranial pressure transducers have been fabricated and subjected to in vitro testing. Most of these function well during the first two months of in vitro testing but develop a baseline drift after this period. This is not satisfactory for in vivo monitoring of pseudotumor or hydrocephalus and a totally implantable long-term intracranial pressure monitoring system remains an El Dorado.

Significance to the NINCDS Program and Biomedical Research: A system capable of detecting increases in intracranial pressure before clinical signs manifest themselves would permit better and faster treatment of patients with intracranial lesions.

Proposed Course: This contract has been extended on a no-cost basis until December 29, 1978. A decision will be made then on whether there will be any further no-cost extensions for further in vivo evaluation of the transducers.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
October 1, 1977 -- September 30, 1978

CORNELL UNIVERSITY MEDICAL COLLEGE (NO1-NS-4-2328)

Title: Prediction of Outcome of Patients with Coma

Project Director: Fred Plum, M.D.

Current Level of Support: 0

Objectives:

1. To test the accuracy and validity of a semiquantitative system for grading coma.

2. To assess the predictive power of each indicant with regard to short term prognosis (3 months).

Major Findings: Results of this contract have been published in an article in the Annals of Neurology, 1977, entitled "A Prospective Study of Non-Traumatic Coma: Methods and Results in 310 Patients," by D. Bates, et al. The summary of this article states: "Neurological signs and outcome are compared in the first 310 patients from a continuing prospective study of coma not caused by trauma or drugs. Sixteen percent of the patients achieved an independent existence within a month; severe diability or the vegetative state developed in 25% of the patients comatose for six hours, and in 79% of those still in coma after a week. The chance of regaining an independent existence was greater in patients who, by one day, obeyed commands or moved limbs appropriately in response to noxious stimuli or who had attained any of the following: orienting eye movements, normal responses to oculocephalic or oculovestibular stimulation, or normal muscle tone. Conversely, the chance of regaining an independent existence fell in patients who, after one day, had either extensor responses of the limbs or failed to move them in response to noxious stimuli or who lacked eye opening, pupilary reactions, corneal responses, or any eye movement in response to oculovestibular or oculocephalic stimulation. Beyond these general guidelines, numbers of patients with particular signs are presently too small for confident prediction of outcome."

Significance to NINCDS Program and Biomedical Research: The large number of cases in this study permits further testing of a semiquantitative system for grading the severity of coma. The general acceptance of such a scale will facilitate neurologic communication and aid in discussions of stroke and head injury.

Proposed Course: This project terminated on June 30, 1978; however, analyses of the results will continue.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
October 1, 1977 -- September 30, 1978

UNIVERSITY OF PENNSYLVANIA (NO1-NS-5-2316)

Title: Computerized Axial Tomography in Acute Head Injury

Project Director: Robert A. Zimmerman, M.D.

Current Level of Support: 0

Objectives:

1. To determine the ability of computerized axial tomography to detect and distinguish lesions in acute head injury.

2. To test the ability of CAT to predict delayed cerebral manifestations of closed head injury.

3. To determine the optimal time for CAT in management of acute severe head injuries.

Major Findings: Four hundred and sixty-three (463) patients have been admitted to the study. X-ray CT has been used 689 times and radionuclide scanning 100 times. These include follow-up scans as well as the initial scans. Analyses of results have continued with particular interest in three categories of brain injury: (1) generalized cerebral swelling of children, (2) diffuse cerebral white matter injury, and (3) hemorrhagic contusion.

Significance to NINCDS Program and Biomedical Research: CAT is one of the most sensitive techniques available for localization and indentification of intracranial lesions. It is, however, the latest tool in the armamentarium of the neurologist and neurosurgeon and as such needs evaluation and comparison with more established techniques, such as angiography. For decades, cerebral aniography has offered the most reliable information in ruling out intracranial hematomas. If comparable information can be obtained from CAT, a definite service will have been rendered to the head-injured patient. Since CAT does not involve arterial puncture and in many cases does not involve the use of contrast agent, it carries significantly less risk than angiography. Initial evaluation of the patient would, therefore, be safer and the physician would more readily order serial examinations to follow the patient's course.

Proposed Course: This contract terminated on June 30, 1978. Results continue to be analyzed.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
October 1, 1977 -- September 30, 1978

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-NS-7-2371)
UNIVERSITY OF CALIFORNIA AT SAN DIEGO (NO1-NS-7-2370)
UNIVERSITY OF MIAMI (NO1-NS-7-2368)
UNIVERSITY OF MINNESOTA (NO1-NS-7-2369)
UNIVERISTY OF TEXAS MEDICAL BRANCH (NO1-NS-7-2372)
UNIVERSITY OF VIRGINIA (NO1-NS-7-2373)

Title: Feasibility Studies for a Program of Comprehensive Central Nervous System Trauma Centers

Project Directors: Kenneth Shulman, M.D. (Albert Einstein)
Lawrence F. Marshall, M.D. (U.C. at San Diego)
Hubert Rosomoff, M.D. (U. of Miami)
Robert Maxwell, M.D. (U. of Minnesota)
Robert Grossman, M.D. (U. of Texas Med. Branch)
John Jane, M.D. (U. of Virginia)

Current Level of Support:

$116,127	(Albert Einstein)
$ 91,938	(U.C. at San Diego)
$100,155	(U. of Miami)
$ 85,000	(U. of Minnesota)
$111,300	(U. of Texas Med. Branch)
$115,519	(U. of Virginia)
$620,039	

Objectives: These studies have as their goals:

1. Development of community resources to evaluate the treatment of CNS injured patients in the community and the effects in the community of progress in CNS trauma research.

2. Encouragement of clinical research in the field of CNS trauma.

3. Establishment of methods to bring results of research in CNS trauma rapidly and effectively to the general community.

Major Findings: Work on the feasibility studies has progressed very well. Epidemiologists and biostatisticians are participating actively in all the studies. Numerator and denominator figures are being obtained. Community agencies have been receptive and with their participation guidelines have been produced for long range cooperation.

Significance to NINCDS Program and Biomedical Research: As research in the Head and Spinal Cord Injury Clinical Reseach Centers has progressed, questions have arisen regarding the applicability of their efforts. Do any of the techniques developed at a particular clinical research center reach the surrounding community hospiatls? If so, does their application

there produce the same results as it does at the Center? Does the presence of a Center affect the distribution of care of CNS injured patients in a community? Does the care given in the Center affect mortality or morbidity for a given type of trauma? Should emphasis be on rapid transportation to a Center or to development of local facilities? To answer such questions as these, the Comprehensive CNS Trauma Center Program has been initiated. The results of such community oriented research should be of great value to all organizations involved in the care of the CNS injured patient.

Proposed Course: These contracts are scheduled to terminate September 30, 1978. If final reports indicate the likelihood of further benefit from this effort and if the NINCDS budget permits, an RFP will be issed this fall for three to four Comprehensive CNS Trauma Centers.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
October 1, 1977 -- September 30, 1978

UNIVERSITY OF MARYLAND (NO1-NS-7-2358)

Title: Functional Recovery in Rats after Spinal Cord Lesion

Contractor's Project Director: Lloyd Guth, M.D.

Current Level of Support: $86,337.00

Objective: To replicate and evaluate the research on regeneration and recovery of function of the severed rat spinal cord which was performed by L. A. Matinian and A. S. Andreasian, Orbelli Institute of Physiology, Academy of Sciences of the Armenian Soviet Socialist Republic, Yerevan, USSR, in 1973. Enzyme Therapy in Organic Lesions of the Spinal Cord. Akademia Nauk Armenian SSR, 1973 pps. 1-94. In Russian with English summary.

Major Findings: Extensive efforts by the Contractor over a period of more than a year to verify the published claims of beneficial effects of enzymes in recovery of function in the transected rat spinal cord by Matinian have been completely unsuccessful.

Significance to NINCDS Program and Biomedical Research: The work of Matinian and Andreasian, if confirmed, has immense potential benefits to the treatment of the severely traumatized spinal cord, and therefore to the possible recovery of paralyzed victims of CNS injury.

Proposed Course: The contract has been extended into a second year to permit the completion of experiments, preparation of reports and publication of results.

ANNUAL REPORT
October 1, 1977 through September 30, 1978
Communicative Disorders Program
National Institute of Neurological and
Communicative Disorders and Stroke

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ANNUAL REPORT
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Introduction

The Communicative Disorders Program of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) is concerned with research directed toward improving the diagnosis, treatment, and prevention of diseases and disorders which affect the ear, nose and throat and cause problems related to hearing, speech and language. Some of the disorders involving the special senses of taste, smell, touch and pain may also relate to human communication and thus are included in the Communicative Disorders Program.

Because the diseases and disorders of human communication are not usually the primary cause of death they do not attract the same attention as do cancer, stroke or heart disease. However, in number of individuals involved, the communicative disorders are one of the most frequent disabilities in our society. Approximately fifteen million individuals in this country have a hearing loss severe enough to impair their ability to function in everyday activities and ten million persons do not speak normally. An additional number do not have adequate language to communicate effectively. These groups with communicative disorders create a real economic impact on the Nation when their habilitative, rehabilitative, and special education costs are considered. An even more important consideration is the effect of such handicaps upon the quality of life of these persons and their families.

Communicative Disorders Staff and Environment

On March 10, 1978, Dr. Wesley H. Bradley resigned as Program Director to accept a position in Albany, New York where he has a joint appointment with the Department of Otolaryngology at the Veterans Administration Hospital and the Albany Medical College. Leadership in this position has been provided by Dr. Eldon Eagles, Deputy Director of the Institute, and present staff members while a search committee is seeking a permanent replacement. Subsequently, Dr. David Hanson resigned his position as staff Otolaryngologist in July to assume a position as Assistant Professor in the Division of Head and Neck Surgery at the University of California at Los Angeles. The absence of botn of these key professionals has had a negative effect on the otolaryngological portion of the Program activities. Nevertheless, the Program was fortunate to be able to add Dr. Rolf Ulvestad, Otolaryngologist, to the staff in June. A recent graduate of the Medical School of the University of Minnesota, Dr. Ulvestad is assuming the position that Dr. Hanson vacated.

The Program is still hopeful that a position for a professional with research experience and expertise in the chemical senses will become available in the very near future. Without a staff member in this area of Program responsibility, it is very difficult to provide adequate grant and contract direction.

Dr. Christy Ludlow, Speech Pathologist, continues to direct the speech and language portion of the Program. In addition to her extramural activities, she maintains an active clinical research program with the assistance of Mrs. Celia Cardano, a full-time research Speech Pathologist.

The areas of communicative aids, effects of noise on hearing, and management of hearing disorders are the responsibility of Dr. Earleen Elkins, an Audiologist. She also supervises the Audiology Service of the Clinical Center and directs clinical research with the assistance of Ms. Pamela Mason, a consultant Audiologist.

Dr. Irving Woods, Health Scientist Administrator, monitors the entire grant portion of the Communicative Disorders Program.

The acquisition of additional space in the Federal Building has alleviated the rather crowded environment of previous years. This was made possible when the Epilepsy Commission completed its work. The research and clinical activities continue to be hampered by inadequate space and facilities. Since no testing space is available for use in the Clinical Center, patients must be accompanied by staff to and from Building 36 for testing in one of the two rooms available for speech and language research. Although this setting is safe for conducting patient speech and language testing, it is often distracting to subjects, and a great deal of time is lost since the staff must continuously dismantle and reassemble instrumentation each time a subject is tested due to severe space limitations. A sound controlled room which can be permanently set up for conducting speech and language patient testing is badly needed in the Clinical Center.

Clinical needs for otolaryngology and audiology at the Clinical Center have remained largely unimproved for reasons beyond the control of the Communicative Disorders Program. The Clinical Center has agreed to build, equip and staff at an appropriate level, an adequate facility to meet present clinical testing needs in audiology. Construction is yet to begin. There has been no administrative clarification of responsibility for otolaryngological care at the Clinical Center beyond the present situation. Program staff have laid the groundwork for closer alliance with the Otolaryngology Service at the National Naval Medical Center which would substantially improve the ability to provide competent otolaryngologic care at the Clinical Center. However, the present unsatisfactory situation has not been resolved.

Program Activities

Some of the activities of the Communicative Disorders Program this year include:

1) Ad Hoc Program Advisors Meetings
2) Research Grants and Contracts
3) NANCDS Council Subcommittee for Development of National Research Strategies Participation
4) Clinical Research Activities
5) Program Development Conferences
6) Improvement of Bibliographic Services for Clinicians and Investigators in Communicative Disorders

Ad Hoc Program Advisors

The Ad Hoc Program Advisors met twice this year, in December 1977 and May 1978. The members of the group include:

Katherine S. Harris, Ph.D.
Graduate School of University Center of the City University of New York

Ira Hirsh, Ph.D.
Central Institute for the Deaf, St. Louis

Marcel Kinsbourne, M.D., Ph.D.
Hospital for Sick Children, Toronto

David J. Lim, M.D.
University Hospital, Ohio State University, Columbus

Ralph Naunton, M.D.
The Pritzker School of Medicine, University of Chicago

James B. Snow, Jr., M.D.
University of Pennsylvania, Philadelphia

The Advisors have been very helpful in evaluating directions for future research and areas needing special emphasis. They have also evaluated critically staff suggestions for new or special initiatives and approved or suggested alternatives. At the December 1977 meeting, Dr. Hanson presented a comprehensive review of the Vestibular System area. He traced the grant support and activity since 1975 so that the Advisors would be better informed about Program needs in this area. Reviews of a similar nature will be presented by staff members in the future. The next meeting is scheduled for September 1978 when new contract and Program projections will be presented.

Research Grants and Contracts

A general picture of the research grant program is presented by the accompanying outline and figures. All currently active research grants are shown by program area along with the respective funds committed in FY '78. Contract work has proceeded at a very active pace with the successful completion of two contracts in the hearing area. In addition, one new contract was awarded in the otological area; two are in the process of

being awarded in speech and language; and one amendment to a Heart, Blood, and Lung Institute contract for hearing assessment was awarded this year. Details of these activities are included in subsequent portions of this report.

FY '78 COMMUNICATIVE DISORDERS GRANTS CLASSIFICATION AND FUNDING
NUMBER OF GRANTS 272; FUNDING (Direct Costs Only): $15,215,134

I.	DISEASES OF THE EAR, NOSE AND THROAT - 23.4			1,748,364 = 12%
	A. Ear-19.2			1,491,850
	1. Basic-12.5		906,424	
	2. Applied-4.7		585,426	
	B. Larynx and Pharynx-4.2			256,514
	1. Basic-.5		143,143	
	2. Applied-3.7		113,371	
II.	VESTIBULAR SYSTEM - 21.4			1,015,701 = 7%
	A. Normal-13.5			455,940
	1. Basic-13.5		455,940	
	a. Structure-5	122,872		
	b. Function-8.5	333,068		
	2. Applied-0		0	
	B. Disorders-7.9			559,761
	1. Basic-1		45,073	
	a. Structure-0	0		
	b. Function-1	45,073		
	2. Applied-6.9		514,688	
III.	HEARING - 109.8			6,654,759 = 44%
	A. Normal-79.9			4,292,447
	1. Basic-58.6		3,275,883	
	a. Psycho-acoustic-13.5	534,927		
	b. Anatomy, Physiological & Biochemical-45.1	2,740,956		
	2. Applied-21.3		1,016,564	
	B. Disorders-29.9			2,362,312
	1. Basic-17.7		1,053,388	
	a. Psycho-acoustic-6.3	294,727		
	b. Anatomy, Physiology & Biochemical-11.4	758,661		
	2. Applied-11.6		1,155,715	
	a. Psycho-acoustic-11	914,234		
	b. Anatomy, Physiology & Biochemical-.6	241,481		
	3. Aural Rehabilitation-.6		153,209	

(Cont'd)

FY'78 COMMUNICATIVE DISORDERS GRANTS CLASSIFICATION AND FUNDING (Cont'd)

IV.	SPEECH AND VOICE	33.6			2,035,871 = 13%
	A. Normal - 25.3			1,232,400	
	1. Basic - 8		341,826		
	2. Applied - 17.3		890,574		
	B. Disorders - 8.3			803,471	
	1. Basic - 1.3		32,042		
	2. Applied - 7		771,429		
V.	LANGUAGE	15.7			631,643 = 4%
	A. Normal - 4			37,844	
	1. Basic - 2		14,816		
	2. Applied - 2		23,028		
	B. Disorders - 12.7			593,799	
	1. Basic - 0		0		
	2. Applied - 12.7		593,799		
VI.	NOISE EFFECTS	2.6			591,151 = 4%
	A. Auditory - 2.6			591,151	
	1. Basic - 2.1		570,411		
	2. Applied - .5		20,740		
	B. Non-Auditory - 0			0	
VII.	COMMUNICATIVE AIDS	13.8			944,771 = 6%
	A. Sensory - 13.8			944,771	
	1. Basic - 4		465,341		
	2. Applied - 9.8		479,430		
	B. Expressive - 0			0	
	1. Basic - 0				
	2. Applied - 0				
VIII.	SPECIAL SENSES	51.7			1,592,874 = 10%
	A. Vision - 4			155,052	
	1. Basic - 4		155,052		
	2. Applied - 0		0		
	B. Pain - 3			135,677	
	1. Basic - 1		52,703		
	2. Applied - 2		82,974		
	C. Touch - 13			436,093	
	1. Basic -12		419,209		
	2. Applied - 1		16,884		
	D. Smell - 16			464,806	
	1. Basic - 16		464,806		
	2. Applied - 0		0		
	E. Taste - 13			332,929	
	1. Basic - 13		332,929		
	2. Applied - 0		0		
	F. Multisensory - 2.7			68,317	
	1. Basic - 2.7		68,317		
	2. Applied - 0		0		
	TOTAL	272			$15,215,134

National Research Strategies Participation

All members of the extramural staff have had an active role in the development of a research strategies plan for the Institute. Primary responsibilities have been with the Communicative Disorders Panel which is one of seven selected to cover all areas of research supported by the Institute. Under the Chairmanship of Dr. Paul Ward, the following scientists have contributed many hours assembling data to represent our knowledge in communicative disorders, the areas that need to be addressed in the next ten years, and the resources in both dollars and manpower that will be necessary to accomplish these goals: Dr. Robert Ruben, Dr. Vincente Honrubia, Dr. Murray Sachs, Dr. Harry Levitt, Dr. Charles Berlin, Dr. Katherine Harris, Dr. Linda Swisher, Dr. Bruce Halpern, and Dr. Christina Leske.

A unique opportunity was provided in May 1978 to obtain testimony from private and public individuals concerned with communicative handicaps. The Communicative Disorders Program staff have provided data regarding current and past support of both grants and contracts and will be responsible for developing a plan for implementing the suggestions of the Panel. A preliminary document will be presented to the NANCDS Council in October 1978.

Clinical Research Activities

Clinical studies in speech and language include the testing of patients in the Clinical Center as well as outpatients, linguistic and acoustic analyses of responses, and data compilation, reduction, and analyses. The major focus of this research is to examine the effects of various neuropharmacological treatments on speech and language disorders associated with Parkinson's disease, Huntington's chorea, impaired language development, hyperactivity, Gilles de la Tourette's syndrome and tardive dyskinesia. The general goal of this research is to develop an understanding of the neuropharmacological bases of speech and language functions in different diseases which will provide indications for treatment. During the last year the following studies have been completed and are being written up for submission to scientific journals:

"The Effects of the On-Off Phenomenon on Speech Production in Parkinson's Disease," C. L. Ludlow, G. Geoffrey, R. Kartzinael, and D. Calne; "A Comparison of Acoustic and Perceptual Methods for Assessing Dysarthria," C. L. Ludlow, C. B. Cardano, and B. L. Cullison; "Language and Speech Correlates of Vocal Tics in Gilles de la Tourette's Syndrome," C. L. Ludlow, E. Caine and M. Ebert; "Characteristics of Vocal Tics in Gilles de la Tourette's Syndrome," C. L. Ludlow, E. Caine and M. Ebert; and "The Effects of Dextroamphetamine on the Language Performance of Hyperactive Children With and Without Impaired Language," C. L. Ludlow, L. Brown, M. Ebert and E. Mikkelson.

Laboratory work in hearing has been addressing the problem of non-sensitivity measures to predict ototoxicity due to chemotherapeutic agents used by the National Cancer Institute in patient treatment protocols. A detailed description is provided later in this report.

The Communicative Disorders Program will continue to press for a full intramural research program in communicative disorders. There are tremendous needs in clinical otolaryngology, audiology and speech pathology research that could be addressed in the unique setting of the NIH Clinical Center. It would seem shortsighted not to fully exploit the potential of an intramural communicative disorders program.

Program Development Conferences

With the assistance of the Ad Hoc Program Advisors and a detailed analysis of grant support, certain areas of Program concern were identified for special attention this year. To provide expert advice for the Program, three conferences were planned and carried out. A brief description of each is provided.

The Neurological Bases of Language Disorders in Children: Methods and Directions for Research. This symposium was organized by Dr. Christy L. Ludlow and held at the NIH on January 16 and 17, 1978. The purpose of the symposium was to review selected methods of studying neurological and behavioral development and to determine the potential of each for use in future research on the etio-pathology of specific language disorders, autism and dyslexia. The symposium was aimed at defining directions for research and determining research training needs. The participants, each engaged in research using different methodologies, reviewed significant research findings provided by using such approaches. Before the meeting, each participant prepared a manuscript covering significant research methodologies and discussing the potential of those methods for studying the neurological bases of language disorders in children. At the meeting, each participant resumed his/her major conclusions with some background information, although the participants did not present the entire contents of their manuscripts at the symposium. In some areas, two presentors were selected to provide different approaches to the same topic.

The group discussed the suggestions made by each participant, drawing on their own experience with similar or different methodologies. The participants were asked to discuss the following:

1. Which research methodologies have the greatest potential for providing new knowledge on developmental language disorders?

2. What questions should first be addressed to provide improved understanding of the bases for language disorders in children?

3. What methodological and technical advances are needed before certain areas of research can be initiated, and

4. What skills need to be taught to new investigators in the field?

Following the symposium, the staff transcribed and edited the discussion and participants were asked to review their remarks. The proceedings will be published in FY '79 by the NINCDS and disseminated free of charge to scientists in the biomedical and behavioral neurosciences to inform them concerning the need and potential for research in this area.

Otitis Media and Child Development. Under the direction of Dr. David Hanson, this workshop assessed the hypothesis that otitis media in its recurrent or chronic state significantly disturbs the learning process in the developing child. On March 16 and 17, participants from many disciplines assembled at the NIH to provide papers and discussion on the current state of knowledge and research design factors involved in: a) Identification and assessment of hearing loss, b) significance of temporary hearing loss as applied to otitis media, c) hearing loss and early linguistic development, d) assessment of intelligence in the affected age group, 3) assessment of speech and language disability, f) assessment of cognitive development, and g) ethical and epidemiological factors.

In general, the group concluded that many of the areas of concern need additional work to provide assessment tools with high reliability and validity before the major hypothesis regarding otitis media may be addressed. The proceedings of the meeting are being processed for distribution and publication in a scientific journal.

Effects of Noise on Hearing: Research Directions and Designs. Dr. Earleen Elkins developed and organized a small working group of scientists to critically review the current state of knowledge on noise and make suggestions for the directions and methodologies that future research should employ. In Silver Spring, Maryland, on June 6 and 7, 1978, the participants presented papers on the following sub-topics: Anatomical status and noise exposure, physiological effects of noise, temporary and permanent threshold shifts, loudness and annoyance as related to noise, psychological aspects of noise exposure, diagnosis and management of noise-induced hearing loss, speech discrimination by noise-exposed listeners, and the effects of noise exposure on the hearing of animal and human laboratory subjects.

A strong input by other Federal agencies concerned with noise effects in general was provided by representatives of the National Institute for Environmental Health Sciences, the Environmental Protection Agency, and the Occupational Safety and Health Administration. The participants concluded with a number of specific and general recommendations to guide the Program in stimulating research in the scientific community in general and identifying areas for investigation that will be developed for contract support.

The proceedings of the workshop are being transcribed, edited, and typed for timely publication and distribution to the scientific community and other Federal agencies.

Improvement of Bibliographic Services for Clinicians and Investigators in Communicative Disorders

Work has continued on this project which was initiated in FY '76 for the purpose of improving the coverage of literature in the communicative sciences and disorders by the National Library of Medicine (NLM), improving the ease with which bibliographic material in the communicative sciences and communicative disorders can be retrieved from the National Library of Medicine data base, and teaching professionals and investigators how to use the NLM Literature Retrieval System to meet their bibliographic needs with maximum efficiency.

The Project was initiated following a detailed review of the coverage of the communicative sciences in the bibliographic data base of the NLM. A conference was held to identify the needs for improving services to scientists and clinicians in the communicative disorders fields, recommendations were integrated for an improved indexing system for bibliographic material, and information was gathered on the MEDLARS retrieval system operated by the NLM by the staff of the Communicative Disorders Program. Specific recommendations were developed for improving the retrieval of bibliographic material from the MEDLARS system.

Dr. Ludlow is staff coordinator for the project and Dr. Barbara Reiner has been hired under professional services contract to work in close collaboration with the National Library of Medicine staff. In FY '78, recommendations for 250 new indexing terms to be added to the MEDLARS system were made to the National Library of Medicine. Consequently, Dr. Ludlow and Dr. Reiner have been working with the NLM staff to determine how much terminology can be added to the system in a compatible manner. In addition, the staff are presently drafting a user's manual on how to use the MEDLARS system to serve their bibliographic needs. The manual and training materials will be used for conducting workshops to train specialists in communicative disorders on how to use the MEDLARS system.

Activities of the Professional Staff

Members of the staff have participated in numerous professional activities outside of the NINCDS Program, some of which are noted below.

Dr. Bradley continued to have an active role in otolaryngological societies and participated in meetings of the American Academy of Otolaryngology and Ophthalmology; the American Association of Medical Colleges; the American Laryngological, Rhinological, and Otological Society; and the Association for Research in Otolaryngology. He also served as a Trustee of the American Otological Society and was a speaker at the Scientific Inauguration and Dedication of the Neurosensory Center of Houston, Texas.

Dr. Hanson presented a paper on otitis media at the Annual Otology-Audiology Workshop in March and attended the Spring Meetings of the Combined Otological Societies. This spring he was awarded the United States Public Health Service Commendation Medal "for sustained high quality performance in initiating and developing a program involving clinical otolaryngology within the Communicative Disorders Program."

Dr. Woods attended the Society for the Neurosciences and the Eastern Psychological Association meetings as part of his professional activities.

During the last year, Dr. Ludlow served on the Convention Program Committee for the American Speech and Hearing Association and as an Editorial Consultant for the following scientific journals: The Journal of Speech and Hearing Research, the Journal of Speech and Hearing Disorders, and ASHA, the official journal of the American Speech and Hearing Association.

In addition, Dr. Ludlow was appointed Chairman of the Committee on Scientific Affairs for the American Speech and Hearing Association. She was invited to serve as a member of the Task Force on Speech, Language and Hearing Science of the American Speech and Hearing Association which was formed to address the issues pertinent to the speech, language and hearing sciences and to the scholars who perform scientific research in these fields. Also, Dr. Ludlow was reappointed the Liaison Representative of the American Speech and Hearing Association to the American Association for the Advancement of Science.

The following scientific presentations were made at meetings by Dr. Ludlow:

"The Communication Behavior of Aphasic Adults." Chairperson and discussant, symposium held at the Annual Meeting of the Academy of Aphasia, Montreal, Canada, October 1977.

"Characteristics of Vocal Tics in Gilles de la Tourette's Syndrome," (with E. Caine and M. Ebert). Paper presented at the Annual Convention of the American Speech and Hearing Association, Chicago, November 1977.

"Effects of Dextroamphetamine on Hyperactive and Normal Children's Language Behavior," (with J. Rapoport, G. Brown, and E. Mikkelsen). Presented at the Annual Convention of the American Speech and Hearing Association, Chicago, November 1977.

"Problems in Diagnosis and Treatment of Language Disorders in Children." Presented at the conference, Understanding Minimal Brain Dysfunction, State University of New York, Upstate Medical Center, Syracuse, New York, November 1977.

"Language Rehabilitation in Aphasia: An Examination of the Process and Its Effects." Chairperson and discussant. Presented at the Annual Meeting of the American Association for the Advancement of Science, Washington, D. C., February 1978.

"Language Impairments in Learning Disability." Presented at the Conference on Learning Disabilities, Bowman-Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina, March 1978.

"The Differential Effects of Dextroamphetamine on the Language and Communicative Skills of Hyperactive and Normal Children," with J. Rapoport, G. Brown, and E. Mikkelsen. Presented at the International NATO-sponsored Conference on Rehabilitation, Treatment and Management of Learning Disorders, Mont Ste. Marie, Ottawa, Canada, June 1978.

Last fall, Dr. Elkins was selected to participate in the Grants Associate Program of the NIH. She was also appointed to the Committee on Speech Audiometry of the American Speech and Hearing Association and will aid in developing national guidelines on that area of hearing assessment.

She attended a conference at the University of Chicago on Evoked Electrical Responses of the Auditory System and chaired a session on Speech Perception by the Hearing-Impaired at the Annual Meeting of the American Speech and Hearing Association.

Her activities related to management of the hearing-impaired have included a seminar on Advancements in Hearing Aid Design in Washington, D. C., and attendance at the Annual Hearing Aid Research Conference in New York City where she chaired a session. She is also on a panel to review research proposals on hearing aids for children under the auspices of the Bureau for Education of the Handicapped. In a continuing effort to coordinate Federal support of sensory aids, Dr. Elkins presented a paper entitled, "Prostheses for the Hearing-Impaired," at a conference co-sponsored by the National Institute of Aging and the National Aeronautics and Space Administration. She also works with the Smithsonian Institution with a group addressing the concerns of the blind and physically-handicapped.

Dr. Elkins continues to serve as consultant on hearing assessments for the United States Public Health and Nutrition Examination Survey of the National Center for Health Statistics and acts as an alternate member of the Committee on Hearing and Bioacoustics of the National Academy of Sciences. In addition, the emphasis by Congress to address the effects of noise on the population has stimulated inter-Federal agency cooperation. She prepared a report of NINCDS-sponsored research which was published by the Environmental Protection Agency for the Noise Effects Research Panel.

Publications by members of the staff are listed below:

Hanson, D. G., Juhn, S. K., Giebink, G. S., and Paparella, M. M. Lactate Dehydrogenase as a Measure of Inflammation in Experimental Otitis Media. Archives of Otolaryngology, 104: 333-335, 1978.

Hanson, D. G., The Bionic Ear. Receiver, Deafness Research Foundation, pp. 1-3, Fall 1977.

Rapoport, J. L., Buchsbaum, M. S., Zahn, T. P., Weingartner, H., Ludlow, C. L., and Mikkelsen, J. J. Dextroamphetamine: Cognitive and Behavioral Effects in Normal Prepubertal Boys. Science, 199; 560-563, 1978.

Ludlow, C. L., Rapoport, J. L., Cardano, C. B., and Mikkelsen, E. G. Differential Effects of Dextroamphetamine on Language Performance in Hyperactive and Normal Boys. In R. M. Knights and D. J. Bakker, (Eds.), Rehabilitation, Treatment and Management of Learning Disorders. Baltimore: University Park Press, in press.

Elkins, E., Causey, G. D. and Roberts, J. Development of Speech Reception Test. U. S. Department of Health, Education, and Welfare Publication No. (HRA) 78-1345. Washington, D. C., U. S. Government Printing Office, 1978, 16 pp.

Grants Activity Summary
Communicative Disorders Program

The Communicative Disorders Program of the NINCDS is continuing to support research in the biomedical sciences and in clinical investigations leading toward the goal of better understanding of the mechanisms of normal communication and those diseases which impair it. The current year has been highlighted by many important contributions. Some selected examples follow.

Diseases of the Ear, Nose and Throat

A group of investigators are establishing an animal model to quantitatively study normal and abnormal Eustachian tube (ET) function and its effect on middle ear (ME) effusions. Models of 1) atelectasis of the middle ear, 2) acute and chronic sterile ME effusions, and 3) acute bacterial ME effusions, resulting from moderate and severe functional ET obstruction, were studied to isolate the parameters of different types of tubal function. The model also will test various non-surgical and surgical methods of ME disease management. In addition, a reversible functional ET obstruction will be created in an attempt to investigate the efficacy of restoration of normal ET function. Sixteen (16) Rhesus monkeys were studied. Post-operative tubal function studies during the six-month follow-up showed total lack of muscle function, indicating that severe functional ET obstruction remained.

Another research group has as its objective to provide basic information on the normal ventilation processes of the middle ear and sinuses, differentiation of abnormal from normal ventilation rates through the use of Xenon-133, a radioactive gas, and the development of a safe, accurate and rapid technique to aid in the clinical assessment of the middle ears and sinuses. They are continuing to obtain data from normal subjects to improve the validity and reliability of the quantitative analysis of the rates of egress from each area. Also, environmental and physiological variables are being introduced during measures on normal subjects so that their effects on the ventilation rates may be evaluated. For example, rates of egress are being assessed for changes due to body position, coughing, warming of the tympanic membrane, vasal constrictors, and deep breathing. Finally, ventilation rates are obtained from as many abnormal subjects as possible and especially subjects with a history of otitis media, serous effusion, completely obstructed eustachian tube, or atelectasis of the middle ear. An insufflation procedure is also being monitored by the use of equipment designed to measure bilateral vacuum and pressure on each subject.

Vestibular System

A continuing study is attempting to increase our understanding of the functional contributions made by various components of the vestibular system by describing the information encoded in peripheral vestibular neurons and its modification in the central vestibular pathways. These investigators describe the origin, trajectory and peripheral action of the

efferent vestibular nuclei by working out the representation of individual endorgans within the medial vestibular nucleus. Their most important finding is that vestibular efferents exert a predominantly excitatory action on afferent discharge. This is contrary to the situation existing in lateral-line and auditory systems. Efferent effects are larger in thick vestibular afferents than in thin afferents. The former receive a post-synaptic efferent innervation, the latter a presynaptic innervation.

In a study of human postural sway trajectories, a grantee is studying characteristics of the human vestibulo-spinal control systems in the normal and vestibular-deficient human. Normal subject data from 200 adults were obtained and analyzed and 100 children from ages 8 to 13 years have been tested. He is comparing the findings with subjects having Meniere's disease, Canalicular acoustic neuromas, and unilateral cerebellar hemisphere lesions. The results from power spectral analyses show a clear quantitative differentiation between the two groups with peripheral lesions (Meniere's disease and acoustic neuromas limited to the internal auditory canal) and with the group with central lesion (cerebellar) by their disturbances of postural control.

In a research study on spatial organization of the vestibular nuclei in the cat, an attempt is being made to determine the locations of motoneurons projecting to each of the extraocular muscles and the location of vestibular neurons projecting to ipsilateral and contralateral extraocular nuclei. After receiving only limited results using antidromic stimulation of extraocular nerves and the recording of motoneuronal field potentials, this investigator revised his approach by mapping motoneuronal locations and now is using microstimulation to activate the motoneurons with EMG recording of the responses. In addition, a mathematical model was developed that describes the relative probability of detecting neurons using extracellular recording techniques as a function of cell size.

Hearing

A team of investigators are studying the psychophysical characteristics and underlying physiological processes of biological sensory systems including auditory, tactile and visual systems. Loudness summation of pairs of tone bursts, loudness as a function of tone duration, and intersensory summation studies have helped to identify methodological problems. By employing S. S. Stevens' modified method of magnitude estimation without designated reference standard, it is expected that absolute values of sensory magnitudes will be obtained. Verification of this hypothesis was shown by comparing loudness scaling and direct loudness matching experiments. However, effects of experience and stimulus range were not systematically investigated, and the intermodal validity of the method remains to be tested.

Because of the considerable potential for sensory research, this group undertook three extensive series of experiments concerning these questions. One of the series was concluded. In separate sessions, magnitude was obtained for brightness of white light, loudness of 1-kHz and 4-kHz tones, and subjective tactile intensity of 80-Hz vibration. In the first two series, the results obtained with magnitude estimation and force matching

were used to predict direct matches between the subjective magnitudes of the light and sound stimuli and of the sound and vibrotactile stimuli. Data indicates predicted values were within ± 2 dB of the directly measured values. This held for both individual data and group medians. The investigators interpret these results to prove that an individual subject can use the same numerical units for judging stimuli in different sense modalities. In addition, the investigators feel that the functional relationship between assigned numbers and sensory magnitudes is independent of sense modality. Experiments on additivity of loudness performed earlier in this project and others suggest that this relationship amounts to direct proportionality. In general, the results indicate that intermodal comparisons of subjective magnitudes can be made not only with respect to their rate of growth as suggested by S. S. Stevens, but also with respect to their absolute values.

Studies on the measurement of basilar membrane displacements in cats with the use of a laser interferometer are being continued. Cochlear microphonic measurements before and after surgical opening of the cochlea have revealed pronounced microphonic losses. The surgical technique altered by carefully drilling away the osseous shell and keeping the mesothelial lining intact until the perforation has the required size. This prevents bone dust from falling into the cochlea and eliminates the use of suction for its removal. The method of transferring the gold-crystal mirrors from storage onto the basilar membrane was also improved. This transfer can now be done with good reliability of success. An electrostatic drive system with built-in probe microphone (design: G. Sokolich) was also completed. Currently, cadaver experiments are done periodically to improve the surgical procedure. Live animal experiments should commence shortly. This group has also completed a study on the shape of the external ear canal directly in front of the tympanic membrance. They found that in a number of species of rodents and bats, the space is almost of capillary dimensions. As the next step, a model of this finding will be simulated by computer to determine its functional significance.

Continuing research on temporal factors in the masking of sound, has addressed detection and recognition, non-simultaneous masking, and basic intensity and frequency discrimination. Two basic studies employing extensive measurements on intensity and frequency limens reported that just-noticeable difference for intensity and frequency are a function of both sound pressure level and frequency. A group at the University of Chicago published a theorem that predicts a quantitative relation between detection and recognition performance. This theorem was tested in an auditory detection task involving a number of pure tones. The subject reported whether or not he believed the pure tone was presented (there may be no signal presented--just noise alone) and then was asked to guess the frequency of the stimulus tone. The researchers were able to predict with reasonable accuracy the subject's ability to identify the tone from the detection data.

In non-simultaneous masking studies, they investigated the growth of masking and the shape of the auditory filter, and compared simultaneous and non-simultaneous masking. Clear evidence for suppression was found in the non-simultaneous masking conditions. A final study of the temporal relation between the suppressor stimulus and the masker in both backward and forward masking is underway using an amplitude-modulated noise.

In an investigation of auditory masking in listeners with hearing loss due to cochlear (sensorineural) pathology, it was found that abnormally prominent upward spread of masking observed in these subjects provides insight into the nature of the underlying pathology. It also serves as a partial explanation of why impaired listeners report experiencing great difficulty understanding speech in the presence of competing background noise. These masking results have been incorporated into an audiological test for predicting speech perception handicap in noise. A hearing prosthesis is being developed, utilizing infra-red light transmission of the acoustical signal. Initial audiological evaluation of the device indicates that it has some qualities that are superior to the conventional hearing aids currently used by many listeners.

In another study, investigators sought to establish a relationship between abnormal spread of masking and speech intelligibility in noise. The purpose was to provide empirical confirmation of the well-known clinical observation that listeners with high-frequency sensorineural hearing loss have difficulties understanding speech in noise far beyond that which would have been predicted from clinical measures. This observation is closely related to the claim of hearing-aid users that they derive little benefit from their aid in noisy environments. The pattern of tonal masking as well as speech intelligibility in noise was evaluated on a sample consisting of listeners having normal hearing, presbycusis, noise trauma and ototoxic poisoning. In general, masked speech intelligibility thresholds for the impaired listeners were about 10 dB higher than for the normal control group which confirms the findings of earlier studies.

In an investigation of auditory neuropsychology and precursors of language, a team of investigators are studying the performance of both normal and brain damaged monkeys in regard to auditory temporal processing. They found that animals with left unilateral ablations of the superior temporal gyrus are deficient in their ability to remember the nature of a previously-heard sound. However, the deficit cannot be detected for monkeys with lesions of the same cortical area in the right cerebral hemisphere. Apparently, the deficit is one of retrieval, engendered by internal interference at the time of discriminative response. Normal (non-brain damaged) monkeys demonstrate unequivocally the processes of pro- and retroactive interference with memory when forced to remember a sequence of sounds. These phenomena are similar to those observed in verbal experiments with humans.

In an anatomical study of the internal ear, the investigator's goal is to contribute to the body of scientific information concerning comparative anatomy and evolution of the vertebrate inner ear. Investigations of the basilar and amphibian papillae in urodele amphibians, and of the papilla neglecta in the lizard, turtle and laboratory mouse show that the fine structure of the receptor is essentially like that in the anuran basilar papilla, except for a divergent polarization pattern of the sensory cells and the presence of efferent innervation in the urodele end-organ.

Other investigators are studying stimulus encoding in the auditory system of birds. The object is to determine how biologically relevant information is represented in the acoustic waveforms of an avian vocalization;

how this representation is transformed into a sensorineural representation at the various stages of the auditory system; and finally, how it is transformed into a measurable behavioral response. The significance of the bird studies lies in the potential insights they will produce for the processing of speech-like sounds by the central nervous system.

Language and Speech

Developmental Language Disorders. Ongoing research supported by the NINCDS is focusing on the early detection of language impaired children at the preschool age to allow for their treatment prior to entering school. Language screening procedures are being developed for use with Anglo, Black Dialect and Spanish speaking children. Further, language assessment tools are being developed for evaluating the skills of language impaired children and designing improved treatment methods.

Recent advances have been made in teaching language to mentally retarded and autistic children. Investigators supported by the NINCDS have determined that such children could often learn to associate concepts with visual symbols more easily than with spoken words. They found that language development could be stimulated by teaching such children signs to represent objects and activities with which they are familiar. Recently, it has been demonstrated that once communication is established, either with visual symbols in the mentally retarded, or with manual signs in autistic children, speech may emerge spontaneously.

A long-range goal of the NINCDS is to determine the etio-pathology of developmental language disorders not associated with mental retardation or deafness. This knowledge is needed for future prevention and treatment. Since few high quality researchers are presently addressing this problem, the NINCDS sponsored a symposium held at the NIH in January 1978 entitled, "The Neurological Bases of Language Disorders in Children: Methods and Directions for Research." This symposium drew together scientists from many different fields to address the problem of impaired language development and to determine which research techniques have the greatest potential for providing some answers. The proceedings of the symposium will be published by the NINCDS and disseminated free to neuroscientists to interest them in this area for research.

One language impaired population which has long been neglected is the autistic. A directed research program on infantile autism is being developed within the NINCDS. A first step will be to study the auditory perceptual skills of these children at different periods in their development and to determine if perceptual disorders are present which could account for their difficulties in developing receptive and expressive language.

The NINCDS supports experimental studies of the auditory, visual and perceptual skills of dyslexic, language impaired and speech impaired children. These investigations are aimed at determining whether relationships exist between the primary impairments of these groups of learning disabled children and their perceptual and cognitive skills. The information derived from this research will provide the knowledge base from which appropriate methods of diagnosis and treatment may be developed.

Adult Language Disorders. Significant advances have been made in the development of tools for the diagnosis and assessment of language disorders, aphasia following stroke, head injury or surgery. Investigators supported by the NINCDS developed a set of diagnostic tests which classify aphasic patients' language rehabilitation needs. Other tests measure the severity of patients' impairments in speaking and understanding relative to normal adults of the same age, sex and education.

Additional tests can be used to estimate the degree of language recovery which a patient would have during the first six months following the onset of aphasia due to a stroke. Such instruments are useful to the physician and family for planning a patient's rehabilitation program and eventual needs for assistance.

Although significant advances have been made in assessment, until very recently treatment of aphasia has not been promising and received little attention from researchers. In the past three years, work has begun on the learning and memory skills of aphasic patients, studies of their ability to regain speech through alternate communication modes and the development of new types of language therapy.

One group of investigators found that speech intonation is the aspect of language which is best retained by aphasic adults. Subsequently, they developed a treatment program using intonation as the basis from which to retain sentence production. They administered this treatment to patients who had been mute for at least six months following a stroke with aphasia. With this new approach, the patients regained fluent production of meaningful sentences in less than two months.

On an NINCDS directed research project, an investigator has developed a method for recording all the events which occur during treatment sessions with aphasic patients. The coding system provides records of which types of treatment techniques have the best results for improving patients' language skills. This system is already allowing the investigators to develop improved methods of treatment.

The NINCDS has also initiated research on the communicative needs of aging adults and aphasic patients. In an effort to determine the everyday needs of aphasic adults, patients living in chronic care institutions, as well as others living at home were observed in their daily lives. It was found that individuals who were aphasic and living in nursing homes or similar institutions were significantly more limited in their communicative ability in contrast with similar patients who continued to live at home. This research is being continued to examine the effects of aging, living environment, condition and mild hearing loss on the communicative competence of adults.

Speech Disorders. Recently, NINCDS grantees have developed new devices which allow experiments to track movements of the speech articulators in real-time. The output of such devices is digitized by computers and displayed visually allowing patients to observe representations of their articulator movements and self-correct their errors. Systems have been

developed which can continuously display the exact positions of the lips and the lower jaw as well as the points where the tongue makes contact with the palate, all at one moment during production of a speech sound. From such displays, the point at which an error occurred in speech production can be located. Although such systems are still in the development stage and only being used with patients in laboratory settings, they have been demonstrated to be far superior to traditional methods for teaching speech to the deaf, and providing patients with dysarthria with biofeedback for self-correcting their speech production.

Approximately three percent of all school children who are without dysarthria or hearing problems have difficulties developing speech articulation skills and require speech therapy. Recently, investigations have begun to determine the process by which normal children learn to perceive speech sounds, begin to imitiate them and consolidate their production of them--a process which is complete in normal children by age seven. If the bases for normal and disordered speech development could be determined, the prevention of such disorders could be attained.

Stuttering, which afflicts one million persons in the United States has long been the subject of a great deal of speculation. NINCDS grantees have recently discovered that during stutterers' speech blocks there is simultaneous and opposing contraction of the muscles which open and close the vocal cords. Additional research has also implicated poor laryngeal control as a basis for stuttering. The NINCDS plans to initiate research on the development of stuttering in young children to determine if certain characteristics of laryngeal functioning can be identified which are precursors to the development of stuttering in childhood.

Laryngeal Disorders. Since it is believed that with early detection and treatment, 90 percent of the cancers of the larynx can be cured, the NINCDS has recently initiated research aimed at developing methods for massive screening in high risk populations to detect early signs of laryngeal pathology. Investigators are evaluating at least three different recording devices and different analysis techniques to determine the most sensitive method for screening large numbers of persons for early signs of laryngeal pathology. In addition, NINCDS is collaborating with the National Cancer Institute to identify factors which are associated with a high incidence of laryngeal cancer. Such information is of importance for determining the cause of the disease and for defining which populations are most in need of screening programs.

Recently, computer based technologies have been developed by NINCDS grantees which provide methods for studying the physiological, anatomical and acoustic aspects of vocal pathologies due to laryngeal neoplasms, laryngitis, or neuromuscular disease. The NINCDS plans to bring together scientists and medical practitioners to develop a system of terminology for classifying voice disorders on the basis of several measurement criteria. The development of such a system is long overdue and necessary for developing standardized methods of diagnosis and assessment of these disorders.

Voice Prostheses and Rehabilitation. NINCDS grantees have refined new surgical reconstruction techniques which will provide voice production in patients who have undergone removal of the larynx as a treatment for laryngeal cancer. These techniques were first developed in Europe in the 1970's and allow patients to produce voice without needing to learn esophageal speech or use a prosthesis. Following removal of the larynx, the upper portion of the patient's trachea is stretched upwards and sutured to the base of the tongue. After six days of recovery, patients can begin to learn to regulate air flow and the amount of pressure necessary for voice. The learning process only takes a couple of days and depends mostly on practice. The only disadvantage to the procedure is that patients must learn a new method of swallowing. Although this technique can only be used with patients who have carcinoma confined to the intrinsic part of the larynx, it should eventually become common in this country, and reduce the number of persons who must rely on voice prostheses.

Noise Effects

Principal findings in a study of noise effects concern the relations between an anatomically determined loss of hair cells and behaviorally demonstrated loss of sensitivity following noise exposure. A comparison of the effects of exposure of animal models for two to nine days to an octave band centered at 500 or 4000 Hz, shows that while shifts in auditory threshold do not change between the second and the ninth day, hair-cell loss increased significantly during the same period. Further exposure at 65 dB SPL (500-Hz octave band) extended to 27 days revealed no acute injury in any of the ears. Higher-level exposure (95 dB, 500-Hz octave band) varied from 18 to 54 days show that the 18 day exposure was not different from the nine day exposure, but after 27 days there was clearly more basal-turn damage. It appears that a noise that produces no threshold shift will not produce any anatomical damage.

Another research group is continuing progress on the effects of noise on hearing and speech. In psychophysical perception and speech processing, earlier work on the discriminability of frequency changes in one component of a ten-tone pattern has now been extended to discrimination of changes in intensity and duration. All three stimulus dimensions appear to depend heavily on the uncertainty that characterizes the listener's task. Like frequency, duration is best discriminated near the end of such patterns and for high frequency target tones, but the dependencies on position in the pattern and on frequency range are not so clear for changes in intensity. Symphony musicians can indeed discriminate smaller frequency changes than non-musical listeners, and their excellent performance in detecting frequency changes in patterns is matched only by unselected listeners who have had long periods of training in psychophysical tasks.

Work is progressing on the analysis of cochlear microphonics. New measurement techniques permit work with lower sound pressure levels. Preliminary findings suggest the effect of the cochlear microphonic on responses of single units in the cochlear nerve may be similar to its effect on the whole nerve action potential.

Communicative Aids - Cochlear Prosthesis

The program of investigating non-human primate model potential of a prosthesis as a treatment for cochlear deafness has accomplished the following goals and technical aims: Further development of implant construction techniques, the completion of certain equipment requirements for behavioral studies, the development of additional software for on-line and off-line data analysis, and the further development of histopathological techniques. This team also acquired basic parametric behavioral data on threshold and dynamic range of hearing associated with specific electrode placement along the cochlear partition, e.g., variation in radial versus longitudinal placement and inter-electrode distance, and the form of electrical stimulation, including variation in intensity, frequency and waveform. They have acquired data concerning stability of hearing performance and data on intensity discrimination and frequency discrimination performance. Data was acquired on the form and intensity functions of the brainstem evoked response associated with electrode placement. They have also evaluated single unit responses at the inferior colliculus to cochlear stimulation in guinea pigs and monkeys at the termination of behavioral experiments. Data has been acquired on cortical evoked potentials in animals by cochlear stimulation. They have performed chronic inferior colliculus single unit studies and investigated stapedius reflex activity in monkeys with cochlear stimulation. In addition, an evaluation of a battery of vestibular tests were performed. Completion of normative data related to spiral ganglion cell counts in guinea pigs and monkeys was accomplished. These studies continue to provide the science base upon which further cochlear prosthetic work is being built.

In another study, a group of investigators seek to answer whether a well-considered multi-electrode stimulation of the auditory nerve in deaf persons becomes a better communication aid than either single-electrode or conventional hearing aid systems. They have just begun the anticipated transition from exclusively animal trials to human experimentation. If all goes well, animal work is behind them. If human work fails, they will go back to animal studies. On the basis of many years of engineering, hardware research and development and animal studies, they decided on human trials following previously outlined protocols. They performed the same pre-implant training and many of the intra-surgery stimulation experiments. One subject has all four electrodes functioning, and the other, three. One electrode in all of the subjects is, however, closer to vestibular fibers than auditory fibers. A second electrode produces auditory sensations, but at subthreshold intensities he receives pressure sensation with discomfort. At higher stimulus intensities the sounds produced by stimulating the electrode are unpleasant grating, screeching noises. Much additional work in this area will need to be supported before a viable cochlear prosthesis is possible to supplement the lack of auditory sensation in deaf or deafened individuals.

Olfaction

In a study on olfactory cortex, a research team did an analysis of the cells of origin of the association projections within the olfactory cortex, which has been completed with an analysis of the fiber projections which

arise in the olfactory peduncle. Several cell groups have been distinguished in the peduncle, based on their axonal connections and cytoarchitectonic structure. The projections of each of these cell groups to the olfactory bulb and to other parts of the olfactory cortex on both sides of the brain have been demonstrated using HRP as a retrograde axonal tracer. These results clarify the key position of the anterior olfactory nucleus and other areas within the peduncle in the olfactory system in mediating interaction between the olfactory bulb and more caudal parts of the cortex.

Touch

A group of researchers are investigating the role of the haptic system in communication. Progress has been made in four areas. Basic psychophysical studies, analysis of saltatory induction, dimensional analysis of tactile patterns and computer generation of tactile patterns. In the psychophysical area, work extended the effects of skin-contactor coupling on absolute thresholds for vibration in humans. By means of a modified chemical balance and an optical monitoring system, careful measurements of the relationship of contactor force, skin indentation, and absolute threshold for vibration were taken at two frequencies, 20 and 250 Hz, under several conditions of static surround. Findings were to some degree at odds with those in the current literature, and the conclusion was drawn that the complex mechanical impedance function for the skin requires the study of force as a possibly better measure of sensitivity than amplitude of vibration. These studies are important basic consideration to the use of vibratory and tactile prosthesis for the deaf and blind.

In regard to dimensional analysis of tactile patterns, previous studies involved the use of discriminative reaction time as the index of information processing capability, the present experiments requires the observer to discern the temporal order of sets of multiple stimuli to the skin. It is thought that adding redundant features to simple stimulus elements should improve the ability of the observer to perceive the order of the embellished stimulus elements, as demonstrated by an increase in the percent of correct responses for a constant stimulus onset interval. By examining the ability to identify correctly the temporal order of four stimuli as the stimulus onset interval was varied, it was thought that one could determine the effects of redundancy in coding if the tasks were performed with no redundant dimensions, with one such dimension, and with two such dimensions. In condition (a), only stimulus site was varied, i.e., four sites having the same frequency magnitude, duration, and onset time were presented in various orders to observers at different intervals, and judgments were obtained. For condition (b), each site had a different frequency, but constant loudness. Condition (c) had a certain frequency and given loudness. Results showed increasing redundancy does improve accuracy of temporal order by small but regular increments, with occasional reversals.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

VETERANS ADMINISTRATION HOSPITAL, MINNEAPOLIS, MINNESOTA (YO1-NS-4-0019)

Title: Development of a Research Tool Concerning Speech and Language Therapy for Aphasic Adults

Contractor's Project Director: Robert H. Brookshire, Ph.D.

Current Annual Level of Support: $0

Objectives: To develop descriptive and quantifiable systems for coding the content of speech and language treatment sessions with aphasic adults. Coding results will differentiate between various types of therapeutic approaches on the basis of differences in treatment content. The system is needed as a tool for conducting research contrasting the efficacy of various therapeutic approaches for the treatment of aphasia.

Major Findings: Two coding systems have been developed and demonstrated to be valid and reliable methods for recording the content of language rehabilitation with aphasic adults. One system contains 39 categories for recording the types of events, demands made on a patient, request complexity, the expected patient response, the patient's success and the feedback provided. This system can be learned by speech pathologists and research technicians following 24 hours of work with self instruction materials. Coders work with video tapes off-line to code the content of treatment. A short form contains only 26 categories and allows on-line coding of live treatment sessions.

Forty-five individuals were trained at seven different field evaluation sites; 23 were trained on the long system and 22 on the short coding system. Reliability coefficients were calculated for the long and short systems to examine site-by-site reliability, category-by-category reliability, and individuals' reliability. Reliability coefficients of coders in each of the sites were strikingly similar substantiating that the coding system is applicable for use in various aphasia treatment settings.

No consistent differences in reliability were observed between experienced and inexperienced aphasia clinicians for either the long or short system. No meaningful differences in category-by-category reliability were observed between field trial sites which were supervised or unsupervised during training. Acceptable mean category-by-category reliability was demonstrated by all field trial sites for the short system and for the long system with the exception of the category "Normal Response". This category fell slightly below the cut-off point for acceptable reliability (80 percent) for four of the six field trial sites.

Individuals using the long form were not only reliable on the events which they did code but they coded an average of eight to nine out of every ten events in the treatment samples.

On the short system, seventeen of the twenty-two coders met the criterion for acceptably sampling the contents of the treatment segments. In addition, it was determined that coding twenty percent of the events (every fifth event) provided an acceptable sampling of the contents of treatment sessions.

Significance to NINCDS Program and Biomedical Research: This new procedure will provide a needed research tool. Not only will it quantify and differentiate among therapeutic approaches, but also, it will provide a great deal of information on how variations in patient symptomatology, types of clinicians, and settings affect the treatment process.

Cooperating Units: None

Proposed Course of Contract: The contractor has completed the data collection phase of the field evaluation and will complete the data analyses and report writing over the next few months. The agreement has been extended without additional funds to allow the contractor time to complete revisions of the training materials for use in non-supervised training of coders who need to learn the system with adequate reliability. The Final Report will contain several research articles suitable for publication in recognized scholarly journals. The agreement will terminate in June 1979.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

CITY UNIVERSITY OF NEW YORK GRADUATE CENTER (NO1-NS-4-2323)

Title: Prescriptive Fitting of Wearable Master Hearing Aids

Contract Project Director: Harry Levitt, Ph.D.

Current Annual Level of Support: $83,323 (Completed January, 1978)

Objectives: The goal of this project was to determine the degree to which wearable master hearing aids (WMHA) used as prescriptive laboratory devices or modifiable training devices could enhance the final fitting of hearing aids and improve the communicative efficiency of the wearer.

Major Findings: The contractor performed the work in two stages. The first stage was primarily diagnostic in nature to attain the following objectives: a) to arrive at a combination of settings of the WMHA which gives good performance for the patient, and b) to provide information on the likely effects of changing the WMHA parameters. The contractor developed and standardized a test for measurement of communicative efficiency known as the Nonsense Syllable Test (NST). With this test, they successfully investigated the following WMHA parameters: (1) slope of the frequency response, (2) upper and lower cutoff frequencies, (3) saturation sound pressure level, (4) amplitude compression, and (5) type of clipping. Their results indicate that parameters (1) and (2) have a significant effect upon patients' NST scores. The second stage of the contract evaluated the WMHAs with a clinical population. An adaptive procedure was employed to arrive at an optimum setting of the WMHA for each patient. The patient's performance with the WMHA final prescription and his own conventionally fitted hearing aid were compared by means of several speech discrimination tasks. Several of these measures indicated that the WMHA provided superior speech perception for the contract population.

Significance to NINCDS Program and Biomedical Research: It is important to identify the electroacoustic parameters of hearing aids which significantly affect a person's communicative efficiency. This will provide direction for clinical treatment of hearing impairments which have been resistant to help from currently available amplification systems.

Cooperating Units: The prototype WMHAs were developed under contract to the Biomedical Engineering program, C&FR.

Proposed Course of the Contract: Further development of a prototype model is anticipated to include miniaturization and additional modification of frequency response slope and low frequency cutoffs. Following a developmental phase, clinical evaluation will be performed with the adaptive procedure from the current contract.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

UNIVERSITY OF FLORIDA (N01-NS-5-2313)

Title: Study of Auditory Sensitivity and Discrimination in Young Children

Contractor's Project Director: W. Keith Berg, Ph.D.

Co-Principal Investigator: Donald C. Teas, Ph.D.

Current Annual Level of Support: $84,200

Objectives: This contract was awarded to study auditory sensitivity and discrimination in children 0 to 6 years of age. The goal is the development and evaluation of a battery of tests which can be used to characterize the hearing ability of young children not suspected of having hearing deficits and to examine the feasibility of using such a battery to assess the hearing of infants and young children who are suspected of, or at-risk, for hearing dysfunction. Of particular concern are the developmental aspects of the hearing ability of this population. The following techniques for measuring sensitivity are being investigated: brain stem evoked responses (BSER), auditory suppression of startle blink responses, and modified conventional behavioral responses. The discrimination portion of this study will not be pursued under the current contract.

Major Findings: The adaptive behavioral technique has been assessed and found successful with children 3 to 6 years for the octave frequencies from 250 to 8000 Hz. BSER procedures are complete for infants up to one year of age and include one-third octave bands of stimulus presentation at five octave levels. The startle reflex measures are presently being refined but appear useful for children 0 to 3 years of age.

Significance to NINCDS Program and Biomedical Research: Procedures are needed for assessing the hearing of young children who are incapable of providing conventional responses. Without a battery of tests to assess hearing sensitivity at different developmental stages, evaluation of degree of impairment followed by treatment for this population is not possible at the present time.

Cooperating Units: None

Proposed Course of Contract: With completion of the development of measures of auditory sensitivity, the contractor is beginning to collect data for both cross-sectional and longitudinal studies of the target population. Three years are intended for this purpose following a Technical Merit Review in July, 1978.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

UNIVERSITY OF PITTSBURGH (NO1-NS-5-2317)

Title: Study of Estimators of Aphasic Patients' Communicative Performance in Daily Life

Contractor's Project Director: Audrey L. Holland, Ph.D.

Current Annual Level of Support: $99,777 (FY 78)

Objectives:

a) To develop a measure of communicative ability in daily life activities for use with aphasic adults,

b) To determine the validity of the new measure when compared to observer information and informant information on the communicative behavior of aphasic adults in their natural living situation,

c) To determine the validity of presently available tests of aphasic language impairment as estimators of communicative ability and performance in daily life, and

d) To conduct a field evaluation of the test of Communicative Ability in Daily Living to determine the range of communicative abilities among normal adults between the ages of 40 and 80: and, the effects of the following factors on communicative adequacy of adults with

- Wernicke's aphasia, Broca's aphasia, mixed aphasia and Global aphasia,
- moderate to severe hearing loss acquired after 21 years of age,
- moderate mental retardation since childhood,
- institutional living environment,
- aging (between 40 and 80 years) and
- educational level, sex and occupation.

Major Findings: The test of Communicative Abilities in Daily Living (CADL) was found to be a valid measure of aphasic patients' performance in everyday living. In comparison with three of the presently available tests of aphasic language, the CADL was found to be most highly related to observations of patients' communicative abilities. In addition, the CADL was most predictive of the superior communicative abilities of non-institutionalized aphasic patients living at home. Only the CADL had acceptable validity for assessing

the daily life function of patients living at home. The CADL was also found to have the highest construct validity for differentiating between patients with different types of aphasia in contrast with the PICA, Functional Communication Profile and the Boston Diagnostic Aphasia Examination.

Aphasic adults with the same degree of language impairments were found to differ in their communicative abilities dependent upon whether they were institutionalized or non-institutionalized.

Significance to NINCDS Program Biomedical Research: A measure of the degree and type of communication handicap is needed for evaluating the efficacy of various types of treatment for aphasic adults. Such a measure will indicate the degree of dependence of such patients on others for meeting their daily needs. The measure developed for assessing the degree of communicative impairment in aphasic adults will also be adapted to determine the degree of communicative handicap of adults with various types of hearing, speech and language disorders, and cognitive impairment and the effects of aging on communicative ability.

Cooperating Units: Memphis State University, Memphis, TN; The Veterans Administration Hospital, Pittsburgh, PA; Western Restoration Nursing Home, Pittsburgh, PA; Butler Veterans Administration Hospital, Butler, PA; Eye and Ear Hospital of Pittsburgh, PA; Rochester General Hospital, Rochester, NY; The Western Pennsylvania Gerontology Center, Pittsburgh, PA; VA Hospital, Minneapolis, MN; Holmes House, Pittsburgh, PA; Western Restoration Center, Pittsburgh, PA; St. Francis General Hospital, Pittsburgh, PA; VA Hospital, Madison, WI; and Concerned Care Inc., North Kansas City, Missouri.

Proposed Course of Contract: The contract has been extended for one final year to allow for field evaluation of the new measure. Over these twelve months, the measure will be standardized on aphasic adults and aging adults of various educational, socio-economic, age, sex, physical, ethnic, and living characteristics. In addition, the effects of hearing impairment, mental retardation and aging on communicative ability will be assessed. The contract will terminate in FY 79.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA (NO1-NS-5-2322)

Title: Measures of Children's Language Performance

Contractor's Project Director: Janice E. Laine, Ph.D.

Current Annual Level of Support: $0

Objectives: The objectives of this project include:

a) To develop a composite of measures of language performance in children which will assess small changes in the language performance of neurologically impaired children,

b) To prepare an administration and scoring manual which will train speech and language pathologists to be reliable examiners, and

c) To demonstrate that the composite of measures is both valid and reliable for assessing small changes in the language performance of children delayed in language development.

Major Findings: During the pilot study over 1,000 language performance items were administered to sixty language impaired subjects. Those 565 test items found to have the greatest validity and reliability for assessing language impaired subjects were selected for development of the Measures of Children's Language Performance (MCLP). The test is designed as a two-stage test; the Level Detector component and the Second Stage component which tests a subject at an appropriate level of difficulty. A subject's score on the Level Detector stage determines which of the subtests included in the second stage items --grouped into levels according to difficulty--the child should receive. This design effectively places a child at a level of language proficiency in each of the eight subtests. The eight subtests assess language performance in four different language systems, using both the receptive and expressive modes. Phonology is only tested in the expressive mode: expressive imitation has been included as a strategy to obtain a clearer assessment of the child's more complex syntactic features. The subtests are: Receptive Semantics, Receptive Syntax, Receptive Morphology, Expressive Syntax, Expressive Morphology, Expressive Imitation and Expressive Phonology.

The test has since been administered to another 70 language impaired subjects and item, subtest and component analyses of validity and reliability completed. Preliminary statistical analysis yielded measures of content validity, criterion related concurrent validity, and construct validity which were satisfactory.

However, inter-examiner and test-retest reliability were low and indicated a need for increased specificity in the test administration manual and examiner training materials.

Significance to NINCDS Program and Biomedical Research: Measures are needed for assessing change in language performance in neurologically impaired children. Those instruments which are presently available are based on language development in normal children and are not sufficiently fine-grained to be sensitive to the small changes that occur in the language impaired populations. This new research tool will be used in future research to determine the process by which language impaired populations develop language and the similarities and differences from the normal process of language development. This information is needed for developing treatment methods for language impaired chidren. Also, the measures will be used for investigating the efficacy of various types of treatment for different groups of language impaired children.

Cooperating Units: Los Angeles County Schools; Los Angeles City Schools.

Proposed Course of the Contract: The contractor is currently involved in analyzing the data from test and retest of two groups of children. Group A were tested on admission to a language training program in the fall of 1977 and retested in May 1978. These data will be analyzed to determine whether the MCLP is sensitive to small changes in language impaired subjects' language performance. Group B subjects were readministered the test within one month for assessing test-retest reliability. The data analyses will be completed and written up as journal articles by October 31, 1978. Also, the test administration and examiner training manual is being revised and will be submitted to the NINCDS upon termination of the contract on October 31, 1978.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

THE JOHN F. KENNEDY INSTITUTE (NO1-NS-5-2323)

Title: Study of Sensory and Perceptual Functioning of Young Children with and without Delayed Language Development.

Contractor's Project Director: Rachel E. Stark, Ph.D.

Principal Investigator: Paula Tallal, Ph.D.

Current Annual Level of Support: $225,488 (FY 78)

Objectives: The objective of the research is to conduct an experimental study of the sensory, perceptual and cognitive performance of different groups of children with specific learning disabilities including: impaired language development, dyslexia, verbal dyspraxia and normal controls. The study will determine whether there are any direct relationships between patterns of task performance on the one hand, and children's primary problem, on the other hand. In addition, the study will determine whether a particular level of performance on any of the tasks is associated with a certain degree of impairment in language development, reading or speech articulation.

Major Findings: The contractor has completed the first series of data analyses on the results of testing the language impaired and the normal control subjects. Contrasts between the language impaired and normal groups on the tests of auditory and visual perception have yielded the following results.

The language impaired subjects at all ages were severely impaired in contrast with normal controls on those auditory perception tasks requiring rapid processing of speech sounds. The language impaired subjects were also impaired in contrast with the normal controls on tests of visual perception although their difficulties were less marked than in the auditory modality. The language impaired subjects performed better on tasks requiring cross-modality integration of visual and auditory information than on similar tasks when only auditory information was provided. These subjects could perform speech perception tasks better when several redundant cues were provided simultaneously than when only single cues were provided for the discrimination of speech sounds. Thus the language impaired subjects' difficulties in speech processing were not due to problems with handling large amounts of information but rather due to difficulties discriminating rapid changes in signals.

In addition, the contractor has completed the following studies: a determination of the effects of language development on mental age test scores, the identification of test score criteria which are valid for selecting language impaired children based on language assessment procedures; and, examination of the relationship between speech perception impairments and speech misarticulations in language impaired children.

Significance to NINCDS Program and Biomedical Research: Many treatment programs of language impaired children have been based on the assumption that auditory processing difficulties are associated with impaired language development in many children. However, research results have been conflicting and have not always demonstrated that this is the case. There is need for a comprehensive study of the sensory, perceptual and cognitive abilities of each of these groups so that comparisons can be made across groups as well as with normal controls. If different performance characteristics in sensation, perception and cognition are found to be typical of different types of impaired children, such information will be directly relevant to improving the diagnosis, assessment and treatment of these children.

Cooperating Units: Howard County Public School System, MD; Dasher Green Cooperative Nursery; Columbia, MD; Chatsworth School, Baltimore County, MD; Baltimore County Public School System, MD; Baltimore City Public School System (the Woodhome School).

Proposed Course of the Contract: The contractor is continuing to conduct the analyses of the results of the language impaired and normal control subjects to determine which subject characteristics are related to visual and auditory perceptual impairments. Multivariate analyses will be conducted to determine whether age, language reception skills, language expressive ability and/or the speech production of language impaired and normal subjects are related to abilities in visual, auditory and oral stereognostic functioning. Identical sensory, perceptual and cognitive testing procedures are being administered to groups of children with dyslexia and speech dyspraxia. The data analysis for these groups begins in the late fall of 1978. The contract will terminate in 1979, following completion of the data analyses for all four groups and the submission of several reports in journal article format contrasting the perceptual and cognitive functioning of the four groups.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

SPECIAL SCHOOL DISTRICT OF ST. LOUIS (NO1-NS-5-2324)

Title: Possible Effects of Hearing Aids on Auditory Sensitivity in Children

Contractor's Project Director: Robert L. Huskey

Current Annual Level of Support: No Funds (Completed March, 1978)

Objectives: This contract had the dual objectives of (1) determining whether hearing aids, as traditionally fitted to young children, produce significant amounts of temporary threshold shift and should such shifts be observed in a child, (2) determining optimal strategies for simultaneously minimizing observed shifts while providing the necessary auditory amplification.

Major Findings: The contractor developed a protocol for measurement of relative threshold shift. Data were collected on groups of impaired children with mild and moderate losses who wore their hearing aids most of their waking hours. The results indicated that up to two years of wearing amplification did not significantly affect their thresholds for pure tones throughout the range of frequencies tested. Secondary findings have documented the use-gain of hearing aids worn by these children and the electroacoustic characteristics of these instruments over two years of use.

Significance to NINCDS Program and Biomedical Research: Continued use of hearing aid amplification has been hypothesized as a cause of permanent hearing threshold depression. This preliminary approach to the problem provides a model for evaluating the problem with a population of hearing aid users who demonstrate severe to profound hearing impairments.

Cooperating Units: None

Proposed Course of Contract: This longitudinal study was completed in March 1978. The results are being integrated into a future study of relative threshold shifts and hearing aid amplification with a severely impaired population of children.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

BOSTON UNIVERSITY MEDICAL CENTER (N01-HV-5-2971)

Title: Framingham Heart Study - Hearing Assessment of Subjects

Contract Project Director: M. Stuart Strong, M.D.

Current Annual Level of Support: $25,000

Objectives: The goal of this project is to assess the hearing sensitivity of subjects enrolled in the Framingham Heart Study and evaluate any observable relationship between sensorineural hearing loss and cardiovascular status.

Major Findings: Almost 400 of the 3500 subjects have been evaluated. Using the criteria of 40 dB HL at 250-3,000 Hz and 50 dB HL at 4,000 and 8,000 Hz in the poorer ear, the following groups have been identified: 1) normal hearing (34%); 2) borderline hearing (19%); and 3) hearing-impaired (47%). The contractor is continuing examination of the experimental cohort.

Significance to NINCDS Program and Biomedical Research: This group of subjects will provide a unique opportunity to relate hearing sensitivity and known cardiovascular findings. Vascular disease is known to be associated with some types of hearing losses but these studies have not had access to such a large and well-documented population.

Cooperating Units: National Heart Lung and Blood Institute, Biometry and Epidemiology Program of NINCDS.

Proposed Course of the Contract: Upon completion of this contract and the identification of those persons with significant hearing loss, an in-depth follow-up study will be conducted to determine etiology and degree of loss by a subsequent contract. Cardiovascular data will be used in the analyses.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

WAYNE STATE UNIVERSITY (NO1-NS-6-2353)

Title: Evaluation of Procedures for Screening Preschool Children for Signs of Impaired Language Development

Contractor's Project Director: Lynn S. Bliss, Ph.D.

Current Level of Support: $180,000 (FY 78)

Objectives:

1) To evaluate the validity and reliability of items contained in presently available language screening procedures for detecting preschool children with signs of impaired language. The validity will be determined when the instruments are administered by speech pathologists to English, Black dialect and Spanish speaking preschool children. Comprehensive speech and language assessments by senior speech and language pathologists using standardized measures will be the criteria for determining concurrent validity.

2) On the basis of the results, select procedures and/or items which are valid for screening preschool children for signs of impaired language development in English, Black dialect and Spanish.

3) To develop reliable instructional materials for training paraprofessionals to administer the language screening procedures.

4) To assess the validity and reliability of the selected screening items, when administered by paraprofessionals to English, Black Dialect and Spanish speaking preschool children.

5) To develop materials for distribution to health and educational service administrators, providing recommendations on how to implement and administer preschool language screening programs.

Major Findings: Data collection was completed on 570 children including: 250 Anglo speakers, 250 speakers of Black dialect, and 70 Spanish speaking children. Additional Spanish speaking subjects are presently being tested under a subcontract with the University of Arizona. Analyses of the data collected on Anglo and Black dialect subjects identified which items have adequate sensitivity and specificity for the selection of language impaired preschool children. These items form six different language screening instruments: one for Anglo children between the ages of 30 to 36 months, another for Anglos between 37 to 42, and a third for 43 to 48 months. Similarly, 3 different tests were developed for Black dialect speakers between 30-36 months, 37-42 months, and 43-48 months. Field evaluation of these six instruments will begin in July 1978 following training of the paraprofessional examiners on test administration to within adequate levels of reliability.

On testing 70 Spanish speaking subjects in San Antonio, Texas, difficulties were encountered in obtaining valid responses from subjects when screening instruments were administered only in Spanish. Most of the subjects attempted to respond in English whenever possible even though the examiners were native Spanish speakers from the same community. Therefore additional subjects are being tested on the same instruments both in English and Spanish by bilingual speech pathologists in Tucson. After these results are complete in September, data analysis will be conducted to select those items found to be valid and reliable for detecting language impaired Spanish speaking subjects.

Significance to NINCDS Program and Biomedical Research: Children impaired in language development cannot progress at the normal rate in school. If such children were detected at preschool age, treatment could begin prior to their entering school. Valid and reliable language screening procedures are needed for conducting language screening under the provisions of the Social Security Amendment to Title XIX supporting early periodic screening for children eligible to receive Medicare funds. Similarly, under the new Education for the Handicapped Act, 94-142, child "find" procedures are needed for identifying language handicapped children. This project was designed to meet these needs. The results will provide the procedures, examiner training materials and program organization for conducting preschool language screening.

Cooperating Units: University of Arizona, Tucson, Arizona

Course of Contract: Those Anglo and Black dialect procedures found to be valid and reliable are being field evaluated with administration by paraprofessionals to preschool children for detecting language impaired subjects. On completion of the continued pilot testing of Spanish and English instruments with Mexican-American children, data analyses will be conducted in the fall of 1978 to identify valid items for screening. Field evaluation of the resulting Spanish-English items will be conducted and data analysis completed in June 1979. Final reports will be submitted prior to expected termination of the contract early in FY 80.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

NAVAL RESEARCH LABORATORY (Y01-NS-7-0033)

Title: Feasibility of Use of Acoustic Analysis for Detecting Signs of Vocal Pathology

Contractor's Project Director: David C. Coulter

Current Annual Level of Support: $28,000 (FY 78)

Objectives: The goal is to specify equipment and methods for a rapid, high-patient-volume screening capability. In doing so, the contractor will meet the following objectives:

a) Determine the applicability of inverse filtering techniques for use as as pre-processor for larynx pulse enhancement from acoustic signals.

b) Evaluate several alternate sensor devices and metric and parametric transformations which appear most promising for large scale screening based on pilot testing with pathologic samples.

c) Determine the most desirable characteristics for the microphone(s), recorder, and environmental setting to be used when recording.

d) Specify which items should be recorded when testing, for immediate screening decisions and for building data bases to provide normative information on various subpopulations.

Major Findings: During the current year, the contractor has had the following results:

a) The accelerometer and laryngograph have been found to have good potential for use in screening for laryngeal pathology since they are portable devices which can be used in any acoustic environment regardless of high noise levels. Both instruments provide stable pick-up of laryngeal signals.

b) Replication of the results of Dr. Steven Davis of the Speech Communications Research Laboratory using inverse filtering techniques to pre-process the laryngeal pulse with autocorrelation techniques to identify degree of jitter have been disappointing. Laboratory tapes of different degrees of jitter have not been well differentiated using these techniques. Further modifications are being made to determine how this system's accuracy might be improved.

c) To process the signals from laryngeal sensors including the accelerometer, the laryngograph and the throat microphone, a jitter detector has been developed which can differentiate between different degrees of jitter with accuracy. Since the use of such a system seems to have greater sensitivity to jitter and these sensor instruments are more stable for use in high noise environments, at this point the preferred method of screening does not seem to be acoustic analysis.

Significance to NINCDS Program and Biomedical Research: The prognosis for survival from laryngeal cancer is significantly improved (close to 90 percent survival beyond five years) with early detection. Figures published by the National Cancer Institute indicate that laryngeal cancer is increasing in incidence in recent years and that certain portions of the populations are at high risk of developing the disease which can be fatal unless detected in the early stages. The immediate need for a method of screening to detect persons with early signs of laryngeal disease was recognized in 1973, at a conference supported by the NINCDS. The results of this feasibility study are needed to determine the equipment specifications for developing a method of screening for early signs of laryngeal pathology.

Cooperating Units: None.

Proposed Course of the Contract: This interagency agreement is for two years and will terminate in May 1979, following the submission of a report providing the pilot study results and the specifications for constructing a screening system.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

MINNEAPOLIS MEDICAL RESEARCH FOUNDATION, MINNEAPOLIS, MINNESOTA(NO1-NS-7-2378)

Title: A Comprehensive Study of the Language Recovery Process in Adults with Aphasia Following a Cerebrovascular Accident

Contractor's Project Director: Alan B. Rubens, M.D.

Current Annual Level of Support: $272,500 (FY 78).

Objectives: The purpose of the research is to develop increased understanding of the neurophysiological and behavioral bases of the language recovery process in aphasic adults, during the first six months following a CVA. This study will determine the following:

a) the feasibility of predicting the outcome of aphasia from information concerning the size and location of brain pathology, cerebral blood flow and neurophysiological activity occurring in each hemisphere,

b) whether changes occurring during the first six months following onset in the location and size of brain pathology, cerebral blood flow and neurophysiological activity of either hemisphere, are associated with the degree of language recovery,

c) whether changes occurring during the first six months in the pattern of performance on dichotic tests of speech perception are associated with the degree of recovery from aphasia, and

d) whether changes occurring in patients' verbal learning/memory deficits during the first six months following the onset of symptoms are associated with recovery from aphasia during the same period.

Major Findings: During the first phase of the research the contractor has developed procedures for studying changes in aphasic patients using the following:

a) studies of regional blood flow at equivalent points over the two hemispheres during language and non-language functioning,

b) measures of size and location of brain pathology from CT scans administered within a few days following admission, between 14 and 20 days post onset and at six months post onset,

c) tests of cognition which do not require normal linguistic ability for successful performance,

d) tests of language performance,

e) methods for studying patterns of performance on dichotic tests of speech performance, and

f) methods for studying patterns of verbal learning/memory deficits.

Normal volunteers and aphasic adults have been pilot tested on all procedures except #b (the CT scans) to determine intra-test form equivalency, retest reliability, testing time and the validity of the measures for differentiating between normals and aphasic adults with moderate language performance deficits.

Significance to NINCDS Program and Biomedical Research: The process of language recovery in aphasic adults is not well understood. Recovery is most rapid during the first nine weeks following the onset of symptoms--when the remission of diaschisis is most likely to occur. Recovery which occurs following this period may be attributed in part to language relearning or the acquisition of compensatory methods of communication.

The size and location of brain lesions, the regional blood flow in the dominant hemisphere and the physiological response of each hemisphere during verbal behavior will be reassessed throughout recovery to determine the association between dominant hemisphere status and level of recovery from aphasia. If recovery is not highly associated with changes in the left hemisphere, and the right hemisphere is found to be involved in verbal functioning, both the right and left hemispheres may be involved in language recovery following a CVA. The results will be useful for determining appropriate approaches for developing effective treatment techniques.

Cooperating Units: University of Minneapolis Medical Center.

Proposed Course of the Contract: During the second phase(3 years), 60 aphasic adults will be studied during the first six months following the onset of aphasia.

Following the first two years of data collection, a Technical Merit Review of the contractor's progress will be held as a site visit. The NINCDS will then determine the length of time that subject testing should continue to provide sufficient data for examining each of the hypotheses statistically. Phase II of the research will be completed when these requirements have been met satisfactorily.

The final phase of the research(6 months) will be required for data analysis and report writing.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

UNIVERSITY OF ILLINOIS (N01-NS-7-2380)

Title: Evaluation of a Test of Speech Perception in Noise

Contractor's Project Director: Robert C. Bilger, Ph.D.

Current Annual Level of Support: $90,000

Objectives: The purpose of this contract is to conduct experimental work to determine the inter-list equivalency, performance by signal-to-babble (S/B) functions, and validity of the Speech Perception in Noise (SPIN) Test.

Major Findings: Preliminary work is establishing the inter-list equivalency for all 10 recorded forms of the SPIN Test on a large sample of hearing-impaired subjects. Stimuli are being presented at a sensation level that corresponds to that afforded a normal-hearing subject for an overall level of 60 dB SPL. Half of the subjects are receiving earphone and half are receiving sound-field presentation. In addition, half are evaluated in one test session and the other half in two sessions separated by several weeks. In addition, subjects are being tested non-auditorily to assess their ability to use semantic and redundant cues in ascertaining the final words of highly predictable sentences. Analyses of the data will provide a group of lists to be used in the following phases of this contract.

Significance to NINCDS Program and Biomedical Research: Assessment of supra-threshold speech perception in noise would provide a valuable tool for the practicing clinician in managing hearing-impaired patients. Hopefully, the SPIN Test may also be employed as a predictive measure of the degree of benefit that persons with acquired sensorineural hearing loss may appreciate from a properly selected hearing aid.

Cooperating Units: None

Proposed Course of Contract: Completion of Phase I (described above) should be completed this fiscal year. Phases II and III will follow in progression.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
October 1, 1977 through September 30, 1978

CHILDREN'S HOSPITAL OF PITTSBURGH (N01-NS-8-2384)

Title: Evaluation of Decongestant Therapy for Otitis Media with Effusion

Contract Project Director: Charles D. Bluestone, M.D.

Co-Principal Investigator: Jack L. Paradise, M.D.

Current Annual Level of Support: $100,746

Objectives: This project is a tightly controlled double-blind clinical trial of the efficacy of antihistamine-sympathomimetic amine drug therapy for the treatment of otitis media with effusion. As a prospective study the work will also provide valuable data on the natural course of and key variables in etiology of otitis media with effusion.

Major Findings: This contract has just been awarded. However, substantial data has already been produced on the ability to establish interobserver and interobservation reliability in the assessment of middle-ear effusion. The contract is currently in a pilot phase.

Significance to NINCDS Program and Biomedical Research: Twenty to fifty percent of the children in the U.S. are afflicted with otitis media at some time. The disease, if neglected, can progress to chronic and serious sequelae. In its acute, subacute and chronic forms, otitis media causes hearing loss in the developing child. The first line of treatment usually involves treatment with decongestant drugs. However, there is no solid scientific data that indicates that these drugs, dispensed each year by the thousands of gallons, are efficacious. This controlled research is designed to provide a definitive answer to whether or not these drugs are useful. It will also provide valuable information about the disease itself.

Proposed Course of the Contract: The study is expected to take three years to complete, depending primarily on the rate of acquisition of randomized subjects.

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 02185-04 CDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Characteristics of Dysarthric Speech Associated with Neurologic Disease

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

P.I. C. L. Ludlow, Speech Pathologist, CDP, NINCDS
C. B. Cardano, Speech Pathologist, CDP, NINCDS
D. B. Calne, Clinical Director, IR, NINCDS

COOPERATING UNITS (if any)
IR, NINCDS

LAB/BRANCH
Communicative Disorders Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.25	.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) The long range goal is to determine the characteristics of different speech production disorders, dysarthrias, associated with various neurological diseases. Objective testing, analysis and measurement procedures have been developed and demonstrated to be valid for differentiating the speech production of normal aging adults from that of patients with Parkinson's disease. In addition, these assessment procedures have been demonstrated to be valid measures of changes in speech symptoms associated with hypokinesia and drug induced dyskinesia in Parkinson's disease, Shy Drager's Syndrome, Tardive Dyskinesia and Huntington's Chorea.

The testing procedures include calibration controls and a uniform testing format. Acoustic analyses include spectrographic tracings and graphic sound intensity level recordings. Ongoing studies include: a) A comparison of acoustic and perceptual methods for assessing dysarthria, b) the effects on the on-off phenomenon on speech production in Parkinson's disease, and c) the acoustic characteristics of laryngeal pathology in Shy Drager's Syndrome.

Project Description:

Objectives:

1) To develop valid and reliable methods for determining and assessing the speech production problems of patients with neurologic disease.

2) To determine the speech production characteristics of patients with neurologic disease in contrast with those of normal aging adults.

3) To evaluate the effects of L-Dopa and Bromocriptine on the speech of Parkinson patients, and

4) To determine the speech production disorders associated with Shy Drager's Syndrome, Huntington's Chorea, and Tardive Dyskinesia.

Methods Employed: Identical testing conditions are maintained by presenting the task instructions, calibration tone settings and models from a stimulus tape recording presented at the same intensity level. Speech recordings are made on tasks of extended phonation, loudness and pitch variation, pause and rate control, and rapid speech initiation. Measurements are made from the 34 sound spectrograms and graphic level recordings.

Subjects: Patients with Parkinson's disease were examined at two-week intervals over a six month period in a double-blind study of changes with administration of L-Dopa versus Bromocriptine. Normal aging adult volunteers were examined by the exact same procedures. In addition, patients with Huntington's Chorea have been tested without medication.

Major Findings:

A. Both the acoustic and perceptual methods were found to be valid for differentiating dysarthric from normal speech. However, inter-rater reliability was low on five of the seven discriminating perceptual dimensions. On within patient contrasts between the two states of hypokinesia and dyskinesia only the acoustic method was void. Thus the acoustic method should be used when assessing the degree of speech impairment in dysarthric patients.

B. The speech production characteristics of patients with Parkinson's disease differed significantly from those of normal aging controls in the following ways:

1) Average fundamental frequency of vocal cord vibration production was significantly higher as well as reduced in degree of variation in patients.

2) The ability to offset phonation was significantly impaired in the Parkinson patients, due to an inability to cease phonation at the end of syllables indicating impaired laryngeal abductor muscle control.

3) During drug induced dyskinesia, patients' laryngeal control was improved although not to the normal level. In particular, dyskinesia reduced mean fundamental frequency, increased variation in fundamental frequency and improved control of phonation cessation during speech. However, during dyskinesia the rapid onset of lingual articulation which requires fine motor control was worsened. Therefore, drug induced dyskinesia was found to improve patients' range of motion but disrupted very fine motor control.

Significance to NINCDS and Biomedical Research: The development of objective procedures for the assessment and differential diagnosis of various types of dysarthria in adults with neurological diseases, will enable researchers and clinicians to evaluate different treatments for these patients. The information being gained on which speech production processes are particularly impaired in each of the neurological diseases, will contribute to our understanding of both normal and disordered speech production as well as providing directions for treatment.

Proposed Course of Project: The following manuscripts are in various stages of preparation for publication, "The Effects of the On-Off Phenomenon on Speech Production in Parkinson's Disease," C. L. Ludlow, G. Geoffrey, R. Kartzinel and D. Calne; "A Comparison of Acoustic and Perceptual Methods for Assessing Dysarthria," C. L. Ludlow, C. B. Cardano, and B. L. Cullison. The later paper will be presented at the 1978 Annual Convention of the American Speech and Hearing Association.

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 ZO1 NS 02247-02 CDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
The Characteristics and Treatment of Vocal Tics and Gilles de la Tourette's Syndrome

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

P. I. C. L. Ludlow, Speech Pathologist, CDP, NINCDS
other E. D. Caine, Staff Fellow, IR, NIMH
M. H. Ebert, Chief, Section on Experimental Therapeutics, IR, NIMH
C. B. Cardano, Speech Pathologist, CDP, NINCDS

COOPERATING UNITS (if any)
Section on Experimental Therapeutics, NIMH

LAB/BRANCH
Communicative Disorders Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.25	.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)

A series of research studies are being conducted to determine the following:

1) Whether Speech and Language Disorders are associated with the type and severity of vocal tic phenomena in Gilles de la Tourette's Syndrome.

2) The characteristics of vocal tic phenomena in Gilles de la Tourette's Syndrome.

3) To determine the differential effects of various neuropharmacological treatments on the type and severity of vocal tics in patients with Gilles de la Tourette's Syndrome.

4) The relationship of vocal tic production to propositional language behavior.

The following is a description of the results of the first two projects in this series. Work is currently ongoing on the next 2 objectives.

Purpose and Procedures: The purpose of these studies of patients with Gilles de la Tourette's syndrome were to characterize their vocal tics and to determine whether disorders of language and speech are associated with these phenomena. Thirteen subjects meeting the five diagnostic criteria of Shapiro et al. (1976), were admitted for study. None received medications for at least two weeks prior to examination. Standardized tests were employed to assess: muscle power of the lips and tongue; oral and lingual praxis; speech articulation; and rate of repetition of oral and speech movements. Seventeen subtests of the Neurosensory Center Comprehensive Examination for Aphasia, NCCEA (Spreen and Benton, 1969) were administered. A head set microphone was used to record speech during picture descriptions, oral reading and while communicating to a listener behind a screen on how to construct a block design. These three tasks were performed under three different test conditions: one without competing stimuli, and during two speech stressor states; binaural presentation of white noise (WN) and binaural presentation of delayed auditory feedback (DAF).

Results: Subjects' tapes were analyzed to determine the rate and place of occurrence of vocal tics and dysfluencies during speech and silence. Seven categories of vocal tics were found: respiratory types including belches, quick inhalations, and exhalations; laryngeal squeaks, barks, and hums; lingual clicks; nasal snorts and sniffs; labial smacking and popping; and verbal tics including partial and complete words and phrases of coprolalia. These categories were found to represent a continuum across subjects, depending on the kinds of tics each subject exhibited. The subjects with only three types of tics had lingual, laryngeal and respiratory tics, while those with four kinds of tics also had nasal tics. The patients with five tics produced coprolalia in addition to all preceeding tic types, while only those with six and seven tic types had labial tics and jargon.

The place of occurrence of vocal tics was tabulated from each subject tape. For all subjects most tics occurred on the initiation of speech and/or sentences or at constituent boundaries. Graphic level recordings and speech spectrograms confirmed that tics occurred when speech intensity level and fundamental frequency decreased. These findings indicate that tics appear during breathing for speech and speech pauses.

During both of the speech stressor conditions (WN and DAF), the rate of vocal tics increased in some subjects, while it decreased in others. However, speech dysfluencies decreased during WN in all subjects and increased during DAF in all subjects except the three who stuttered. The differential response of subjects' tic productions to stressor stimuli may indicate differences between subjects with Tourette's Syndrome.

All subjects scored greater than two standard deviations below their age level on two or more NCCEA verbal subtests. The number and severity of subtects' verbal deficits were positively related to the number of different tic categories they exhibited. Each of those subjects exhibiting all types of tics, including jargon, were severely impaired in the construction, repetition, and comprehension of sentences, word finding, reading, and writing. Conversely, the subjects with only lingual and laryngeal tics only had deficits in word finding, word fluency, and sentence repetition.

These results indicate that patients with Gilles de la Tourette's Syndrome demonstrate a continuum of linguistic impairment with the kinds and severities of their vocal tics related to the severity of their language performance deficits. A linguistic analysis of this disorder provides an objective and valid method for investigating patient characteristics and assessing treatment efficacy.

Proposed Course: The above findings were presented at the American Speech and Hearing Association Annual Meeting in Chicago in November, 1977 under the title, "Characteristics of Vocal Tics in Gilles de la Tourette's Syndrome," (with E. Caine and M. Ebert).

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 02336-01 CDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Early Identification of Drug-Induced Ototoxicity

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

PI:	Earleen Elkins	Audiologist	CDP	NINCDS
Other:	Pamela Mason	Audiologist		

COOPERATING UNITS (if any)

Radiation Oncology Branch, NCI
Division of Cancer Treatment, NCI

LAB/BRANCH
Communicative Disorders Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.80	.05	.75

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)

Certain pharmaceutical agents used in the treatment of cancer are known to have toxic effects upon the hearing of patients. Cochlear damage is manifested by a high-frequency hearing loss and general difficulty in understanding normal speech conversation. Periodic assessment of puretone thresholds and suprathreshold speech perception are being conducted to evaluate and relate degree and progression of ototoxicity to drug dosage and frequency of administration.

Project Description: Z01 NS 02336-01 CDR

Objectives: To develop techniques for the assessment of ototoxicity related to the administration of certain chemotherapeutic agents employed in cancer treatment. The following areas are being addressed: a) type of hearing loss, b) onset and degree of loss relative to dosage and preexisting hearing condition, c) documentation of onset of tinnitus and/or vertigo, d) predisposition to ototoxic effects, e) unilateral/bilateral symmetrical loss, f) suprathreshold speech perception, g) prediction of probable ototoxicity, and h) possible reversibility of ototoxic effects.

Methods Employed: In addition to routine procedures, monosyllabic speech stimuli are presented at various signal-to-noise ratios (S/N) and at different levels above puretone or speech reception threshold levels.

Major Findings: Preliminary data on 32 subjects indicate that a +10 dB S/N using consonant-nucleus-consonant monosyllabic stimuli did not significantly degrade their speech perception when compared to normal performance on the same task. More difficult S/Ns are being explored. Multiple correlations of all measures are being performed in an attempt to develop a predictive profile of patients particularly suseptible to ototoxic effects of the drugs under study.

Significance to NINCDS and Biomedical Research: The development of repeatable and valid methods of assessing hearing impairment following the administration of known drug dosages would provide the clinician with documentation for the managing physicians who in turn may reassess their drug protocols. A reliable method of predicting ototoxicity is definitely needed if prevention of impairment is to be realized.

Proposed Course of Project: Further investigation will be given to the problems and procedures identified above with a patient population available through cooperation with the National Cancer Institute.

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 ZO1 NS 02337-01 CDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
The Effects of Dextroamphetamine on the Communicative and Language Skills of Hyperactive Subjects

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

P. I. C. L. Ludlow, Speech Pathologist, CDP, NINCDS
J. L. Rapoport, Psychiatrist, IR, NIMH
G. L. Brown, Psychiatrist, IR, NIMH
C. B. Cardano, Speech Pathologist, CDP, NINCDS

COOPERATING UNITS (if any)

Section on Experimental Therapeutics, NIMH

LAB/BRANCH
Communicative Disorders Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50	.25	.25

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 the research is to determine whether dextroamphetamine--a stimulant drug treatment--has a beneficial effect on the language performance and communicative skills of different groups of normal and learning disabled boys: hyperactive boys with impaired language development, hyperactive boys with normal language development and impaired communicative skills and normal boys with superior language and communicative skills.

Dextroamphetamine was found to have a beneficial effect on the language and Communicative skills of all three groups studied, although the effects differed in each group. The group most benefited by drug administration were the normal subjects whose task directed communicative speech increased in fluency and complexity. Both hyperactive groups were benefited. Those impaired in language development increased most in their linguistic complexity and language task performance while those only impaired in communication were only aided by a decrease in their non-task directed speech. Their task directed communicative speech fluency was not increased as in the normal subjects.

Project Description: Z01 NS 02337-01 CDP

Objectives: The purpose of the research is to determine whether dextro-amphetamine--a stimulant drug treatment--has a beneficial effect on the language performance communicative skills of different groups of normal and learning disabled boys: hyperactive boys with impaired language development, hyperactive boys with normal language development and impaired communicative skills and normal boys superior language and communicative skills.

Subjects: Subjects were accepted for study in two hyperactive groups on the basis of their admitting diagnosis of hyperactivity, CTRS scores and standardized language test scores (the Illinois Test of Psycholinguistic Abilities and the PPVT). Twelve normal boys constituted a control group (Group One). The Group One subjects and Group Two subjects (12 hyperactive boys) scored within the normal range on the language tests while Group Three subjects (8 language impaired hyperactive boys) scored in the impaired range and had a history of delayed language development.

Methods: Subjects were trained on three language sampling tasks--picture description, story telling and a communication task requiring instruction of a "blind" listener on block design construction. On the two experimental days, either d-amphetamine (.5 mg./kg.) or placebo was administered in counter-balanced double blind fashion. The speech samples were transcribed and the frequency of the following language behaviors relative to one minute of the child's speech was computed: task directed speech, descriptive speech, complex sentences, grammatical sentences, child initiated speech commands, questions, refusals, echolalia, stereotypic speech and perseverative speech. Language complexity measures included mean length of utterance, grammatical complexity and speech rate. Measures of communicative speech included the proportion of task directed commands, descriptive task directed statements and story telling length.

Major Findings: Although Group One and Two subjects had similar scores on verbal tests, Group One subjects were superior to Group Two on linguistic complexity measures on placebo. The normal subjects had a greater mean length of utterance, speaking rate and grammatical speech rate. With dextroamphetamine these aspects of expressive language did not increase in either the normal or Group 2 subjects. The Group 2 subjects were also impaired in communicative skills in contrast with the normal subjects. These hyperactive subjects produced more interruptive and child initiated speech, less task directed speech, had a slower speech rate and markedly shorter stories. Medication had similar beneficial effects on Group One and Group Two subjects. In Group Two, subjects' interruptive and child initiated speech decreased while speaking rate increased. In the normal subjects, non-task directed speech, such as questions, decreased on the drug while story telling length, (task directed speech) increased.

On medication, the hyperactive subjects in Groups 2 and 3 significantly increased in their expressive language skills. Also, their disruptive and noncommunicative speech (echolalia, questions and perseverations) decreased while the frequency of production of complete and longer grammatical sentences increased. However, differential effects were found between the hyperactive subjects with and without language impairments. The language impaired subje

showed the greatest increases in linguistic skills on medication; their mean length of utterance increased significantly while there was no increase in Group Two subjects even though they were impaired on this measure in contrast to the normal subjects. With medication, Group Three subjects, the language impaired hyperactive children, were those with the smallest improvements in their behavior (on the ABCTRS) even though they initially had the highest CTRS Factor I scores.

Dextroamphetamine was found to have beneficial effects on the language and communicative skills of all three groups studied, although the effects differed in each group. The group most benefited by drug administration were the normal subjects whose task directed communicative speech increased in fluency and complexity. Both hyperactive groups were benefited. Those impaired in language development increased most in their linguistic complexity and language task performance while their communication was only aided by a decrease in their non-task directed speech. Their task directed communicative speech fluency was not increased as in the normal subjects.

Significance to NINCDS and Biomedical Research: The efficacy of stimulant drug therapy for different groups of learning disabled children is an issue of national significance. The finding of a differential response to such treatment is dependent upon the language, communicative and behavioral characteristics of importance to physicians and therapists responsible for treating these children.

Proposed Course of Project: This project has resulted in two publications in the last year as follows:

Rapoport, J. L., Buchsbaum, M.S., Zahn, T. P., Weingartner, H., Ludlow, C. L., & Mikkelsen, E. G. Dextroamphetamine: Cognitive and behavioral effects in normal prepubertal boys. Science, 1978, 199, 560-563.

Ludlow, C. L., Rapoport, J. L., Cardano, C. B., & Mikkelsen, E. G. Differential effects of dextroamphetamine on language performance in hyperactive and normal boys. In R. M. Knights and D. J. Bakker, (Eds.), Rehabilitation, Treatment and Management of Learning Disorders. Baltimore: University Park Press, in press.

ANNUAL REPORT
October 1, 1977 - September 30, 1978
Fundamental Neurosciences Program
National Institute of Neurological and
Communicative Disorders and Stroke

The Fundamental Neurosciences Program (FNP) provides broad support of basic research in the neurosciences through Individual Research Grants, Program Project Grants, Training Grants and Contracts.

Grants

It has been estimated that about 70% of all NINCDS Research Grants are truly fundamental in nature and some of these, because they relate more or less directly to specific disease categories, are supported by the other three Extramural Programs.

The number of grants currently assigned to the FNP are: 350 Individual Research Grants; Nine Program Project Grants; 22 Research Career Development Awards and no Teacher Investigator Awards. The 359 FNP grants compare with a total of 1,355 for all four Extramural Programs, or 26%.

Funds allocated to support FNP grants were $21 M in FY 78 or 19% of funds allocated to all extramural grants.

During FY 78, 307 competitive grant applications (Types I and II) were assigned to FNP. Of these 268 or 87% were approved by Study Section and 111 were funded, for a funding rate of 41% of approved grants. This is in sharp contrast to the 19% funded by FNP in FY 77.

About 30 applications were brought up for Special Consideration by the Advisory Council during the year. Of these seven were for approval of 4 or 5 year awards as recommended by Study Section, and 27 were funded in FY 78.

Policy Recommendations

The current assignment of grants to FNP is much smaller than the number of grants which are truly fundamental in nature. This practice has arisen in an attempt to show a larger investment of research in specific disease categories at the expense of basic research. Thus, consideration of the size of the FNP in relation to other extramural programs gives a completely false picture of the NINCDS effort in basic research.

Last year, over 2,000 neuroscientists wrote to their Congressmen in support of basic research. Narrative Statements from Congress, speeches by President Carter, Secretary of HEW Califano, and the Director of NIH,

Dr. Fredrickson, all have spoken out in favor of basic research and have urged an increase not only in dollars but in percentage of funds devoted to basic research.

FNP urges that the misrepresentation referred to above be rectified by increasing the number of basic research grants assigned to FNP. This would not only tend to correct the false impression given by the current assignment ratio, but would appear to satisfy the demands for more basic research.

More important than numbers of grants assigned to FNP is the level of dollar support. The average quality of FNP grants, as indicated by Study Section priority scores, is considerably higher than that of the other programs while the funds allocated to FNP grants are not proportionately higher than in the other programs. As a result, it is a distict disadvantage to a grantee to have his grant application assigned to FNP. This is basically wrong and should be rectified. Basic research continues to be the most cost effective investment of research funds.

The sudden payline cutoff of grant support from full funding of grants one point better than the payline to nothing for grants one point below payline is unfair. The "noise" in priority scores makes all grants within 25 points essentially equal in scientific merit.

Various practices have been used in the past in attempts to alleviate this "sudden death" phenomenon, such as funding only of salaries for a terminal year, etc. However, these have been dropped as funds got tighter. A solution which would not cost more would be to establish a "pay zone" rather than a payline. Grants with priority scores near one edge of the "pay zone" would receive nearly the full budget recommended by Study Section, while less good grants at the other "edge" would get only minimal support. While this scheme would be a little mor difficult to administer, it would alleviate a great deal of suffering due to the present abrupt termination of research--much of it very good research. The increase of flexibility of grants management thus provided would improve the effectiveness of our grant program.

Program Project Grants

Current policy in NINCDS on the advice of the Council calls for phasing out Program Project Grants in three years with a shift of the component projects to individual research grant support. Approximately four such Program Project Grants of long standing have been phased out by FNP during FY 77. This will terminate some of the most fruitful, multidisciplinary team research in the country. Large, modern research undertakings are requiring the cooperation of more and more specialists from different disciplines. Such research projects often cannot be broken down into individual unidisciplinary sub-projects which are successful in competing for scarce funds. There are many cases in which "the whole is more valuable than the sum of its parts." Also, where expensive core equipment is required, its cost usually cannot be divided up among a number of individual grant applications, if only one in five is funded. NINCDS policy on Program Project

Grants should be modified to permit continuation of at least the very best of such teams.

Program Announcement: Local Neuronal Interactions

In the Annual Report for 1976-1977, the subject of "Local Circuit Neurons" was proposed as a particularly promising area for research. This year, after considerably consultation with many neuroscientists working in this and in related fields, and after thorough discussion with our FNP Scientific Advisory Board and NINCDS senior staff, it was decided to issue a "Program Announcement" indicating our interest in, and encouragement of, grant applications in the field of Local Neuronal Interactions. The text of this announcement follows:

ANNOUNCEMENT

Research Grants in the Field of Local Neuronal Interactions.

The Fundamental Neurosciences Program (FNP) of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) is encouraging the submission of applications for research grants in the field of local neuronal interactions.

Purposes:

a. This announcement indicates the interest of the FNP in stimulating new research and encouraging present research in this potentially significant area.

b. Such research may lead to a better understanding not only of basic neurobiological mechanisms, but also of "higher" brain functions such as learning, memory or complex behavior.

Definitions:

"Local neuronal interaction" deals with:

a. The activity of local circuit neurons (LCN's): cells with short axons or no axons such as Golgi Type II neurons, Renshaw cells, horizontal and amacrine cells in the retina, granule cells in the olfactory system and many other interneurons;

b. local neuronal circuits: describing the transfer of information in a neural network confined to a localized area in the nervous system; impulses limited to part of a cell surface or to a single dentritic tree or to one or a small group of LCN's;

c. electrotonic synapses and electrically coupled cells;

d. reciprocal and two-way synapses;

e. ephaptic transmission and other localized electric and electromagnetic field effects;

f. localized chemical field effects involved in information transfer;

g. central information processing in very restricted areas of the nervous system, possibly involving feedback and reverberating circuits;

h. low voltage (below action potential threshold) information transfer, within cells and among cells; including the translation of "noise" into meaningful signals;

i. interactions of these local chemical and electrical circuits;

j. localized interactions between glia and neurons.

Method of Applying:

Applications should be submitted in the same manner as with any research grant application. However, the face page should indicate that the application is in response to this announcement and one additional copy of each application should be sent to the address below. Applications to be judged more responsive to program interests of other funding units will be assigned accordingly.

Deadlines are the same as for all other research grants.

Specific Review:

Applications will be judged solely on scientific merit, in accord with NIH policy and procedures involving peer review.

Applicants should contact:

Novera Herbert Spector, Ph.D.
Health Scientist Administrator
Fundamental Neurosciences Program
NINCDS, National Institutes of Health
Federal Bldg., Room 120
Bethesda, Maryland 20014
Tel: (301) 496-5745

We expect to set aside one milllion dollars for support of grants in this field in FY 1979, about 1.3 million dollars for FY 1980 and 1.7 million dollars in FY 1981. These figures are subject to change, depending upon (a) budget, (b) quality and number of proposals received, and (c) possible bandwagon effects of research successes and publications in this field.

This Announcement has been published in the NIH Guide for Grants and Contracts, as well as in various professional society newsletters, journals, and other information services. In addition, at least 100 individuals and laboratories known to be presently active in related research have been notified.

The FNP has as its goal the support and stimulation of high quality research in the field of brain and nervous system function.

It is felt that this project will enhance the possibilities of more rapid scientific progress in the difficult but very promising field of local neuronal interactions.

Because this is the first venture of the FNP into "Program Announcements", this is viewed as an experiment in science administration and the staff is optimistic about the outcome. It is estimated that at least three years will be needed to evaluate the degree of its failure or success.

Other Fundamental Neuroscience Developments

Progress reports from FNP grantees reveal a wealth of new scientific knowledge. Two examples are presented:

Nerve cell bodies ensheathed by myelin membranes occur in the sensory ganglia of various species. They have also been observed more recently in autonomic ganglia and in the granular layer of the cerebellum of several species including man. They have often been recorded in tissue culture, especially of cerebellum but also in material derived from other areas of the CNS. Current studies have focused upon the dorsal horn of the monkey lumbar spinal cord where these formations have also been observed. Perikaryal myelin is occasionally derived from extensions of axonal myelin, but for the most part has a different, obscure origin. A few tentative generalizations have been offered with regard to the occurrence of this phenomenon. It may be seen in areas of high cell density or in cells that have few axosomatic synapses. From a functional viewpoint, perikaryal myelin is of unknown significance.

The peripheral chemoreceptor cells are located in the carotid and aortic bodies, attached to major arteries which branch to provide them with a rich blood supply. One to two millimeters in diameter, weighing approximately 2 milligrams, these structures receive the highest blood flow reported for any tissue--approximately 2000 ml/100 grams/minute, ensuring that the chemical composition of the arterial and venous blood are virtually identical. These cells, sensitive to arterial partial pressures of oxygen and carbon dioxide and to hydrogen ion concentration, are more closely concerned than any other

regulatory neurons with the interface between the external and cellular environment. Activation of these receptors leads to stimulation of the brain stem respiratory and vasomotor centers. Recent evidence suggests that deficiencies in chemoreceptor function may be in part responsible for "Sudden Infant Death Syndrome."

The functional unit consists of the chemoreceptor cell or glomus I cell, the sustenticular or interstitial cell and the nerve fiber. The intimate mechanism of stimulation is not known although the complexity of data obtained clearly suggests that several different modes of excitation are operative. A single glomus cell will increase its rate of discharge in response to a decrease in oxygen, an increase in carbon dioxide and a decrease in ph. The system is also sensitive to many drugs, metabolic inhibitors and poisons, and while it has been shown that a variety of stimuli depolarize, hyperpolarize or alter the resistance of the glomus cell membrane, other effective agents do not appear to produce these biophysical changes in the chemoreceptive cell itself.

Currently, it is hypothesized that acetylchcline is the neurotransmitter involved in afferent nerve activation, since the substance can be isolated from the carotid body and the enzyme required for its synthesis and hydrolysis are present. The close arterial injection of acetylcholine or the administration of anticholinesterase agents increase the effects of carotid body stimulation. However, dopamine is also present; its origin and function is currently under investigation.

Recent research in this area has demonstrated that the petrosal ganglion of the glossopharyngeal is the origin of nerves terminating on the glomus cell of the cat carotid body; that the glomus cells are also sensitive to temperature changes as well as changes in osmotic pressure; that similar chemoreceptor cells may be found in other parts of the body such as the viscera; and there may be very significant species differences in glomus cell morphology and physiology. Studies in this area should be aided by the development of a tissue slice technique which will enable the impalement of individual cells under direct visual control. The discovery of relatively large chemoreceptor cells in necturus should also prove advantageous. The proliferation of studies in peripheral chemoreception has resulted in a monograph detailing the current status of research, "Chemoreception in the Carotid Body." Considering the neurovascular integrative and regulatory functions of this system, it is logical that this research is supported by both NINCDS and NHLI.

Neural Prosthesis Program

About 10% of the FNP budget allocation is in support of the contract program and most of this is devoted to the Neural Prosthesis Program (NPP). This program is composed of 14 research and development contracts aimed at solving the basic problems standing in the way of development of neural prostheses to aid the neurologically handicapped. The descriptions of these contracts, their goals and major findings are presented in the attached Contract Narrative Statements.

The NPP is the most important part of the FNP. It is the firm conviction of the FNP Director that the future development of neuroscience will inevitably lead to the interfacing of the nervous system with ever more complex devices which will at first merely alleviate deficiencies in the natural organism, but ultimately will provide an extension of man's capabilities far beyond his present limitations.

It is hoped that the importance of these developments will be recognized so that this program can grow and will receive the support it needs to become an independent program of the NINCDS. This will require the allocation of additional funds and staff positions and the development of a grants arm to complement the growing R&D contracts.

Other FNP Contracts

In addition to the NPP contracts, FNP supports two information exchange contracts, the Brain Information Service (BIS) and Neurosciences Research Program (NRP) and one contract for the study of Neuroanatomical Asymmetry in the Human Temporal Lobes in Relation to Psychological Characteristics.

The BIS provides an important extraction of current references directly relevant to several specific areas of neuroscience. These include several Current Alerting Bulletins, Recurrent Bibliographies, Conference Reports, Updated Reviews, Special Reports, Demand Searches and a Computer Data Base of about half a million citations.

A Technical Merit Review and other in-house review committees have recommended modest reductions in budget for this contract during the current year and for FY 1979. After that, the need for this service will be reconsidered and, if it is found to be necessary, a new Request for Proposals (RFP) will be advertised and bids considered. New information products which should result from such contracts are currently being considered.

The Neurosciences Research Program at MIT continues to provide a unique review of the most important neuroscience developments which have the greatest potential for new breakthroughs in understanding. A large body of the best neuroscientists in the world, the Associates, are brought together periodically to re-examine various areas of neuroscience. Their goal is to identify what is known fact, what is false and which questions most urgently need answering. The results of these deliberations are made available to the scientific public and to the FNP through publication of the NRP Bulletin, the NRP Science Newsletter and other publications. Specific accomplishments of the NRP are detailed in the attached Contract Narrative.

FNP Advisory Committee Meeting

The FNP Advisory Committee met October 19, 1977 to review the current Fundamental Neurosciences Program and to consider future directions for FNP-sponsored research. The following have been extracted from the Advisory Committee Minutes:

Average priority ratings of some of the research projects proposed:

100 High Priority

200 Moderate Priority

300 Low Priority

1. Dr. Lansdell's proposal to use a "co-citation cluster analysis" to show the relation of basic neuroscience to biomedical applications. Priority Rating: 160

2. Drs. Lacey and Bullock suggested a contract with, for example, Dr. Ted Melnechuk to supervise a telephone survey for identifying basic research which has led to important discoveries in applied neuroscience. Priority Rating: 100

3. Assuming the acceptance by DHEW and Congress of the Report of the Commission for the Protection of Human Subjects in Biomedical and Behavioral Research and its recommendations about the scientific investigation of psychosurgery, Herbert Lansdell has suggested that we might become involved in sponsoring some phase of this research such as the effectiveness of unilateral psychosurgery. Priority Rating: 180

4. Dr. Hambrecht's plans for the Neural Prosthesis Program.

   a. New contracts for the development of electrode materials and packaging materials for insulating electrodes and their leads and for encapsulating implanted prosthetic devices. Priority Rating: 140

   b. A new contract or grant for studying the mechanisms of activation of cortical neurons by applied electrical fields produced by arrays of implanted electrodes. Priority Rating: 120

   c. A new contract or grant for the development of methods for producing reversible inhibition of peripheral and CNS neural activity. Priority Rating: 140

   d. A new contract or grant for studying the changes which occur in the motor cortex as a result of long-term spinal cord injury. Priority Rating: 180

   e. A new contract for the development of arrays of the presently available floating microelectrodes for outward information transfer including the development of necessary interface electronics. Priority Rating: 160

f. New contract for the exploration and development of sources of feedback signals for neural control. Priority Rating: 180

g. New grant or contract for the study of the effects of electrical stimulation on local, cerebral bloodflow. Priority Rating: 160

5. Dr. Spector's proposal to issue RFAs for stimulation of research on mechanisms of local neuron interactions (sometimes referred to as "Local Circuit Neurons"). Priority Rating: 180

6. Dr. Reswick pointed out the need for a method to selectively activate individual fasciculi in mixed peripheral nerves. Priority Rating: 200

7. Dr. MacNichol's proposal to transplant an embryonic retina onto the visual cortex of rats which, if it formed functional connections with cortical nerve cells, might provide a rudimentary visual organ responding to direct stimulation by light patterns applied through a transparent cranial plate. Priority Rating: 180

8. Exploration of regeneration of dorsal root fibers, after section between dorsal root ganglion and spinal cord, into various peripheral nerves or muscles. Priority Rating: 200

9. Dr. Cooper suggested a technique for implanting a reservoir of drugs which could be slowly released to modulate the excitability of a small region in the central nervous system. Priority Rating: 180

10. Dr. MacNichol described the implantation of rat pancreatic cells contained in a permeable membrane bag to protect them from phagocytosis. Further research along this line might be stimulated either by grant or contract. Priority Rating: 230

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: HUNTINGTON INSTITUTE OF APPLIED MEDICAL RESEARCH (NO1-NS-0-2275)

Title: Studies to Determine the Feasibility of a Sensory Prosthesis

Contractor's Project Director: William Agnew, Ph.D.

Current Annual Level of Support: $193,958

Objectives: The effects of long-term electrical stimulation of the nervous system in animals are being studied with various electrode designs, stimulus wave forms, and stimulus parameters. These studies include the effects on the blood brain barrier and regional cerebral blood flow. Following stimulation, meninges, nervous tissue and associated vasculature are examined histopathologically with both the light and electron microscope.

Major Findings: 1) In vivo neural toxicity studies continue to indicate rhodium is as safe as platinum when used as a stimulating electrode. 2) Charge densities below 10 microcoulombs/cm^2 at one microcoulomb/phase result in no neural damage when stimulation is applied for 35 hours to cat cerebral cortex. 3) Neural damage was seen at 30 microcoulombs/cm^2 and a positive correlation was found between the extent of damage and charge density above 30 microcoulombs/cm^2.

Significane to Biomedical Research and to the Program of the Institute: These studies are important for determining the safety and efficacy of various forms of stimulation of the central nervous system utilized in neural prostheses for the neurologically handicapped.

Proposed Course of Contract: This work will continue to develop and evaluate safe, effective means of electrically stimulating neural tissue.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: MASSACHUSETTS GENERAL HOSPITAL (NO1-NS-0-2276)

Title: Studies to Determine the Feasibility of a Sensory Prosthesis

Contractor's Project Director: Daniel Pollen, M.D.

Current Annual Level of Support: 0

Objectives: The mechanisms of neuronal activation resulting from electrical stimulation of the central nervous system are being studied. Methods of reducing the latency of activation of neurons and methods of preventing after discharges are being developed. Also, the subjective experiences of human patients during stimulation of the visual cortex are being studied.

Major Findings: 1) Neurons within 10 microns of an intracortical stimulating electrode can be excited at currents of 8-50 microamps. 2) Cells within 170 microns can be fired in the 50-60 microamp range. 3) At separations in the 300 micron range, currents of greater than 600 microamps are necessary to excite cells. 4) Some cells were activated with latencies of 0.35 - 0.5 msec. These short latencies are felt to indicate direct activation of the cell. 5) Attempts to use the NIH floating microdrive to record intracellular potentials during surface stimulation were unsuccessful.

Significance to Biomedical Research and to the Program of the Institute: An understanding of the mechanisms of activation of neurons by electrical stimulation and the subjective experiences of such activation are important for the development of neural prostheses for the neurologically handicapped.

Proposed Course of Contract: The Principal Investigator is leaving MGH and the contract will terminate.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: UNIVERSITY OF ROCHESTER NO1-NS-0-2279)

Title: Development of a Sensory Prosthesis

Contractor's Project Director: John Bartlett, Ph.D.

Current Annual Level of Support: $89,770

Objectives: The stability of the threshold of excitation of nerve cells of the visual cortex during long-term electrical stimulation is being studied in monkeys. The mechanisms causing increases in threshold for detection of stimulation and means of preventing the increase are being evaluated. The effects of chronic blindness and various stimulus modulation schemes on information transfer rates are also being studied.

Major Findings: 1) Access resistance measured in the macaque during stimulation was demonstrated to be essentially independent of applied current substantiating the theoretical basis of access resistance. A highly flexible electrode lead was developed based on segmented wire pieces within a salt solution held in polyethylene tubing. 2) Studies to determine whether "kindling" seizures might be due to toxic electrolytic products, have shown that such is not the case. 3) Gradual increases of the stimulation level over the period of hours or days can result in some protection against threshold elevations due to chronic stimulation. However, this effect is neither strong nor predictable from one locus to another.

Significance to Biomedical Research and to the Program of the Institute: This work will be useful for developing safe and efficient methods of stimulating the central nervous system for use in neural prostheses for the neurologically handicapped.

Proposed Course of Contract: 1) Continued evaluation of techniques of electrical stimulation which are effective but noninjurious even when applied continuously for long periods of time. 2) Development of a simple electrophysiological test for detection of neural injury. 3) Definition of the laminar extent of threshold elevation by electrical stimulation. 4) Evaluation of a possible neural damage cause for the "kindling" effect.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: University of Florida (NO1-NS-1-2286)

Title: Electrode Materials Study

Contractor's Project Director: William W. Dawson, Ph.D.

Current Annual Level of Support: $22,000

Objectives: The development and evaluation of electrode conductors and insulators for biomedical implantation.

Major Findings: 1) Further in vivo testing of tantalum pentoxide capacitor electrodes has shown that when used as stimulating electrodes on the surface of the cerebral cortex they are safe or safer than any other available electrode system. 2) The access resistance of a stimulated electrode falls during the first few minutes of stimulation and the impedance of nearby non-stimulated electrodes (within 2 mm) also falls. 3) Tantalum pentoxide stimulating electrodes have been custom -made and some improvement in charge storage capability over commercially available electrodes has been achieved.

Significance to Biomedical Research and to the Program of the Institute: The evaluation and development of electrode materials is necessary for all devices that utilize electrical stimulation of excitable tissues.

Proposed Course of Contract: This contract is being terminated.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: BOWMAN GRAY SCHOOL OF MEDICINE (NO1-NS-4-2304)

Title: Study and Test of Ultrasound Techniques for Diagnosis of Cerebral Disorders

Contractor's Project Director: Ralph W. Barnes, Ph.D.

Current Annual Level of Support: 0

Objectives: One of the major problems in effectively studying intracranial arterial echo activity, either in range or amplitude, has been isolating the arterial echo and measuring its activity during the cardiac cycle. The isolation of intracranial artery echoes and their activity has been achieved using moving target indicator (MTI) techniques originally developed for radar and sonar use. The contractor is extending the basic MTI technique in order to develop and clinically evaluate instrumentation for non-invasive assessment of cerebrovascular dynamics.

Major Findings: This period has been dedicated to writing a final report which has been received.

Significance to Biomedical Research and to the Program of the Institute: The diagnosis and treatment of stroke and other intracranial disorders is a major concern to NINCDS and to the medical fields related to the Institute's mission. The non-invasive diagnostic techniques being investigated under this project will, if successful, provide valuable tools for non-invasive diagnosis of major forms of cerebrovascular disease.

Proposed Course of Contract: This contract has been terminated.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: University of California at Los Angeles, Brain Information Service (UCLA-BIS) (NO1-NS-3-2306)

Title: Operation of Specialized Information Center in Brain and Other Neurosciences

Contractor's Project Director: Michael H. Chase, Ph.D.

Current Annual Level of Support: $365,000

Objectives: The contractor operates a specialized information center which serves as a national focal point for the identification, collection, storage, retrieval, analysis, repackaging, and dissemination of information on non-clinical neurosciences. The major thrusts of this information center are information analysis products and services using the identified and stored information. The contractor makes available comprehensive information services, including: a) current alerting bulletins, b) demand searches of the data base, and c) other products and services mutually agreed upon by the contractor and the Institute.

Major Accomplishments: During the current contract period, the Brain Information Service is carrying out the following activities:

Current Alerting Bulletins
- Biogenic Amines and Transmitters in the Nervous System
- Neurochemical Transmitters and Modulators
- Neuroendocrine Control Mechanism: Hypothalamic-Pituitary-Gonadal System
- Sleep Bulletin including Sleep Reviews
- Index to Current (EEG) Literature
- Memory and Learning--Research in the Nervous System
- Memo of Current Books in the Brain Sciences(Incl. Author Index)
- Developmental Neurobiology

Recurrent Bibliographies
- Neuroimmunology
- Proteins in the Brain
- Cerebral Evoked Potentials

Bibliographic Cumulations
- Bibliography on the Hypothalamic-Pituitary-Gonadal System
- Biogenic Amines in the Central Nervous System, A Bibliography
- A Bibliography of Electrical Recordings in the CNS and Related Literature
- Sleep Research

Reference Bibliographies

Endorphins: Endogenous Morphinomimetic Ligands and Opiate Receptors in the Central Nervous System

Enzymes Involved in Neurotransmitter Synthesis and Metabolism

Conference Report

Seventh Annual Meeting of the Society for Neuroscience

During the last twelve month period approximately 50,000 citations were added to the data base which now contains over half a million citations. About 70 demand searches of the data base were carried out in response to requests from neuroscientists.

Significance to Biomedical Research and the Program of the Institute: The Brain Information Service performs an important service to the biomedical community by the maintenance of a very extensive data base devoted to the fundamental neuroscience literature. In addition, a number of important synthetic and analytic information products are produced by the staff for distribution to the neuroscience community.

Proposed Course of the Contract: The program is under the continuing surveillance of the NINCDS Project Officer and the BIS National Scientific Advisory Committee. Each product receives detailed review.

It is proposed to continue the contract at a reduced funding level for one more year (January 1, 1979 to December 31, 1979). The future need for such a service will then be reevaluated and if desirable, it will be re-advertised for competitive procurement with a new scope of work and a new RFP.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: STANFORD UNIVERSITY (NO1-NS-5-2306)

Title: Transdermal Stimulation Electronics for an Auditory Prosthesis

Contractor's Project Director: Robert White, Ph.D.

Current Annual Level of Support: $110,370

Objectives: The design and development of transdermal stimulators to be used in the evaluation of multi-channel cochlear implant auditory prostheses.

Major Findings: 1) A four-channel transdermal stimulator has been fabricated and tested by applying the output through a percutaneous plug in a deaf human volunteer with electrodes implanted in the modiolus. The stimulator functioned as designed. 2) A suitable technique for encapsulation of the transdermal stimulator has still not been achieved. The latest failure was Hysol encapsulation. 3) An eigt-channel bipolar transdermal stimulator and a 12-channel monopolar transdermal stimulator have been designed and bread-boarded.

Significance to Biomedical Research and to the Program of the Institute: The Institute is presently supporting under the grant mechanism the evaluation of multi-channel auditory prostheses. This contract will provide electronic stimulators to these grantees.

Proposed Course of Contract: Eight and twelve channel transdermal stimulators for auditory prostheses will be fabricated and tested in vitro and in vivo.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: UNIVERSITY OF CALIFORNIA, SAN FRANCISCO (NO1-NS-3-2307)

Title: Studies of Urinary Bladder Evacuation by Electrical Stimulation

Contractor's Project Director: Emil Tanagho, M.D. .

Current Annual Level of Support: $85,833

Objectives: Studies are being conducted in animals with upper motor neuron lesions to determine the feasibility of urinary bladder evacuation by electrical stimulation of the sacral spinal roots.

Major Findings: 1) Electrodes have been designed which are suitable for stimulation of the sacral roots in humans. 2) A method of sequential stimulation of sacral nerves for achieving continence by sphincter activation has been developed.

Significance to Biomedical Research and to the Program of the Institute: The ability of a person with a neurogenic bladder to empty his bladder voluntarily is the long-range goal of this work and would eliminate or reduce the major cause of death in paraplegics. The problem of urinary incontinence is of both social and medical significance, especially in the geriatric population. The development of a neural prosthetic implant to alleviate this condition would be a significant advance.

Proposed Course of Contract: The ultimate aim of this work is the development of successful human prosthetic implants for control of micturition and for control of incontinence. Should the present testing of the new peripheral nerve electrodes be successfully completed in animals, they will be evaluated in humans.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: EIC CORPORATION (NO1-NS-3-2313)

Title: Safe Procedures for Electrical Stimulation of the Nervous System

Contractor's Project Officer: Barry Brummer, Ph.D.

Current Annual Level of Support: $103,545

Objectives: The electrochemical properties of both metal and capacitor electrodes are being studied. Potential toxic reaction products and their relationships to electrode design and stimulus parameters are being evaluated.

Major Findings: 1) The addition of methionine or cysteine to inorganic simulated cerebral spinal fluid results in an apparent increase in platinum electrode corrosion during current passage. This is in contrast to the addition of human serum albumin which results in a decrease. 2) Multi-stranded, coiled, stainless steel electrodes supplied by another contractor were found to have real surface areas of about 10 percent of that theoretically predicted. It is not clear whether this effect is due to effective blocking of the interior surface areas from participating in current flow to the surrounding solution or whether some form of surface contamination is responsible. 3) Scanning electron microscopy with x-ray analysis has shown that electrodes supplied by Huntington Institute have their surfaces contaminated with aluminum. This contamination most likely occurred during sandblasting of the platinum electrodes with alumina (sand).

Significance to Biomedical Research and to the Program of the Institute: The development and evaluation of safe stimulating techniques for use in neural prostheses is one of the major goals of the Neural Prosthesis Program of the Institute.

Proposed Course of Contract: 1) Evaluate electrochemical reactions associated with micro-intracortical stimulating electrodes as supplied by the Laboratory of Neural Control, IRP, NINCDS. 2) Develop a method of measuring the oxygen tension in the solution immediately surrounding the stimulating electrodes. 3) Determine whether protein in solution actually reduces platinum dissolution or merely traps dissolution products at the surface of the electrode. 4) Develop electrochemically safe methods for stimulating neural tissue.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: CASE WESTERN RESERVE UNIVERSITY (NO1-NS-2-2314)

Title: Study of Intramuscular Electrical Stimulation of Muscle

Contractor's Project Director: Thomas Mortimer, Ph.D.

Current Annual Level of Support: $225,060

Objectives: Both animals studies and muscle implant studies in humans are directed toward the development of proportional control of the upper extremities in paralyzed individuals. In particular, methods of reversing disuse atrophy, preventing muscle fatigue and providing smooth, coordinated muscle contractions are being investigated. Stimulation of the paravertebral muscles through percutaneous electrodes in patients with scoliosis is being evaluated.

Major Findings: 1) In three cases of scoliosis in which electrodes have been implanted in the paravertebral muscles and stimulated, the spinal curvature has not progressed or is less than the curvature at the onset of stimulation. 2) Single strand electrodes have had a high failure rate and are presently being replaced by multi-strand stainless steel electrodes which are much more resistant to fatigue fracture. 3) No failures of the multi-strand stainless steel electrodes have occurred in periods of up to six months of implantation in human paravertebral muscles. 4) Excitation of the thenar muscles in spinal cord injury patients augments the tenodesis grasp that accompanies wrist extension. This improves lateral pinch (key grip) and release in the C-6 quadriplegic patient. The technique allows the three patients being studied to grasp and hold objects, especially writing and eating instruments, tonically without the need for an external orthosis. 5) Stimulation of the abductor pollicus muscle for 12 hours per day in a human quadriplegic patient over a period of 1 year produced measurably increased fatigue resistance and slowed the speed of muscle contraction. This confirms previous studies in cats. 6) Stimulation periods of less than one hour per day are insufficient to maintain the muscle in this altered state. 7) Modulation of muscle force by recruitment of muscle fibers is more nonlinear than modulation by changes in interpulse interval and therefore harder for patients to control.

Significance to Biomedical Research and to the Program of the Institute: The techniques being investigated are intended to restore lost function in paralyzed individuals and lead to an effective treatment for scoliosis.

Proposed Course of Contract: This is a long-range project for solving basic neuroscience and clinical engineering problems associated with the development of totally self-contained stimulation systems to allow quadriplegic patients to regain control of their paralyzed muscles. Long-term objective

measurements of curvature in patients with scoliosis treated by electrical stimulation of the paravertebral muscles will be pursued.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: UNIVERSITY OF CALIFORNIA, LOS ANGELES (NO1-NS-4-2331)

Title: Studies on the Effects of Electrical Stimulation of the Cerebellum

Contractor's Project Director: Thomas Babb, Ph.D.

Current Annual Level of Support: $112,980

Objectives: The effects of cerebellar stimulation on the electrical and behavioral aspects of seizures produced by alumina cream implants in the hippocampus of monkeys and on the firing behavior of hippocampal singe units are being studied.

Major Findings: 1) Alumina cream in the amygdala of monkeys produces a mixed seizure model consisting of psychomotor and generalized motor components with periods of status epilepticus. 2) Alumina cream confined to the hippocampus results in an essentially pure model of psychomotor epilepsy characterized by head turning and absence. 3) Direct projections from the cerebellar deep nuclei to the hippocampus had been found in the monkey but they are relatively few in number. 4) Cerebellar stimulation has continued to show no significant effect on the seizure frequency or duration in alumina cream models in the macaque.

Significance to Biomedical Research and to the Program of the Institute: These studies should provide information on the mechanisms, if any, by which cerebellar stimulation modifies clinical seizures and movement disorders.

Proposed Course of Contract: The effects of stimulation on cerebellar neurons over longer periods of stimulation will be studied histopathologically. Stimulation of more direct pathways which project to the hippocampus will be studied for their effect on the hippocampal model of temporal lobe epilepsy.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: UNIVERSITY OF MINNESOTA (NO1-NS-4-2332)

Title: Study of the Effects of Electrical Stimulation of the Cerebellum

Contractor's Project Director: Heinrich Bantli, Ph.D.

Current Annual Level of Support: $89,338

Objectives: The effects of cerebellar stimulation on primate models of epilepsy and movement disorders are being evaluated. The neurophysiological mechanisms and anatomical pathways associated with such stimulation are being examined.

Major Findings: 1) The correlation between the amplitude of the cerebellar evoked response and the amplitude of the cerebellar stimulus is relatively weak. 2) The measurement of the H-reflex and the calculation of the H/M ratio is not a practical way to evaluate the effects of cerebellar stimulation on spinal integration in humans due to the large normal variability. The data on seven patients in which the H-reflex was measured was inconclusive. 4) Stimulation of the cerebellar surface does not produce statistically significant changes in the duration of penicillin induced generalized motor seizures, but the effect is far from dramatic.

Significance to Biomedical Research and to the Program of the Institute: These studies should provide information on the neurophysiological mechanisms, if any, by which the cerebellar stimulation modifies clinical seizures and movement disorders.

Proposed Course of Contract: An animal model of spasticity based on bilateral ablation or cortical areas 1-7 will be developed and the effects of cerebellar stimulation on the resultant movement disorders will be studied. The previous work on seizure models will be continued but at a reduced level of effort.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: Massachusetts Institute of Technology, Neurosciences Research Program (NRP) (NO1-NS-6-2343)

Title: Support of the Neurosciences Research Program

Contractor's Project Director: Frederic G. Worden, M.D.

Current Annual Level of Support: $360,000

Objectives: The objectives of this contract are to examine current data and concepts of brain structure and behavior at all levels of complexity and to accelerate progress on crucial problems of neural science.

Major Findings: Five Work Sessions were held during the year, their titles were:

Integration and Modulation in the Brain Stem

Central Core Regulation: Monoaminergic Systems

The Selection and Modulation of Behavioral Programs

Visual-Vestibular Interaction in Motion Perception and the Generation of Nystagmus

Recent Developments in Theoretical Neurobiology.

A Planning Meeting was held on "Mechanisms Regulating Post-synaptic Sensitivity."

The Thirty-fourth Stated Meeting of NRP (Meeting of the Associates) was devoted to the evaluation of research topics for future NRP Work Sessions, Conferences, etc. Inaugural Lectures were given by the eight newly elected Associates.

A "Whither NRP" Committee was appointed to do an in depth study of strengths and weaknesses of NRP and to make recommendations for NRP's evolution over the next 5-10 years. The Thirty-fifth Stated Meeting was on the subject of "Functional Organization of the Cortex." A half-day was devoted to cooperativity theory as applied to neuronal systems. The Associates reviewed the role of phosphorelative processes in neuronal metabolic regulation. The Executive Session of Associates considered the election of four new Associates and discussed the interim report of the "Whither NRP" Committee.

NRP Bulletin issues published included: Neuronal and Neurochemical Substrates of Reinforcement; The Developmental History of the Spinal Motor Neuron; Neuronal Mechanisms in Visual Perception; Depolarization-Release-Coupling Systems in Neurons. Vol. 16 includes a discussion on Pain, Neurobiology of Peptides, Neuron-Glia Interactions, Electrotonic Junctions, and Sexual Differentiation of the Brain. Two books are in press at MIT: The Mindful Brain: Cortical Organization and the Group Selective Theory of Higher Brain Function and, The Neurosciences: Fourth Intensive Study Program.

The F.O. Schmitt Medal and Lectureship went to Dr. Roger C.L. Guillemin, whose address was entitled "The Hypophysiotropic Peptides of the Hypothalamus" which will be published in a supplement to the NRP Bulletin. The Prize provided by a contribution from a private source to the Neurosciences Research Foundation, was increased to $2,500. Five days after Dr. Guillemin gave the F.O. Schmitt Lecture, he was notified by the Karolinska Institute that he would share this year's Nobel Prize in Medicine.

The MIT Graduate Seminar "Seminar in Neuroscience Research Topics" has continued to attract outstanding students who attend all NRP Work Sessions, Stated Meetings and Conferences. The students are required to participate in reviews of Work Sessions with NRP staff and Work Session Chairmen. Each student prepares a written proposal for a possible Work Session which in his opinion would define a growing point in neuroscience, outlining the scope of the topic, the issues around which it would be organized, and a list of scientists who have contributed importantly to the subject area.

The 1977 Intensive Study Program was extremely successful. Some 80 neuroscientists and 50 carefully selected postdoctoral fellows participated and the Proceedings were published by the MIT Press in the summer of 1978. It is hoped that this Intensive Study Program through its effect on the young postdoctoral fellows and the broader influence of the book, will implement the quiet revolution that has for several years been gathering momentum and that it will influence the neuroscience community to pursue new directions in research seeking to clarify the physical, chemical and neuronal bases of higher brain functions such as perception, learning, memory and conscious experience in normal and clinically disordered persons.

Significance to Biomedical Research and to the Program of the Institute: NRP endeavors to identify those research areas in the neurosciences which are ready for exploitation and are most likely to yield important new concepts. This helps FNP to identify the most relevant areas of research for Program Initiatives.

Proposed Course of Contract: The activities of the NRP will be of the same kind as in previous years and of approximately the same scope. Individual research areas to be emphasized will be determined.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: McMASTER UNIVERSITY (NO1-NS-6-2344)

Title: Neuroanatomical Asymmetry in the Human Temporal Lobes and Related Psychological Characteristics

Contractor's Project Director: Sandra F. Witelson, Ph.D.

Current Annual Level of Support: $85,000

Objectives: Patients in good neurological and mental health at a cancer clinic are approached by a trained "clinical coordinator" about granting permission for autopsy and taking psychological tests. The tests are those demonstrated to be affected by cerebral lesions of the temporal lobe on the left or the right; certain variations in anatomical asymmetry in the two normal temporal lobes will be investigated for relationships to the test scores.

Major Findings: As of June, 32 patients signed informed consent forms, and 28 have undergone some psychological testing. Eight patients have died and 6 brains have been obtained. Gross anatomical procedures have begun on the brains and quantitative histological procedures will begin soon, after work on pilot brains is completed.

Significance to Biomedical Research and to the Program of the Institute: The research may solve the mystery of why nearly a quarter of human brains do not show the temporal lobe asymmetry of the two-thirds majority; the results should provide insight into the hemispheric specialization of the human brain.

Proposed Course of Contract: Testing of patients is planned until a total of 60 brains become available for gross measurements and 8 specimens with extreme asymmetry (4 left and 4 right temporal areas larger) will have histological analyses. The prospect of obtaining a sufficient number of volunteers within the contract period is good.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: STANFORD UNIVERSITY (NO1-NS-7-2366)

Title: Development of Multi-Channel Electrodes

Contractor's Project Director: Robert White, Ph.D.

Current Annual Level of Support: $145,000

Objectives: This project is attempting to model the electrical and mechanical properties of the eighth nerve as it passes through the modiolus. The results of these studies will be used to design second generation multi-electrode arrays for stimulation of the eighth nerve in the human modiolus.

Major Findings: This contract was only recently initiated and no major findings have resulted so far.

Significance to Biomedical Research and to the Program of the Institute: Multi-channel electrode arrays for stimulation of the eighth nerve may provide a means of communication for sensory deaf individuals. The NINCDS is committed to determining the feasibility of auditory prostheses for the deaf.

Proposed Course of Contract: The contract will lead to the development of electrode arrays suitable for human implantation in the modiolus as part of and auditory prosthesis for the deaf.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, NINCDS
October 1, 1977 - September 30, 1978

Contractor: UNIVERSITY OF CALIFORNIA, SAN FRANCISCO (NO1-NS-7-2367)

Title: Development of Multi-Channel Electrodes

Contractor's Project Director: Michael Merzenich, Ph.D.

Current Annual Level of Support: $66,297

Objectives: This project is attempting to model the electrical and mechanical properties of the scala tympani and, on the basis of these studies, develop multi-channel electrode arrays suitable for stimulation of the eighth nerve in humans.

Major Findings: This project was only recently initiated and no major findings have resulted as yet.

Significance to Biomedical Research and to the Program of the Institute: Multi-channel electrode arrays for stimulation of the eighth nerve may provide a means of communication for sensory deaf individuals. The Institute is committed to determining the feasibility of auditory prostheses for the deaf.

Proposed Course of Contract: This contract will lead to the development of multi-channel electrodes suitable for implantation in the scala tympani of humans as part of an auditory prosthesis for the deaf.

Annual Report of the Scientific Director
of the
National Institute of Neurological and
Communicative Disorders and Stroke
October 1, 1977 through September 30, 1978

For the NINCDS Intramural Research Program, FY 78 was a year of continuing research accomplishment, coupled with vigorous managerial efforts to improve program efficiency and take maximum advantage of newly emerging investigative opportunities in the neurosciences. Because the maintenance of scientific excellence depends on the ability to recruit, retain and support highly qualified researchers, personnel issues remained of paramount concern.

Throughout FY 78, the Program continued intense efforts to recruit permanent Chiefs for the Surgical Neurology Branch and the Laboratory of Neurophysiology. In both cases, the Program has been extremely fortunate to have the assistance of external Search Committees whose knowledgeable and diligent efforts greatly facilitated the selection process. The search for a new Chief of the Surgical Neurology Branch was recently brought to a most successful conclusion. The Branch Chief designate, Dr. Paul Kornblith, currently of Harvard University and the Massachusetts General Hospital, will initiate a broad-based program of studies on the biology of nervous system tumors upon joining the Intramural Program. Although a new Chief has yet to be selected for the Laboratory of Neurophysiology, several highly qualified candidates are under active consideration, and it is expected that an appointment will be made during the coming year. Despite these successes, recruitment of senior-level scientists remains a difficult task: Civil Service and Public Health Service salaries for senior physicians and scientists differ substantially from those offered by academic institutions, and the ever increasing administrative complexities attending these high-level appointments seriously hinder timely action.

Another continuing problem in the area of personnel management involves the frequent, unpredictable fluctuations in budgeted employment ceilings. Within a twelve-month period, Program ceilings for Full-Time Permanent employees changed three times: In July, 1977, the ceiling was cut from 295 to 282; partial restoration occurred in February, 1978, and was completed in June. Since no effective mechanisms exist to force the marginally productive, tenured researcher from government service, for most of the year the Program could do little more than struggle to achieve the lower ceiling through attrition. This highly undesirable situation creates shortages in certain high turnover support positions and prevents the filling of short-term Associate or Fellow positions when they are vacated, thus closing the primary route for recruiting younger, creative scientists.

Two consequences of chronic personnel stringencies also deserve reiteration. First, the Program remains unable to take full advantage of many new research opportunities. Currently, initiation of important studies in molecular virology, neuroendocrinology, neurotoxicology, and in clinical audiology and speech pathology await only the availability of additional positions. Indeed, the Program finds it increasingly difficult to provide adequate personnel support for several of its most productive, existing

laboratories. Second, the current no-growth situation, coupled with prevailing rigidities in personnel policies and procedures, inevitably results in a rise in the average age of the scientific cadre as well as in the percent having tenure. Due to continuation of severe restrictions on the conversion of younger researchers to regular staff positions, the proportion of tenured scientists within the Program has remained essentially stable at 45%. Nevertheless, it is not difficult to imagine the long-term deleterious consequences the trend toward a predominantly tenured scientific staff would impose on the productivity of the Program.

In an attempt to deal with certain of these personnel contraints, the Program has continued to recruit ceiling-free personnel. In June, 1978, a total of 538 individuals worked in the Program, an increase of 27 over the preceding year. Staff composition included 246 scientists, 208 technicians, and 84 administrative/clerical employees. Among these are 143 individuals who did not occupy budgeted positions--mostly Guest Workers, Visiting Fellows, and university faculty members who come to NIH for one or two years under the Intergovernmental Personnel Act. These ceiling-free appointments, largely filled by talented, younger scientists, provide much needed flexibility, and as a result of vigorous efforts to encourage this type of recruitment, their number has remained essentially constant at about 26% of the total work force.

In order to make optimum use of its scientific staff, the Intramural Program must provide adequate resources for research support. In FY 78, an operational budget of $8.2 million was received. This allocation helped reverse a long-term decline in buying power. With these funds, the Program was able to provide a reasonable degree of per capita support to staff members and keep pace with rapid advances in biomedical instrumentation. Nevertheless, the cost of major equipment purchases is becoming increasingly prohibitive, as each year inflation erodes the Intramural budget. The impact of inflation on the scientific community is particularly severe, since the cost of biomedical research has advanced one percentage point more than the Consumer Price Index, and it is likely that this trend will continue.

Research contracts provide an indispensable extension to the Program's in-house research efforts. In FY 78, total active contracts amounted to $3.1 million, a figure which has remained essentially stable during the past few years. Significantly, the administrative complexities surrounding use of the research contract mechanism continue to grow. It is unclear, however, whether the additional scientific review and managerial control that result offset the excessive delays now attached to contracted research.

In addition to budgetary support, Program productivity is contingent on the availability of laboratory and office space. During FY 78, on-campus space assignments continued to approximate 90,000 net square feet. Unfortunately, use of this space will soon be curtailed due to construction of the Ambulatory Care Research Facility (ACRF). During ACRF construction, several research units must move from Building 10 to other Intramural facilities. These relocations, although temporary, will undoubtedly affect Program efficiency in FY 79 and 80. Eventually, however, the ACRF should provide NINCDS with

sufficient additional space to intensify several high-priority research activities and possibly undertake certain new initiatives.

Several favorable developments regarding off-campus space occurred during the past year. Renovations of Building 376 at Ft. Detrick, Maryland were finally completed, thus providing the Laboratory of Central Nervous System Studies with approximately 17,000 square feet of animal holding and virus containment facilities needed for extended investigations into the pathogenesis of slow viral infections of the nervous system. Also during FY 78, portions of several Intramural Laboratories were transferred to the Park 5 Building in Rockville. It is expected that this facility--providing approximately 5,000 square feet of critically needed space--will be fully operational by the beginning of FY 79. Park 5 will house staff of the Developmental Brain Pathology Section, the Molecular Virology Section, and the Myelin and Brain Development Section.

Although research opportunities in the clinical neurosciences have expanded dramatically during the past two decades, the NINCDS assignment of 52 beds in the NIH Clinical Center has remained essentially unchanged since the Clinical Center opened in the early 1950s. During FY 78, this shortage in research beds, together with chronic nursing overload, continued to prevent NINCDS clinicians from fully exploiting new biochemical, pharmacologic, immunologic, and surgical approaches to the study and treatment of nervous system disease. To address this problem, the Institute sought to borrow beds from other Institutes, as well as to use local commercial facilities to lodge ambulatory patients. While these alternatives offered some relief, they are at best stop-gap measures. Since many NINCDS research patients suffer from chronic, debilitating diseases or are recovering from nervous system surgery, they typically require close and highly specialized nursing care that is only available on neurology wards. Thus, individuals with these disorders cannot easily be assigned to beds borrowed from other Institutes nor participate in special ambulatory programs. As a result, NINCDS research patients frequently wait weeks or even months for admission.

In addition to exploring ways to alleviate the bed/nursing shortage, the Program continued to ensure full and efficient use of its assigned Clinical Center beds and nursing staff during FY 78 by scheduling all patient admissions through an NINCDS Bed Coordinator. This approach has proved very effective, and monthly occupancy of assigned beds remains at or near 100%. Yet, despite these efforts, restricted availability of research beds still constitutes the greatest hindrance to NINCDS clinical research progress. It is hoped, therefore, that completion of the ACRF, together with full implementation of a Clinical Center bed-banking plan, will eventually provide more equitable distribution of this vital resource.

During FY 78, the Intramural Program encouraged scientific inquiry through the direct operation of its sixteen laboratories and clinics; supervision of research contracts; and support of off-campus studies at Ft. Detrick, Maryland; Woods Hole, Massachusetts; and the Guam Research Center. In accord with the Institute's mission, the Program has sought to improve the nation's health by:

- o Investigating the basic structure and functional mechanisms of the nervous system;

- o Exploring the epidemiology and pathogenesis of neurologic and communicative disorders to facilitate prevention and early diagnosis;

- o Developing improved pharmacologic, surgical and other therapeutic approaches through laboratory investigations and clinical trials;

- o Disseminating new research findings to the health care community via timely publications and symposia; and

- o Training highly qualified young physicians and scientists in research methodologies.

In each of these activities, the past year has been one of exceptional accomplishment. During FY 78, 143 Research Projects remained active; 20 new projects were initiated, 9 completed, and 20 terminated. Collaborative efforts between Intramural scientists and other federal and non-governmental institutions characterized many of these studies.

Based on the judgment of Program scientists reporting on the nature of their research projects, 93% of FY 78 Intramural funds was directed toward fundamental (51%) and clinically applied (42%) studies, in which the development of new knowledge contributed to expansion of the science base. Basic work in neurophysiology, neurochemistry and bacteriology/virology/immunology continued to receive the largest budgetary allocations, while applied studies of infectious, demyelinating, metabolic, endocrinological and degenerative disorders were accorded the highest levels of support in the clinical neurosciences. The remainder of FY 78 Intramural funds (7%) was directed toward applications involving clinical trials, primarily drug trials. On the basis of research bed utilization in the Clinical Center, studies of neuromuscular diseases, brain tumors, and metabolic or degenerative disorders were most active.

Based on the record of scientific publications, Intramural scientists enjoyed another productive year. During 1977, over 250 research reports appeared in 131 different scientific journals and books. Those journals publishing the most papers authored by Intramural scientists were, in descending order, Experimental Neurology, Neurology, Brain Research, The Journal of Neurochemistry, Archives of Neurology, The Lancet, Annals of Neurology, and The Journal of Infectious Diseases.

Evidence of the high esteem accorded the Program's scientific performance derived from several sources. During the year, two Intramural scientists received the DHEW Meritorious Service Medal for significant research contributions and administrative leadership. Many other staff members received non-government recognition, such as the Washington Academy of Sciences Annual Award for achievement in biological sciences. In addition, rigorous evaluation of Intramural laboratories by the Institute's Board of Scientific Counselors, augmented by Ad Hoc specialists, yielded highly laudatory comments.

Details of the outstanding contributions made by Intramural scientists appear in the Laboratory and Branch Chiefs' summaries and in the individual Project Reports that follow. Nevertheless, a few of these accomplishments deserve special mention here.

As part of its investigations of the neuronal cytoskeleton, the Laboratory of Biophysics has focused on the ultrastructural features and protein makeup of axoplasmic neural filaments and microtubles. Using moderately thick sections and stereoscopic electron microscopy, an ordered transverse bridge lattice system associated with neurofilaments and microtubles has been observed in the axonal cytoplasm of several marine species. Close stereoscopic inspection of axoplasm from Loligo and Hermissenda indicates an extensive ordered network of thin transverse elements arranged in planes approximately normal to the fiber axis and spaced about 40 nm apart. These elements appear often to bridge neighboring neurofilaments and connect the lattice to axolemmal surfaces. It thus appears that the neurofilaments and microtubles, together with thin transverse elements, form a highly ordered lattice structure or gel intimately connected to the neurolemmal surface.

Research in the Laboratory of Neuropathology and Neuroanatomical Sciences has advanced our understanding of morphologic events underlying synaptic transmission. A rapid freezing technique developed by the Laboratory has shown that each quantal secretory event results from the fusion of one synaptic vesicle with the plasmalemma of the synaptic terminal. Because synaptic vesicles are so small and the initiation of exocytosis so rapid, visualization of these initial stages has been elusive. Using amebocytes from Limulus, which have relatively large secretory granules, it has been possible to visualize the very first sign of exocytosis, a tiny hole in the plasmalemma which subsequently widens. This observation is incompatible with the generally held belief that exocytosis begins as a broad approximation between the secretory granule and the plasmalemma, which subsequently thins and bursts. The present findings suggest, rather, that a local disruption in adjacent lipid bilayers occurs as the initial event in exocytosis.

The Laboratory of Molecular Biology has continued studies of the mechanism of vesicular stomatitus virus replication and autointerference. The chromosome of the vesicular stomatitus virus is a single-stranded RNA molecule that contains five genes. By separating the different messenger RNA molecules, hybridizing them to the genomic RNA, and inspecting the hybrid under the electron microscope, the location and sequence of these genes on the viral RNA have been determined. During the course of infection, vesicular stomatitus virus produces "defective interfering" particles that contain only a part of the viral chromosome (often less than one gene), but all of the viral protein. When these particles coinfect a cell with the homologous infectious particle, they markedly inhibit the replication of the infectious virus. Many different types of interfering particles exist, some of which contain genetic information from one end of the viral genome, while others contain information from the other end. All these particles autointerfere to the same extent. Interference occurs when there is a limited amount of replicase available for which the defective interfering particle genome competes favorably.

The Neuroimmunology Branch, as part of its continuing investigations of host-viral interactions in various models of nervous system infection, has been studying how measles virus under one set of conditions can produce an acute, fatal disease, while under other conditions the infection is subacute with nonlytic involvement of selective populations of neurons. For example, infection of Balb/c weanling mice with HNT strain measles virus produces an acute encephalopathology leading to death. The distribution of viruses is characteristic, primarily affecting limbic structures. No inflammatory response occurs, but electron microscopic studies show viral antigen in nerve cell bodies and in the presynaptic regions of dendrites. Conceivably, the pathophysiology involves a viral-induced dysfunction of synaptic transmission. The acute measles infection can be modified when mice of a different genetic background are used or if the Balb/c mice are given antimeasles antibody after receiving measles virus. In either case, only a few animals become acutely ill and die. Instead, a significant number develop a subacute disorder with onset 2-6 months after infection. Virus is detectable by immunofluorescence and, in contrast to the acute disease, there is a characteristic paravascular inflammatory response.

The Infectious Diseases Branch has induced brain tumors in some owl monkeys inoculated with in vitro cultivated JC virus from a patient with progressive multifocal leukoencephalopathy. These tumors were glioblastomas and contained "T" antigen of primate polyomaviruses. Cells cultured from the tumors also had "T" antigens. No infectious virus or virion antigens were detectable in the tumor or cultured cells.

The Laboratory of Neurophysiology has continued to use tissue cultured neurons as models to study the binding of certain drugs, amino acids, peptides and other substances known to influence the nervous system. This work has shown a variety of operationally distinct effects of opiate peptides including a neurotransmitter action, a neurohormonal action, and a neuromodulator action.

Recent investigations conducted by the Laboratory of Neural Control involve the characterization of the discharge patterns of primary and secondary afferents. The main question of interest is whether the gamma motor system, during normal movement, significantly modulates the sensitivity of muscle spindle receptors. Current results suggest that there is much less gamma bias, or coactivation of alpha and gamma motoneurons, than has been previously thought likely on the basis of reflex experiments. Spindle afferents often appear to behave passively, especially when the parent muscle is active in postural or limb stabilization actions. There is more evidence for coactivation of gamma motoneurons, and consequent enhanced sensitivity of the spindle afferents, when the parent muscle operates as a prime mover in the action in question, especially during normal, unrestrained locomotion.

In the Laboratory of Neuro-otolaryngology, studies of synaptic transmission in the inner ear have now shown that alpha-bungarotoxin reversibly blocks transmission between the crossed olivocochlear fibers and the cuter hair cells in the cat cochlea and possibly also between these efferent nerve fibers and auditory nerve fibers coming from inner hair cells. These observations suggest that cochlear efferent receptors are different from

cholinergic receptors at other vertebrate synapses and strengthens the hypothesis that the cochlear efferent receptors are cholinergic. Biochemical and morphologic study of synapses of cochlear nucleus neurons that receive input from the auditory nerve has provided additional evidence to indicate that glutamic acid, aspartic acid, or both may be neurotransmitters of the auditory nerve.

The Laboratory of Experimental Neurology has recently applied the deoxyglucose method to estimate glucose utilization and thus presumably neuronal activity following penicillin-induced seizures in the monkey. During the thirty minutes following penicillin injection into the right motor cortex, a series of focal seizures took place. With unilateral face seizures, there was a distinct, contralateral increase in neural activity in portions of the globus pallidus, thalamus, and motor cortex. When face-hand seizures occurred, there was a marked increase in contralateral activity, predominantly in the medial and lateral globus pallidus, but also involving the ventral thalamic nuclei, and both the motor and sensory cortex. With an extension to bilateral seizures, this pattern of contralaterally-increased neural activity remained. Little or no activation of cerebellar structures was observed in these studies.

The discovery by the Laboratory of Central Nervous System Studies that Creutzfeldt-Jakob disease is caused by a serially transmissible, self-replicating agent that passes through bacteria-retaining filters and is widely distributed in tissues and fluids outside the CNS of affected patients has produced a growing concern among hospital personnel regarding the potential hazards of caring for patients with dementing disorders. Indeed, there is now documented evidence of the transmission of Creutzfeldt-Jakob disease by corneal transplantation and by neurosurgical procedures with contaminated electrodes. A recently completed worldwide epidemiologic survey of Creutzfeldt-Jakob disease indicates that 15% of cases are familial, thus suggesting a genetic susceptibility to this disorder.

The Developmental and Metabolic Neurology Branch has obtained encouraging results during the past year regarding enzyme replacement therapy for metabolic disorders of the central nervous system. Previously, the Branch discovered a procedure for temporarily altering the blood-brain barrier so that systemically injected enzymes would penetrate into the brain. Now it has been found that central neurons have membrane receptor sites for the glycoprotein enzymes that are involved in metabolic storage diseases, and that these cells are able to effectively endocytose these enzymes. Since neurons possess the necessary apparatus for intracellular localization of exogenous enzymes, enzyme replacement as a treatment for disorders such as Tay-Sachs disease and mucopolysaccharidosis now can progress on a much more rational basis. Other studies conducted by this Branch suggest that the major myelin glycoprotein in the central nervous system is selectively concentrated in membranes that are transitional between the oligodendroglial surface membrane and compact myelin. Myelin-associated glycoprotein antibody titers are produced in rabbits when experimental allergic encephalomyelitis is induced by injecting rat myelin preparations. These findings suggest that myelin-associated glycoprotein is most likely involved in human demyelinating diseases, either as the site of viral attachment to myelin or oligoden-

droglial membranes, or by participating in an autoimmune response to viral modification of the glycoprotein.

Studies in the Laboratory of Neurochemistry to determine whether peripheral nerve graphs between genetically different animals of the same species can be used to repair nerve tissue injuries have continued to advance during the past year. Present results indicate that it is possible to rapidly and functionally repair a large injury to the peripheral nervous system with a nerve allograph that contains only minor transplantation antigens. This finding is of significance since no immunosuppression of the host is required. Apparently allogenic cells survive long enough to permit host nerve fiber regeneration through them, following which they are rejected and replaced by host cells. The results suggest that nerve allographs with only minor antigens may be useful in the repair of human neuronal tissue, for if minor antigens in man behave like they do in the experimental animal, there will be no need for immunosuppression of the allograph.

Administration of an inhalation mixture containing a non-radioactive isotope of oxygen, $^{18}O_2$, by the Experimental Therapeutics Branch to evaluate central transmitter metabolism produced readily detectable labeling of all major monoamine metabolites in cerebrospinal fluid. Using this technique, substantial differences in cerebral dopamine turnover have been identified between patients with Parkinson's disease and Huntington's chorea. Other studies of parkinsonian patients indicate that spinal fluid levels of tetrahydrobiopterin, the cofactor for tyrosine hydroxylase, are substantially reduced. This reduction might explain why tyrosine hydroxylase activity is characteristically diminished in patients with this disorder. Measurement of Substance P levels in human cerebrospinal fluid has shown consistent abnormalities in patients with spinocerebellar degeneration and various peripheral neuropathies, suggesting that central Substance P containing neuronal tracts may be involved in the pathophysiology of these disorders. Controlled trials of muscimol, a selective GABA agonist, failed to improve dyskinesias in patients with Huntington's chorea or psychotic behavior in patients with chronic schizophrenia, but significantly diminished involuntary movements in tardive dyskinesia patients. Observations on the biochemical mechanism of central receptor action have revealed multiple categories of dopamine receptors. An initial dichotomy recognizes that some dopamine receptors are unrelated to adenylyl cyclase, while others are closely linked to this enzyme. Further subcategorization is based on pharmacologic responses. For example, dopamine receptors in the anterior pituitary and those on presynaptic dopamine terminals in the corpus striatum are both adenylyl cyclase-independent, but differ remarkably in their responses to dopamine agonists.

The Medical Neurology Branch has previously reported the identification of a circulating IgG which blocks the binding of alpha-bungarotoxin to human junctional acetylcholine receptors at the normal neuromuscular junction in some myasthenic patients and to extrajunctional acetylcholine receptors of denervated human fibers in most myasthenic patients. The nicotinic acetylcholine receptor has now been ultra-structurally localized, both postsynaptically and presynaptically, suggesting that the pathologic antibody acts at

both sites to cause disease. Related studies have sought the rationale for the clinically observed benefit of thymectomy for myasthenic patients. The Branch's demonstration that epithelial cells in both "hyperplastic" and "involuted" thymus contain histochemically demonstrable acetylcholine receptors is consistent with the hypothesis that the mechanism of myasthenia gravis might include an alteration of thymic epithelial cells which makes them "foreign," in response to which B-cells make anti-acetylcholine receptor antibody that co-reacts with junctional acetylcholine receptors to cause paralysis. Recent findings by the Branch indicate that even in myasthenic thymuses considered "atrophic" by existing histopathologic criteria, there are, nevertheless, small nests of active-appearing cells. Conceivably, these cells contribute to the pathogenesis of myasthenia and their removal may lead to the improvement which follows thymectomy.

The Clinical Neurosciences Branch has re-examined the cortical representation of speech function utilizing electrical stimulation of the cerebral cortex in patients undergoing surgical treatment for epilepsy. Stimulation at the supramarginal and angular gyri produced dysphasia qualitatively similar to that obtained from stimulation of the anterior speech regions in the temporal lobe. Speech arrest was also induced when stimulation was applied to the occipital cortex and to the cortex medial to the supramarginal gyrus. Such stimulation may interfere with a search mechanism in which the non-verbal concept of the visual stimulus is linked to a specific word in memory that is then withdrawn for use. On the basis of autopsy specimens, it would appear that cortex bearing indispensable speech representation extends to within a few centimeters of both the occipital pole and the parietal midline.

Annual Report of the Section on Technical Development

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders and Stroke

October 1, 1977 - September 30, 1978

Theodore R. Colburn, Ph.D., Chief

The Section on Technical Development is a group of engineers, computer specialists, and technicians which provides technical services to the Intramural Research Programs of NIMH and NINCDS. A veterinarian and a group of laboratory animal technicians and animal caretakers provide animal medicine and care services to the Intramural Research Program of NIMH. The major functions of the Section are:

(1) Instrumentation research and development. Design and development of instruments and instrumentation systems which represent advances in the state-of-the-art. Most of the research within the Section falls in this category, and is generally done in collaboration with investigators in the laboratories of NIMH and NINCDS.

(2) Production of custom instrumentation. Design and fabrication of electronic, mechanical, and optical equipment to suit the particular needs of the requesting investigator. These instruments, while often quite complex, utilize rather than advance the current state-of-the-art in design techniques and components.

(3) Computer services. The Section assists the investigators in data collection, reduction, and analysis, by supporting two laboratory digital computers for general use, including real-time on-line applications, and by providing programming service and technical consultation.

(4) Laboratory animal medicine and care. The Section provides veterinary medical care and a centralized program of animal caretaking functions. A health care program for employees who work with animals is being developed.

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

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

When an investigator requires the services of the Section, he first meets with the Section Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether the Section will take on the project. In some cases it is more appropriate that the work be done elsewhere, due to the nature of the project or to an excessive backlog of work.

When the Section Chief agrees to take on a project, the investigator is required to submit a memo, initialed by his Lab Chief, which formally requests the services of the Section and provides complete specifications of the functions which are to be performed by the required instrumentation. Section personnel will respond in writing as to how the instrument is to be built, how much it will cost, and approximately when it will be ready. The Section does not charge for services, but the investigator will be billed for the cost of 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 Office transfer funds from his CAN to the Section's CAN.

Two essential changes have been made in the operation of the machine shop. Short-term mechanical jobs (four hours or less), which have an emergency nature such that an on-going research project is being delayed, are now being handled on a first-come, first-served basis by a technician assigned to that duty for a two-week period. This service has been instituted so that an investigator need not wait two or three weeks (our typical backlog) to have a small job done if his current research project will be delayed. However, we wish to stress that this service is provided only for urgent projects, and must not be abused by persons who do not properly anticipate their instrumentation needs, or simply wish to circumvent the system.

The second change involves the auxiliary machine shop. Although the Section has traditionally provided a facility for investigators to do their own mechanical work in an unsupervised situation, this policy was reconsidered in light of the frequent abuse of the machinery (generally due to inexperience and haste) and disregard for minimal safety and cleanup standards. We now station the technician responsible for small jobs in the auxiliary machine shop; in general, he will do the work formerly performed by the investigator. In certain cases where the investigator insists on doing the work himself, and the technician feels that he is competent to do so, he will be allowed to use the machines under the supervision of the technician.

INSTRUMENTATION

The following are selected instrumentation projects undertaken during the past year. These are chosen from a total of 302 projects, and are representative of the types of electronic instruments and systems developed by the Section.

(1) Patient Activity Monitoring System. The Section has continued to develop a system for monitoring the activity patterns of ambulatory subjects. The system is built around the basic activity monitor, which underwent its

third major redesign this year. As the device is now configured, a piezoelectric accelerometer generates a voltage when the limb to which the monitor is attached moves. If the voltage exceeds a preset threshold, an event is recorded. A standard timing period (generally 15 min.) is divided into 4096 equal intervals of 0.22 seconds, and only one event can be recorded in each interval. At the end of the timing period, the total number of events, divided by 16, is stored in solid state memory; the event counter is cleared and a new timing period is initiated. The memory can store activity totals for 256 timing periods (64 hours) and the range of activity counts for each period is 4096 ÷ 16 = 256.

One major feature of the new design is a complete reworking of the transducer amplifier to make its sensitivity more easily adjustable, more consistent among individual monitors, and less responsive to high frequency vibrations. Another new feature is the use of lithium iodide batteries. The advantages include off-the-shelf availability (the old batteries were custom fabricated); longer life (one year as opposed to two months); and ease of battery change.

We are about to let a major contract to produce miniaturized electronic circuits using thick-film hybrid technology. Using these circuits we will produce a monitor of half the current volume, and four times the current memory capacity. This device will be particularly useful for studies involving outpatients and children.

Stored activity data is extracted from the monitor by reading the device directly into a PDP-11/40 computer through interface electronics. The computers are located in Building 36, Building 10, and the WAW Building at St. Elizabeth's. With the computer handling the read-out, permanent storage, numerical processing, and plotting of the activity data, the need for manual data handling has been essentially eliminated. This development has been a major factor in the success of the activity monitoring programs thus far.

The activity monitor system has been well received by the technical community; over 70 monitors are now being used by eleven different research groups. Applications include research on manic-depressives, hyperactive children, Huntington's and Parkinson's patients, drug addicts, and restrained monkeys. Publicity gained from word-of-mouth and from our two publications has evoked numerous inquiries from investigators all over the country. We have filed a patent application, and several firms have expressed interest in licensing the device for manufacture.

(2) Measurement of Activity of Confined Animals. The Section has continued to innovate techniques of transducing and recording quantitative and qualitative aspects of the activity of confined or restrained animals. The following are examples.

(a) Rat Activity Monitoring Cage. An activity cage has been developed which electronically senses the location of an unrestrained rat within the cage, the amplitude and frequency of gross and fine movements, and the occurrence of rearing. The instrument produces two analog voltages which together contain this spatial and temporal activity information.

One system has been developed which will include sixteen of the activity cages interfaced to a PDP 11-03 microcomputer for data acquisition. Programs in the 11-03 will analyze the output signals from the cages in real time for types of activity pertinent to a particular study, such as gross movement, rears, and rotation. Summaries of that information will be recorded on a digital cartridge tape unit. The cartridges will be transported to a larger computer to be read for further analysis.

(b) Measuring and Recording Long-Term Hamster Activity. A new activity monitoring system was developed to allow an LSI-11 computer to continuously monitor the activity from sixteen animal exercise wheels and record the data on cartridge tape. The data is later transferred to the PDP-11/40 computer for analysis and plotting. The rotational activity of the wheels is detected using photodetector devices. The program continuously polls each input data line, and if a rotation occurs, a 16 bit data word is recorded. After a selectable time interval (usually 15 min.), the program will print out, on the terminal, the total rotational counts for each wheel during that time period. Using the tape recording system for storing the data, experiments lasting a week or more can be recorded without interruption.

(c) Activity of Monkeys in Restraining Chairs. A system has been developed for periodic measurement and recording of activity and temperature data from as many as twelve monkeys in restraining chairs, for purposes of observing physiological rhythms. A thermistor affixed to the cortex transduces temperature, and a specially designed activity monitor mounted to the skull measures head movement. The data from all animals is periodically acquired by a microprocessor and stored on digital cartridge tape for later analysis on a digital computer.

(d) Rat Rotation Monitor. This device transduces and counts the clockwise / counterclockwise rotations of a rat. It is used in pharmacology studies. During the past year the design was modified to allow more accurate use for a larger number of animals by addition of memory circuitry. The memory stores total counts for up to five animals and up to thirty-two time intervals. The time interval can be selected over the range 5-60 minutes, in five minute increments. The summary counts can be retrieved from memory via LED display by switch selectable address (animal and interval) on the front panel.

(3) Activity Monitor of Chorea Movement. An activity monitoring system is being developed for detection of choreiform movements for use in a periodic, clinical testing procedure to help quantify the efficacy of drugs used in treating the disorder. The apparatus is battery powered and portable, and involves placing acceleration transducers on the arm of the patient. The experimenter dials in a time period between 1 and 99 sec. The start button is then pushed and the patient asked to perform a certain task. Every time a choreiform movement is detected (the transducers' output voltage crossing a set threshold level), a counter is incremented, and the total counts displayed. At the end of the time interval, the total count is held on the display until the counter is manually reset.

(4) Computer Controlled Visual Display System. A system is being developed that uses a display oscilloscope to present visual patterns of variable contrast and frequency to normal and operated monkeys, in experiments designed to determine contrast sensitivity thresholds. The behavioral paradigm involves the monkey pressing the proper button in response to the display presented, to receive a juice reward. An LSI-11 computer is interfaced between the display generating circuitry and other logic controlled circuits used to present the display and control the experiment. In addition, the LSI-11 logs the data and computes the sensitivity thresholds for each experimental series.

(5) pH Amplifier. A low noise, fast responding amplifier was developed to be compatible with a standard Beckman pH electrode and to provide a 10 millivolt change for each 0.1 pH unit change. The device has an adjustable baseline allowing the output to be "zeroed" over the pH 1 - pH 7 range. Subsequent changes in pH can be accurately measured by the output voltage change. Calibrated outputs and temperature compensation are included also.

(6) High Voltage and High Current Pulse Generator. A pulse generator capable of delivering $\pm$ 100V pulses at 100 mA has been developed to investigate the effect of electrical stimulation on the cellular development of tissue culture cells. This high level output allows simultaneous stimulation of multiple groups of culture plates, where each group contains five or more series-connected plates. The generator employs digital counting techniques so that all pulse train timing parameters are set by six groups of front-panel thumbwheel switches.

(7) 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.

(8) 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.

(9) Large Screen Display System. A large screen (16" x 12") CRT display system is being designed in order to present complex spatial and temporal visual patterns to monkeys. The circuitry will generate the Y-axis raster, the X-axis sweep, the Z-axis blanking, and the rotation of X and Y. This hardware will receive digital values for Z-axis modulation, angle of rotation

X and Y bounds of D.C. positions via a DMA interface to a PDP LSI-11 microprocessor. The microprocessor, in turn, will be downloaded from a PDP-11/40 minicomputer, which will be controlling the experiment and collecting data.

COMPUTERS

The Section on Technical Development 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. STD 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 the 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. This capability should be used more in the future.

The Section provides software support for the individual investigators. Many procedures have been written 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. System studies are conducted, on request, for possible use of the computer in laboratory experimentation.

The Section conducted a series of training courses in the use of the laboratory computer during the past year. These courses were designed to be used by scientific personnel beginning to use the computer and provided the framework to enable the user to get his hands on a computer and begin working on a specific application, not as academic exercises. Unfortunately, only a very small percentage of the students actually used a computer following the courses. The Section does not plan to offer these courses in the future, but will continue to provide individual training to individuals with specific applications.

The concept of the microcomputer as an integral part of laboratory instrumentation for process control, data logging, timing and coordination of instruments was thoroughly explored during this fiscal year. This concept has resulted in the integration of the functions of the engineering and computer components of the Section. The microcomputer is increasingly being used in connection with instruments developed by the Section, such as the Patient Activity Monitor (PAM) and the rat activity monitoring cages, as well as commercially available equipment such as rat rotation cages. The use of the microcomputer extends the range of application and adds to the versatility of such instruments. This concept will open large areas of application with hopefully low overhead and shortened development time. The Section has explored the available techniques and equipment and has become familiar with

the capabilities, as well as the drawbacks and problem areas, of the use of microcomputer techniques and equipment. This integration of engineering and computer technology should provide continually improving technical support.

Examples of computer-related projects include:

(1) Membrane Activity of Neurosecretory Cells. A computer based system for the digitization and analysis of membrane currents elicited from voltage clamped neurosecretory cells was developed in collaboration with the Laboratory of Neurophysiology. This system provides a means to study the conductance of cell membranes that may exhibit bursting capabilities during clamping. The activity of the action potential may be retained while studying the slower changes in the conductance of the membrane. This system is applicable to all voltage clamp data and should eliminate tedious pencil and ruler processing of current changes.

(2) Continuous Performance Task. A PDP-11/03 microcomputer has been used to implement the Continuous Performance Task, a testing procedure used to measure attention and vigilance. Letters of the alphabet are displayed in random sequence on a dot matrix light emitting diode display. The subject responds by pressing a button when a certain letter sequence (i.e., the letter A followed by the letter X) appears on the display. The microcomputer records the number of correct responses and the response times. Two error scores are recorded: omissions (failure to respond when appropriate), and commissions (responses made when there was not an appropriate stimulus combination). At the completion of the test session, a summary of the results, including the mean and standard deviation of the response times, is printed on a terminal.

(3) Automated Analysis of Autoradiographs. An operator interactive system for the densitometric analysis of autoradiographs has been developed. Under control of a PDP-11/34 computer, the information present in pictorial form in the autoradiograph is converted to digital densitometric data by a scanning microdensitometer and displayed on a video monitor. Image features are isolated using a joystick controlled outlining frame, with area and average density computed on request.

(4) Cell Culture Analysis. The Section developed a prototype system for the study of the electrical activity of neural cell cultures on the PDP-12. This system was designed to provide on line control of artifacts introduced by the measurement systems, a method of interactively determining thresholds and to set parameters. Data may be visually displayed in a variety of formats as the experiment is in progress. The elicited activity is immediately available to enable the investigator to select the proper measures to study different types of cells. The system analyzes excitatory and inhibitory post-synaptic potentials, spontaneous and elicited action potential, as well as spontaneously occurring changes in membrane activities. These routines analyze elicited responses from individually presented stimuli, as well as stimuli presented as trains of pulses and as pairs of pulses.

(5) Membrane Noise Spectrum Analysis Programs. A data acquisition and spectral analysis system has been written for the PDP-11/40 which effectively quantifies the parameters of membrane current fluctuations in cultured spinal

nerve cells. The effects of drugs on the transmitter mechanism of the cell can be studied by using these parameters as statistical measures. The programming system is a continuation and enhancement of a similar system used to study voltage fluctuations in turtle cones.

(6) PAM Continuous Activity Monitoring. Until recently, the data analysis methods used to interpret PAM data have been essentially restricted to studying isolated records and obtaining statistics on a day-to-day basis. A new software system has been developed which allows one to collate all the data for one patient into one "continuous" file in order to carry out a "global" analysis. It also serves obvious organizational purposes. It is hoped that the regular PAM data structure would become only an intermediate step in establishing a patient activity record over periods of time in the order of months. The advantages of this data system include the ability to study long-term trends and cycles, averaging data, frequency analysis, amplitude analysis, as well as data compression for visual inspection and presentation.

ENGINEERING, COMPUTER AND FABRICATION SERVICES

This table shows the distribution of the Section's workload among the various laboratories.

LABORATORY OR BRANCH	HOURS	PERCENT
Division of Special Mental Health Research, NIMH	4591	13.72
Neurophysiology, NINCDS	2507	7.49
Neuropsychology, NIMH	2383	7.12
Clinical Science, NIMH	2300	6.87
Section on Technical Development, NIMH/NINCDS	2109	6.30
Intramural Research Program, NIMH	2083	6.22
Biological Psychiatry, NIMH	2016	6.03
Experimental Therapeutics, NINCDS	2007	5.99
Intramural Research Program, NINCDS	1982	5.92
Clinical Psychobiology, NIMH	1674	5.00
Developmental Neurobiology, NICHD	1458	4.36
Neurophysiology, NIMH	1442	4.31
Brain Evolution and Behavior, NIMH	1016	3.04
Cerebral Metabolism, NIMH	788	2.35
Biophysics, NINCDS	733	2.19
Neuropathology and Neuroanatomical Sciences, NINCDS	718	2.15
Molecular Biology, NINCDS	631	1.89
Neurochemistry, NIMH	435	1.30
Clinical Neurosciences, NINCDS	417	1.25
Neurochemistry, NINCDS	404	1.21
Central Nervous System Studies, NINCDS	372	1.11
Neurobiology, NIMH	335	1.00
Developmental Psychology, NIMH	294	.88
General and Comparative Biochemistry, NIMH	259	.77
Psychology and Psychopathology, NIMH	205	.61
Neuro-Otolaryngology, NINCDS	138	.41
Neuroimmunology, NINCDS	84	.25
Others, NINCDS	70	.21
NIMH (Total)	20,876	62.40
NINCDS (Total)	11,118	33.25
NICHD (Total)*	1,458	4.35
	33,452	100.00

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

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-01526-11-ODIR

PERIOD COVERED
October 1, 1977 to September 30, 1978

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 IRP, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Office of the Clinical Director

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The activity of two individual neurons in the somatosensory cortex of the cat were recorded simultaneously with the cortical surface waves. The distance between the two neurons was less than 0.5 mm. It was found that their spontaneous discharge patterns as a function of time and their responses to stimulation of a peripheral nerve or of the nucleus centrum medianum of the thalamus were different. With the cortex under the effect of strychnine, these neurons became epileptic and their epileptic discharges were not necessarily synchronous. Most of the cells underwent a prolonged depolarization for 100-680 msec with a magnitude of 20-50 mV. In these instances, there was no post-excitatory hyperpolarization suggesting that inhibitory impulses generated from the internuncial cells in the cortex are blocked and that the large and prolonged depolarization of the epileptic neuron is a result of the invasion of "unrestrained" excitatory synaptic impulses. During the period of prolonged depolarization, intracellular stimulation with depolarizing or hyperpolarizing pulses were applied. This did not change the time course of the prolonged depolarization although the intracellular stimulation altered the discharge frequency of the cell.

Project Description:

Objective: To investigate the excitability characteristics of cerebral cortical neurons with particular reference to the mechanism of epilepsy.

Methods Employed: Glass micropipette electrodes were used to record the electrical activity of cells in the pre- or post-cruceate gyrus in anesthetized cats. When the electrode was found inside the cell, depolarizing or hyperpolarizing pulses were applied. In all cases, stimulation of the radial nerve or nucleus centrum medianum of the thalamus were also applied to study the evoked potential recorded from the cortical surface and from the cortical neurons. Thereafter, strychnine was put on the surface of the cortex and similar stimulation and recordings were made.

Major Findings: Strychninized epileptic neurons were found to be different from neurons which were also epileptic but resulted from repetitive stimulation of the cerebral cortex. In the latter case the discharge of the neuron was not accompanied by prolonged depolarization with a magnitude of 20-50 mV and was usually terminated by a post-excitatory hyperpolarization potential indicating that there is a negative feed-back mechanism through the cortical internuncial cells. On the other hand, the strychninized epileptic neuron displayed a prolonged depolarization potential which was not followed by a post-excitatory hyperpolarization potential suggesting that the negative feed-back mechanism was not operative. Without the negative feed-back activit the neuron is likely to respond with excessive discharges. As a result epilepsy occurs.

Significance to Biomedical Research and the Program of the Institute: The effectiveness of clinical treatment of epilepsy depends on our understanding of the excitability characteristics of the nerve cells and the interaction of these cells in the central nervous system. The present investigation was made to further understand the mechanism of epilepsy and eventually develop a means to overcome the mechanism of epileptic discharges.

Proposed Course of Project: To continue the present investigation with particular reference to pharmacological treatment of epilepsy.

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-02010-06 ODIR

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Neurophysiological Mechanisms of Pain

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 IRP, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Office of the Clinical Director

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Mar-land 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	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)

To investigate the excitability characteristics of the fiber components which mediate pain sensation and to study the electrical properties of the cell membranes of the neurons which have synaptic connections with the pain fibers. Special emphasis was made in reference to the response thresholds, conduction velocities and magnitudes of the various fiber components in the peripheral nerve and to the conductance and capacitance of the cell membrane of the neurons, which have synaptic connections with the pain fiber components, in response to various analgesic agents including morphine, beta-endorphin, etc.

Project Description:

Objective: To study the physiological mechanisms of pain and to record the change in responses of the pain fiber component in a peripheral nerve and in CNS nerve cells when other sensory inputs and various pharmacological agents are applied to the nerve or to the nerve cells.

Methods Employed: The saphenous, splanchnic, cervical sympathetic or cervical vagal nerve was placed in a specially constructed stimulating-recording assembly. The nerve was stimulated through a function-generator with a negative Haversine wave of various durations and intensities. After a series of control responses of the nerve was recorded, the nerve was exposed to different temperatures, to saline solutions of various tonicities or to various anesthetics. Under these circumstances, the response thresholds, conduction velocities and amplitudes of the fiber components in the nerve were again recorded. Thereafter, the nerve was re-exposed to a normal physiological solution and the recovery of the nerve was observed. For studying the responses of nerve cells in the CNS, intracellular micropipette electrodes were used. Cells which have synaptic connections with the A-delta and C-fiber components of the nerve were identified and their responses to stimulation of these fiber components were recorded. Thereafter, the change in the discharge pattern of the cell and the change in conductance and capacitance of the cell membrane in response to fiber stimulation were recorded.

Major Findings: Under similar experimental conditions, the amplitude and duration of a given fiber component in the compound action potential of the saphenous splanchnic, cervical sympathetic and vagal nerve were different, but the response thresholds and conduction velocities of these fiber components were almost the same. It was also found that there was a large A-delta component in the sympathetic and splanchnic nerves; and a large C-fiber component in the vagus. These two components are known to mediate pain sensation. Furthermore, there was a power function relationship between the conduction velocity and temperature of the nerve; and within a range of 40 - 20^{0} C, change in conduction velocity for each degree centigrade of the A-beta fibers was 6.4 meters per second; of the A-delta, 0.85 meters per second; and of the C-fibers, 0.08 meters per second. The A-delta fibers, as compared with the A-beta and C-fibers, were found to be most sensitive to temperature change and to analgesic and anti-inflammatory agents. In regard to the change in membrane capacitance and conductance of the neurons in the CNS in response to A-delta and C-fiber stimulation, experimental results are, at present, not sufficient for any conclusion to be made. This has been due to interruption of our experimental activity because of the renovation of our laboratory.

Significance to Biomedical Research and the Program of the Institute: The present study may contribute to our understanding of the mechanism and treatment of pain.

Proposed Course of Project: The present study is to be continued with particular reference to intracellular recordings from neurons with synaptic connections of the A-delta and C-fibers and to the changes of membrane potentials and properties in response to various pharmacological agents.

Publications: None

Annual Report
Oct. 1, 1977 through September 30, 1978
Medical Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke

W. King Engel, M.D.
Chief, Medical Neurology Branch

Introduction: An inter-related multidimensional attack on the chosen target diseases is emphasized in our application of basic research techniques to clinical neurologic problems. The current techniques consist of: histochemistry, tissue culture, 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 main emphasis is on the neuromuscular diseases -- they are considered to be affecting more than 1,000,000 persons in the country.

For the clinical investigations, 308 patients were admitted for a total of about 7,300 patient days, and there were 1,063 outpatient visits. About 460 patient muscle biopsies were processed histochemically and reported out -- about 90 of those were from outside hospitals. Many other outside biopsies are submitted for formal opinion. Neurologic consultations were provided on 1,140 patients of other departments in the Clinical Center, with performance of indicated muscle and nerve biopsies, and electromyograms and nerve-conduction studies (>100 consult electrophysiologic studies). In the past year, 40 were published, 32 are in press, and there were more than 50 presentations to meetings.

The one-year approved residency training program in neurology was continued. Approximately 12 neurologists and other physicians and 8 technicians came this past year to learn clinical research techniques in neurology, especially in neuromuscular diseases and the application of enzyme histochemistry and special clinical electrophysiology thereto. A few residents from various other hospitals rotate through our service.

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

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.

Amyotrophic Lateral Sclerosis (ALS): The cause of ALS is unknown -- dysmetabolic vs. viral are the two main possibilities. We favor the former but are pursuing both.

Therapeutic trials: We have found, and presented, that CSF levels of cAMP and cGMP depressed, 40% and 50% respectively, in a group of 58 ALS patients, due to decreased production within the CNS on the basis of the i.v. probenecid test (with NNMC). Phthalazinol, a cyclic nucleotide (especially cAMP) phosphodiesterase-inhibitor, used to raise cyclic nucleotide levels caused a dose-related increase of cAMP but not cGMP. A single-blind placebo-controlled 4-month-each crossover therapeutic trial with phthalazinol (20-75, mean 43, mg/kg showed no alteration of the steady, carefully quantitated progression of disease in 9 ALS patients; one man with only bulbar lower-motor-neuron ALS steadily progressive for 2.5 yr. prior to the drug has had no further progression for 3-1/2 years on phthalazinol 35 mg/kg/d. (with NNMC and Atherosclerosis Res. Inst. Japan). Technical factors influencing CSF cAMP and cGMP assays have been delineated, including pH, storage, freezing, and CSF-phosphodiesterase-inactivation; no ventricular-lumbar gradient exists. A blood-to-CSF barrier for cAMP in man, resistent to 40-fold increase of serum cAMP (glucagon-provoked) was reported (with NNMC). In the wobbler mouse, a mutant with motor-neuron degeneration, decreased spinal-cord cGMP is being reported (with ID).

Polyinosinic-polycytidylic-acid: (Poly-ICLC) an inducer of interferon (a autogenous intracellular antiviral substance) has been used in 3 ALS patients without therapeutic effect, even though interferon production was moderate (with NIAIDD); several parameters of leucocyte response were concurrently measured v.i. Human leucocyte interferon, was not beneficial in one ALS patient (with Stanford Univ. and Finnish Red Cross).

Adenine arabinoside (Ara-A), an antiviral agent, 10 µg/kg/d i.v. X10d. is being evaluated as a possible therapeutic agent in 10 ALS patients.

To study the possible role of abnormality of parathyroid function and/or calcium metabolism in ordinary ALS, we have studied, now reported and are presenting various parameters of calcium homeostasis. Reduction in retention of oral $^{47}CaCl_2$ was seen in all neuromuscular diseases studied, but in ALS patients was more pronounced than in myopathy and neuropathy patients (>80 retention tests). That reduction was due in part to inactivity because it was presnet in paraplegia regardless of etiology, but was especially low in paraplegics with ALS. That decrease was not due to decreased 25-hydroxy vitamin D levels found to be normal, nor to resistance to dihydrotachysterol because administration of the latter mathematically corrected the decreased retention (but did not cause clinical improvement). Serum-calcium, urinary 24-hr calcium excretion, and renal cAMP clearances were normal. Serum mean parathyroid hormone concentrations, measured, were higher than normal in 40-50% of all patients with neuromuscular disease and similar in all subgroups: ALS myopathy, neuropathy.

Folate (oxidized and reduced forms) and B_{12} in CSF and serum in sporadic and familial ALS were measured and compared to values in neuropathy and myopathy (with Johns Hopkins and Bronx VAH) and no significant reductions found when age and sex-matched groups were compared (being reported). Thus the posterior column degeneration in familial forms (originally described from this Branch) is not directly due to local reduction of folate or B_{12} (however, interference with its action on cells could be occurring). Additional data suggest that probenecid does not cause rise in CSF folate in ALS patients.

In 54 ALS patients, including 6 with a late-post-polio progressive muscular atrophy (LPPPPMA) syndrome compared with matched other-neuromuscular-disease controls also with muscle wasting, Ab-distribution and geometric-mean Ab-titers in sera were not statistically different for poliovirus 1,2,3, Coxsackie B3, B4, influenza A, mumps, varicella, measles, rubella, herpesvirus 1,2, cytomegalovirus, and toxoplasmosis; nor was CSF poliovirus 1,2,3, Ab abnormal. CSF HI-Ab (or HI-Ab-like activity) $\geq$ 1:2 against rubella antigen was present in 10% of ALS patients (also 10% of multiple-sclerosis patients, but none of our 52 neuromuscular-disease controls. The positive ALS patients had somewhat more rapid early atrophy but no history of overt rubella infection involving the CNS. Serum/CSF Ab ratios did not demonstrate local specific Ab synthesis in the CNS against any of the virus antigens tested, in both ALS and non-ALS patients. Thus, if ALS is caused by a virus, its detection will require techniques other than the assay employed here.

Benign Focal Amyotrophy, important because of its excellent prognosis but usual misdiagnosis as fatal ALS, was originally described by us 10 yrs ago as a separate clinical syndrome, and in young-adult males possibly a distinct disease. We have now presented our updated experience. It is a limited form of lower-motor-neuron disease confined to the upper extremities, unilateral or markedly assymetric, gradual in onset, progressive for 1/2-4 years and then clinical stability, or only very minimal progression, for 2-10 yrs to present. We have recently found CSF oligoclonal Ig bands in some, raising a question of a viral/dysimmune pathogenesis. If related to ALS as a spontaneously arresting form, it may hold a clue to treating that disease.

Motor neurons of 15-day fetal-rat spinal cord are now being grown in tissue culture and serving as test objects of possibly toxic fluids or agents related to ALS patients.

CSF and blood cells are being analyzed biochemically seeking an abnormality specific to ALS. Total fatty-acid composition of CSF is being analyzed by quantitative computer-integrated gas-chromatography of fatty-acid methyl-esters purified by thin-layer chromatography; with this technique we have just finished showing no fatty-acid abnormality of erythrocyte membranes in ALS patients, and will also study their platelet membranes. Enolases, neuron-specific and non-neuronal, are now being studied in ALS CSF (with NIMH). ALS platelets show a reduced rate of initial uptake of H-serotonin and of initial release to 0.5 μ/ml thrombin, and normal cAMP- and cGMP-phosphodiesterase.

Histochemical properties of lower motor neurons (LMNs) are being explored, looking for special properties of them and disease-characteristic defects thereof. We have previously shown phosphorylase to be special for LMNs. Now, using the alkaline phosphodiesterase (PDE-I) reaction, α-naphthyl-thymidine-5'-PO_4 as substrate, we find in normal cat cord dark staining of the pia and of large and small vessels within the cord (but not capillaries), and no neuron soma or fiber or glia staining. Five fluoroscein-labelled lectin probes, for various membrane-bound saccharide groups, in normal and ventral-root-section reacting cat cord showed: with all but Soy, gray > white staining, possibly attributable to more surface-membranes; root-myelin > tract-myelin stained with ConA; only Soy selectively stained vessels; a lat posterior zone capping the posterior horn demonstrated only with Soy; only wheat-germ stained the nucleoplasm (not nucleolus or cytoplasm) of motor neurons unchanged in chromatolysis. In ALS cord (autopsy), no abnormal findings with any lectin was detectable.

As an induced animal-model (with LNC and Johns Hopkins), Swiss mice acutely infected with poliomyelitis, which attacks anterior horn cells, are being studied to ascertain whether the time-course of reduction of neurotransmitters, substrates high-energy intermediates, and cyclic nucleotides shows an early drop, e.g., of cGMP, in spinal cord antecedent to paralysis, attempting to determine the sequence of the virus-induced biochemical alterations.

In Shy's Scientific Basis of Neurology, two extensive chapters have been published, one on numerous aspects of the biology of the lower motor neurons as a basis for understanding and investigating diseases thereof (>1000 references), and the other on various aspects of the several motor neuron diseases as well as 3 chapters on other neuromuscular diseases: central core disease, rod disease, and muscle fiber hypotrophies. A clinical-research conference on ALS is being published (with Mayo Clinic and Johns Hopkins).

The hypothetical pathogenesis of central core disease has now been refined on the basis of 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 we propose it to be a paucity of lower motor neuron (LMNs), especially of the hypothetical type-II LMNs occurring during development, either impaired formation or increased normal loss as a part of "neurothanosis" (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).

To study the dyschwannian neuropathies we have developed techniques to grow in tissue-culture human schwann cells, e.g., obtained from diagnostic nerve biopsies. In primary dysschwannian neuropathies (e.g., metachromatic leucodystrophy, familial idiopathic "Charcot-Marie-Tooth" neuropathy the

schwann cells in culture should have the biochemical defect, which can be elucidated and when identified can be treated in culture. They might also in other acquired neuropathies such as diabetes mellitus. Electron-microscopy and electronmicroscopic enzyme- and immunocytochemistry is being done on both the original biopsied nerve and the schwann cells cultured from it for direct comparison. Surface membrane markers being localized electronmicroscopically include concanavalin A (for α-d-mannoside and α-d-mannoside and α-d-glucoside groups), ruthenium red for acid mucropolysaccharides, α-bungarotoxin for nicotinic acetylcholine receptors, and tannic-acid. A variety of biochemical assays are being applied to the cultured schwann cells.

In the majority of patients we see the PN is of undiscernable cause. Those which are non-familial we may treat with LT-HSDAD-prednisone, especially if less than 5-years duration, postulating a dysimmune pathogenic mechanism. We are publishing a summary of our ongoing experience in 25 carefully-documented patients who have had good to outstanding success, most having been given-up on by others and some having come with diagnoses of non-treatable diseases (e.g., ALS or "Charcot-Marie-Tooth" disease). Long-term treatment is required -- too-rapid reduction of dosage too soon results in exacerbation. Excellent results have been sustained for as long as 13 years in an adult and 10-1/2 years in a child (who at age 22 is still regaining motor skills). Our correlative studies indicate that patients most likely to respond have the triad (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 LT-HSDAD-Pred. Prednisone has a broader scope of responsitivity in this disease than previously believed. Virtually all our corticosteroid-responsive patients are cortiosteroid-dependent requiring 5-20 mg single-dose q.o.d. to prevent exacerbation.

In the cultured non-innervated muscle fibers of chick, rat and human tissue, we have now published that α-bungarotoxin demonstrated diffuse plasmalemmal nicotinic acetylacholine receptors (nACHRs) of single myoblasts through multinucleated well-differentiated contracting fibers. They did not appear electron-microscopically to have "hot-spots", as reported by others from light-microscopic autoradiography, -- we suggested "hot-spots" may be artifacts based on subtle plasmalemmal folds.

Correlations of histochemistry with single-fiber-EMG (SFEMG) in patients with various neuromuscular diseases have been published. Ordinary neurogenous deinnervations have significant densification of motor-units (from collateral foreign reinnervation of orphaned muscle fibers) and fiber-subtype grouping histochemically. Chronic myopathies (Duchenne dystrophy, polymyositis/dermatomyositis, limb-girdle morphologically-non-specific myopathy snydrome) had mild densification by SFEMG but no definite subtype grouping histochemically, indicating a greater sensitivity of SFEMG. From study of 8 patients with muscle fiber-type predominance without major evidence of ordinary denervation (5 central core disease, 1 congenital rod disease, 1 type-I-fiber-hypotrophy-with-central-nuclei, 1 type-I-fiber-predominant unclassifiable neuromuscular

disease) it became evident that, in a field of fiber-type predominance, subtype grouping is requisite for a histological diagnosis of neurogenous denervation (although its lack does not, hypothetically exclude an abnormality of LMNs).

In patients with scoliosis, our continuing studies show a wide variety of neuromuscular diseases (by muscle biopsy histochemistry) associated with and probably causing scoliosis, most commonly some form of neurogenic muscular atrophy. Some of our scoliosis cases were previously considered "idopathic", a group we are studying in more detail (with DuPont Inst., Wilmington, DE).

Polyneuropathy (peripheral Neuropathy) (PN): The peripheral neuropathies comprise a group of disorders of various causes, more than half unknown. They always cause serious physical handicap sooner or later in the course of the disease, sometimes associated with intractable pain and ulceration and 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 peripheral nerves.

Dysschwannian neuropathies are ones in which the neuronal-axon defect is considered secondary to Schwann cell abnormality, whereas in dysneuronal neuropathies the LMN neuron soma and/or axon is the major site of abnormality.

The mechanisms of HSDAD-Pred anti-dysimmune effect on circulating blood lymphocytes and its effect on cerebrospinal-fluid (CSF) lymphocytes and immunoglobulins have been studied. We are publishing our method of identifying T- and B-lymphocytes in CSF, and that percentages normally are the same as in peripheral blood (72 and 16%). In 9 chronic idiopathic relapsing polyneuropathy patients, with 2-10X elevated CSF IgM and or G, studied longitudinally, HSDAD-Pred caused borderline reduction in CSF T- and B-lymphocytes and definite reduction of IgM, IgG and IgA (but not total-protein), and serum IgG but not IgM or IgA: thus the accompanying remarkable clinical improvement may have been by prednisone affecting lympocyte Ig production (with IB) directly within the CNS rather than numbers of lymphocyte subpopulations. We showed that high-single-dose alternate-day prednisone did not impair development of antibody in patients, cf. untreated persons, prophalactically immunized with influenza virus.

A new treatment, Polyinosinic-polycytidilic acid poly-L-lysine stabilized with carboxymethyl cellulose (Poly-ICLC), has been remarkably successful in a patient with prednisone-plus-azathioprine unresponsive chronic presumably-dysimmune relapsing polyneuropathy (with NIAIDD). 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 6 months. Although Poly-ICLC is an interferon-inducer, we postulate it is beneficial in this dysimmune by an action we found, marked lymphocytopenia (to 10-20% of baseline) 1-2 days after the drug with return to baseline by 4-5 days (granulocytes actually rose 3-fold at 6-24 hr. and fall only 30% below baseline at 2-3 days and return to baseline by 3-5

days. We propose this to be a new antidysimmune treatment potentially benefical to other dysimmune diseases (if acting through interferon it would provide a new insight into the pathogenesis of relapsing neuropathy). We are extending the trial to other dysimmune neuropathy patients, dermatomyositis/polymyositis, and myasthenia gravis patients. We are also measuring in detail the quantitative and qualitative responses of B- and T-lymphocytes, including killer-T-lymphocytes (with NCI).

Amyloid neuropathy is of particular importance in respect to pathogenic mechanisms. The "idiopathic" form, beginning in mid or later adulthood, we reported last year a male predominance (8/10) and all patients having an associated, and probably causative, plasma-cell dyscrasia, detectable in in 8/10 of our patients as multiple myloma, and/or serum and/or urine "paraprotein" immunoglobulin fragments (IgG-kappa >> IgG-lambda, IgM-lambda). Since we proposed that the neuropathy is due to a systemic metabolic abnormality, possibly related to a circulating abnormal protein fragment (i.e., a "para-Sparafucile" phenomenon, v.s.), rather than to pressure from multifocal amyloid deposits of immunoglobulin fragments or ischemia, we are seeking the topographic lodgement of that putative fragment in amyloid patients and others, especially ones with monoclonal Ig spikes in serum, urine or CSF or with oligoclonal bands.

Substance P in CSF by radioimmunoassay has been found decreased in our neuropathy patients, but in no other neurologic diseases or myopathies surveyed (with CPB and Harvard). Further neuropathy patients are now being studied to seek possible sensory-vs.-motor and/or disease specificity.

Patients with muscle cramps and pains without detectable neurologic deficit were reviewed in detail. Of 63, 47 had abnormal muscle biopsies: 19 denervation (with normal nerve conduction velocities, 15 type-II fiber atrophy, 13 phosphorylase deficiency, 1 phosphofructokinase deficiency, 2 defect in utilizing long-chain fatty acids. This subclinical denervation is the commonest cause of otherwise unexplained muscle cramps and pains.

In multifocal eosinophilic granuloma, multifocal extradural compression of cervical roots responded rapidly and completely to corticosteroid plus radiation therapy (with RRB, NICHHD).

A new principle/model for inducing an experimental allergic neuropathy (EAN) in animals (sheep) has been reported. It utilized immunization with soluble nerve protein fraction (in contrast to lipid-associated protein of myelin used in previous EAN models). This represents a new potential model of some human dysimmune-dysneuronal peripheral neuropathies such as in some patients we have seen with prednisone-responsive neuropathies without demonstrable schwann-cell involvement. It also represents a new approach to studying certain dysimmune disorders of the CNS, such as multiple sclerosis and para-infectious encephalopathies. Since our EAN animals also have a component of blockade of neuromuscular transmission that is responsive to edrophonium, the model may have some relevance to myasthenia gravis or other disorders of the

neuromuscular junction. We now have found two patients who are rather similar, having prednisone-responsive trunk and limb muscle weakness, no cranial nerve muscle weakness, atypical neuromuscular transmission defects, and responses to anticholinesterase, normal nerve conduction velocities and normal CSF protein. Thus, 2 previously therapeutically-overlooked patients have achieved remarkable clinical improvement with prednisone.

We have now established combined studies of patient sural nerve biopsies in vitro -- including in vitro nerve conduction velocities of fast and slow fibers and other electrophysiologic parameters, teased fiber histochemistry, electronmicroscopy, and EM-histochemistry, as well as tissue-culture of the schwann cells, v.s., allowing more precise and direct multidimensional analyses of the afflicted nerves in polyneuropathy patients. This has shown, for example, markedly slowed conduction in small (C) fibers correlated with small-fiber pathology while clinical conduction times (which do not "see" C-fibers) were normal. As a parallel model cat saphenous nerve is being used to work out techniques such that electrophysiologic parameters combined with metabolic manipulations can be utilized to seek normal and abnormal features of human nerve biopsies (Schwann cells and ? axons) in vitro.

Central Nervous System Disorders: 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.

cAMP is thought by some to be a mediator of synaptic transmission of some systems in the cerebellum. Our newly developed histochemical technique for its synthesizing enzyme, adenylcyclase AC, being reported, shows the greatest amount to be in cerebellar blood vessels, and of the neural-associated enzyme the greatest amount was in the basket-cell basket endings at the base of the Purkinje cell, not in the Purkinje-cell soma as previously supposed from tissue-slice biochemistry - this necessitates modification of current hypotheses of Purkinje-cell function. It also shows significant AC in blood vessels, which would influence biochemical assays of homogenates of microdissected tissue samples. If some of the AC, vascular or neuronal, might be deficient (primarily or secondarily) in spinocerebellar ataxias, treatment with a cAMP phosphodiesterase inhibitor would be indicated. Chronic electrical stimulation of the presumably normal cerebellum in patients with intractable epilepsy raised norepinepherine, did not alter cAMP of cGMP, and reduced GABA levels in CSF (with SNB).

Progressive spastic paraplegia: This is a progressively crippling disorder of children and adults. The causes are not known. Now published is our identification of three unrelated patients with a syndrome of chronic adrenal insufficiency from infancy juvenile-onset of progressive spastic paraplegia and dysschwannian peripheral neuropathy, with normal intelligence (with RRB, NINCHD). We postulated a single metabolic defect to underlie the abnormalities in the neural and adrenal tissues (? an adrenoleucodystrophy variant).

This has now been found by others to be reflected by accumulation of ultra-long chain saturated-fatty-acid cholesterol esters in the CNS. We are pursuing expressions of this defect in our patients.

Other CNS Disorders: In epileptic patients, decreased CSF homovanillic acid and 5-OH-indolacetic acid was found, with increased turnover of the latter and normal turnover of the former (with SNB).

An example of chorea associated with subdural hematoma in childhood leukemia has been published.

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" pathogensis are not known; others are not treatable but their cause is known, e.g., genetic deficiencies of phosphorylase, phosphofructokinase, acid maltase or carnitine-palmatyl-transferase; while still others, such as Duchenne muscular dystrophy and other genetic disorders bearing the name "dystrophy", are of unknown pathogenesis and are untreatable.

Our tissue culture laboratory has been rejuvenated, with greatly 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. Methodologically we have achieved techniques for obtaining abundant, reproducible and mature growth of human fibers in culture, including spontaneous twitching, for precisely selecting certain fibers for our enzyme-cytochemistry and immunocytochemistry at light- and electronmicroscopic levels and for various biochemical studies of them. This year we have grown over 100 human muscle biopsies, as well as numerous rat and chick embryo cultures. Specific studies are noted below.

The Muscular Dystrophies

Biochemically-distinct genetic myopathies

Lysosomal defects: In adult-onset acid maltase deficiency caused by a defect of lysosomal acid-maltase, our earlier reported "reincarnation" in cultured muscle fibers of the biochemical, histochemical and electromicroscopic defects characteristic of the disease was a first proof of a muscular dystrophy having its myopathos endogenous in the muscle fiber. Now we have shown similar reincarnation of the severe biochemical and morphologic defects in 5 additional chronic-infantile and 4 adult cases of acid-maltase deficiency, and partial defects in 4 heterozygotes (with Columbia). These results (a) define the cell of origin of the disease and (b) provide a new test system for manipulations directed toward the treatment or prevention of muscle-fiber damage in this disease without risk to the patient.

"Afuelias": We have introduced the term "afuelias" to describe the defects, known and unknown, of (i) glycogen/glucose utilization Institut de Pathologie Moleculaire. The former cause muscle-fiber breakdown during heavy exercise, especially ischemic exercise -- they include phosphorylase, and phosphofructokinase deficiencies, and infantile fatal fasting rhabdomyolysis.

We have now twice reincarnated a glucolytic-enzyme defect of muscle, phosphofructokinase (PFK) deficiency, by demonstrating extremely reduced levels of that enzyme were found in the muscle fibers cultured from a patient with that defect. In the patient, forearm ischemic exercise was reported to produce electrically-silent contracture, preferential damage to type-II muscle fibers (calcium accumulation when mild and frank necrosis when severe) and positive ^{99m}Tc-diphosphonate scan.

In phosphorylase deficiency, a glycogenolytic-enzyme defect of muscle, we have in 6 additional cases confirmed our previous finding that the enzymatic absence from the muscle fibers is "cured" with their growth in culture -- viz., a remarkable and unexpected recovery of quantitatively normal levels of phosphorylase activity, and not an excess of glycogen, is seen in fibers in the regenerative state in culture and in vivo. We have now reported, immunologically and with histochemical staining of isozyme-focused gels, that there is the normal mature "muscle"-type isozyme phosphorylase as well as the immature "brain-type (fetal-muscle-type) isozyme in the muscle cultured from the phosphorylase-deficiency patients, identifical to that of cultured control human muscle. Thus we have demonstrated a true rejuvenation of an enzyme genetically programmed ultimately to be deficient in mature fibers (with Institut de Pathologie Moleculaire, Paris). It becomes evident that a therapeutic thrust will be needed to provoke and maintain that phosphorylase in the mature fibers of the patient. As one approach, because phosphorylase is activatable via cAMP, we are seeking a clinically beneficial effect of an available phosphodiesterase-inhibitor.

"Alternate-pathway-therapy" is another approach to the treatment of afuelias. In two phosphorylase-deficiency patients utilizing customized treadmill exercise to achieve reproducible serum CPK rises and muscle cramps under standard conditions, we have shown that that i.v. triglycerides prevented the CPK rise but not the cramps whereas glucose-insulin prevented both. In one patient, an oral medium-chain-triglyceride ketogenic diet for two weeks also prevented the exercise-induced CPK rise. Thus acute-intravenous and chronic-oral triglyceride therapy can prevent exercise-induced rhabdomyolysis, reflecting plasmalemmal breakdown from failure of ATP supply (since it did not mitigate the cramps as did glucose-insulin, different mechanisms and/or different biological type of muscle fibers must be involved).

In two glycogenglucose afuelias, phosphorylase deficiency and phosphofructokinase deficiency a new diagnostic test has been introduced and reported positive (and negative in many other neuromuscular disorders): ^{99m}Tc-diphosphonate scanning showed increase of calcium in forearm muscle 24 hrs after the standard diagnostic forearm ischemic-exercise test (FIET), and this uptake

approximately parallels serum CPK elevations. Correlated were histochemical and our autoradiographic-technique for localizing the clinically-administered gamma-emitting ^{99m}Tc-disphosphonate, which documented uptake of the tracer into injured, calciumed muscle fibers; they showed preferential injury of, and calcium accumulation in, type-II muscle fibers after FIET. (This is part of our effort to develop techniques for doing autoradiography of patient biopsy samples following injection of some of the short-lived gamma-emitting radionuclides used for patient-scanning, establish direct scanning-histoautoradiographic correlations.)

Another way to seek which fibers are more damaged in phosphorylase deficiency was by our single-fiber EMG study of the patients during and after FIET. It established electrical failure of all motor units during FIET, and later no abnormal jitter and no motor-unit densification (i.e., no foreign reinnervation of type-I muscle fibers) -- thus although both fiber types fail during FIET, the I-fibers were not remarkably damaged (perhaps because of greater supplies of, and ability to use, endogenous non-glycolytic substrates and myoglobin-associated O_2 or possibly their axons could have "protectively" shut off. These findings provide a basis for formulating alternate-pathway -substrate therapy in MPD.

A new model of defects in muscle glycogen/glycose utilization was reported using iodoacetate (which inhibits glyceraldehyde-3-phosphate dehydrogenase) intra-aortically plus repetitive sciatic-nerve stimulation; there was an electrically-silent contracture and 24 hrs later a positive ^{99m}Tc-disphosphonate scintiscan, and the low-oxidative type-II muscle fibers were preferentially injured, evidenced hisotchemically by calcium-uptake early and later frank necrosis. This model makes possible controlled analyses of the stages of intrinsic energy-depletion muscle injury, and subsequent repair, as well as evaluation of various alternate-pathway therapies applicable to human glycogen/glucose utilization defects, e.g., phosphorylase or phosphofructokinase deficiencies.

Tissue-culture of the muscle of a baby with familial rhabdomyolysis and lipid-droplet accumulation in muscle fibers showed abnormal transplasmalemmal (? transtubular) leakage of the MM and MB isozymes of CPK (with CP, CC).

Adenylate deaminase deficiency has recently been reported by others as a biochemical defect of human skeletal muscle present in 2% of their muscle biopsies from various neuromuscular-disease patients. We have improved the histochemical assay of that enzyme but have not found any defect in our first 75 biopsies assayed. We will continue to seek whether the enzyme deficiency is present and pathogenically significant in our neuromuscular-disease patients.

"Hypocyclasias": We have found reduced plasmalemmal adenylate cyclase but normal β-adrenergic receptors 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 (the last two are discussed in the Episodic Weakness and Myotonia Project). The

first two have muscle fiber smallness with central nuclei. Muscle biopsies of affected infants from two families with X-linked recessive infantile-fatal muscle-fiber-hypotrophy-with central nuclei were flown to use from Amsterdam, The Netherlands and from Texas for tissue-culture. Both showed the same abnormalities: (i) cultured muscle cells had a marked, apparently uncontrolled, ability to proliferate, resembling that of neoplastic cells, which has presented through many passages over 8 months to date, and was not controlled by CNS extract or CNS co-culture; (ii) 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 (per ^{125}I-hydroxypindilol-binding). Thus demonstrated was an intrinsic defect of the muscle cell and apparently impaired control mechanisms related to cAMP -- that could, among other effects, have resulted in impaired response to neurogenic maturational mechanisms, i.e., one kind of "myogenous deinnervation". The identified defect now can be the basis of treating such patients with a phosphodiesterase inhibitor to increase cellular cAMP.

Our "Calcium Hypothesis" was published this year, and was detailed in our 1977 Annual Report. In it we proposed that entry of calcium into the muscle fiber following a pathologic breach of the plasmalemma from any cause (e.g., endogenous afuelia, exogenous ischemia toxin, toxic antibody, or toxic T-lymphocyte, etc.), (a) if slight and restricted, is relatively benign, and is the "spark for repair/regeneration" or hypertrophy of muscle fibers, or (b) if severe and unrestricted, begins a lethal cascade of events pushing muscle fibers past their point of no return, i.e., is the "ultimate molecular assassin" or the "messenger of molecular doom". We 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 electron-microscopic histochemistry autoradiography, biochemistry, and clinical scanning.

A detailed study of mechanisms of muscle-cell injury in relation to Duchenne dystrophy has been published as has a survey chapter (>500 references) of the biology of the muscle cell in relation to myopathies.

Duchenne muscular dystrophy (DMD) the most prevalent of the so-called "muscular dystrophies" by old terminology, 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. For example, the defect of erythrocyte morphology reported by others we have previously reported as not present in our controlled studies. We now report a further aspect of erythrocyte normality in our finding that in 7 DMD patients there was no increase of erythrocyte turnover as measured directly by ^{51}chromium-labeled erythrocyte survival (with H,CC) or indirectly by hemoglobin, reticulcyte count, plasma-hemoglobin. The total fatty-acid composition of erythrocyte of DMD patients and carriers was normal by careful cellular preps and quantitative computer-integrated gas-chromatography of fatty-acid methyl-esters purified by thin-layer chromatography (as well as in myotonic atrophy and many other neuromuscular-disease-controls), thus contradicting the "defect" of palmitoleic acid reported by others.

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 (v.i.). Human regenerative fibers have certain fetal isozymes -- e.g., LDH-1,2 has been known, and we now confirm the recent finding of CPK-MB. We have introduced the use of elevations of CPK-MB isozyme in serum (and in muscle biopsies) for detecting DMD carriers. Our other two new methods of carrier detection are elevated serum hemopexin and serum myoglobin (v.i.). These together we use as fine-tuning for DMD-carrier detection, identifying carriers missed by all other carrier-detection methods. Our data have also been reported in the context that elevated plasma CPK-MB is not diagnostic of myocardial infarction as some cardiologists have reported -- we find it elevated in any kind of leaky skeletal-muscle disease, e.g., DMD and dermatomyositis/polymyositis (with CP, CC). Immunocytologic methods to localize the fetal, MB, form of CPK within muscle fibers has demonstrated in DMD and other myopathic biopsies regenerative fibers that are not evident with ordinary stains. Our new test of ^{99m}Tc-diphosphonate forearm scanning 24 hours after ischemic exercise (6 hrs, after isotope injection) did not defect DMD carriers (or patients) like it did phosphorylase and phosphotfructokinase deficiency patients, (with NM, CC).

Hemopexin is an inducible, liver-produced, heme-transport protein. It was first discovered elevated in DMD patients and carriers by Askanas a few years ago. We are reporting our confirmation of that. To support the hypothesis that the elevation is due to subtle myoglobin leakage from minimally damaged muscle fibers, we are reporting that: (a) with a sensitive radioimmunoassay, myoglobin leakage into the serum of nearly all DMD patients and half of the carriers (with Columbia); (b) hemopexin elevation in monkeys with experimentally crushed muscle or injected with myoglobin, which persisted long after CPK levels returned to normal (in the crush studies). A large number of neuromuscular-disease patients were surveyed with the immunodiffusion assay for hemopexin quantitation. Only active myopathies, especially dermatomyositis/polymyositis and DMD patients and carriers, and myasthenia gravis patients, had hemopexin elevations, and all of those groups of patients had elevated serum myoglobin. We are now studying ^{125}I-hemopexin molecular turnover in a variety of neuromuscular-disease patients, especially in DMD patients and carriers and in monkeys with experimentally altered hemopexin levels (with UCSD).

Our new and reported model involving the blockage of leg-muscle anaerobic glycolysis by intra-aortic iodoacetate, which results in the secondary loss of plasmalemmal membrane integrity of the damaged fibers (v.i.), establishes an experimental basis for at least the possibility of hypothesis "b" re. DMD pathogenesis (above, in harmony with our clinical demonstration that in muscle phosphorylase deficiency ischemic exercise precipitates acute breakdown of the plasmalemmal barrier, allowing prominent creatine-phosphokinase (CPK) egress and calcium ingress (detected by scanning with ^{99m}Tc-diphosphonate), v.s.

Regarding our ischemia hypothesis for DMD, our now-published study demonstrates that in known ischemic human muscle, caused by occlusive vascular disease, the histochemical pattern of the regions of lesser involvement of muscle (groups of necrotic or regenerating fibers in fields of normality) is indistinguishable from that of early DMD and of our experimental ischemic myopathy in animals, thus being at least harmonious with the ischemic myopathy in animals, thus being at least harmonious with the ischemia hypothesis (with VAH, DC). To date, none of the studies attempting to detract from our ischemia hypothesis is conclusive, nor even incisive.

Platelet uptake of serotonin, which 10 yrs ago we found impaired in DMD, now remeasured with a new technique, again shows reduction of initial uptake rates in DMD patients and carriers cf. normal and neuromuscular-disease controls (only ALS patients showed similar reduction) and normal serotonin-release rates in DMD.

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.

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 "autoimmune" or "dysimmune". Before the introduction of antidysimmune therapy, all patients were seriously incapacitated and many died.

The special variation of anti-dysimmune treatment we introduced to this disease 9 1/2 years ago, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred) has continued to be, in our series (about 70 cases) and others, the single best available treatment for children and adults (without or with an associated cancer). It has the greatest benefit/side-effect ratio and is easiest to manage. However, because not all patients respond and because the side-effects of LT-HSDAD-Pred can be significant, we are: (a) defining some of the parameters of their immunologic response to prednisone and seeking predictive indicators thereof, and (b) testing other antidysimmune agents.

By use of T- and B-lymphocyte cell markers, T- and B-lymphocyte mitogens, and T-lymphocyte cytotoxicity on tissue-cultured chromium-labeled muscle fibers, we have now published that in PM/DM patients, although HSDAD-Pred is clinically cumulatively effective for months and longer, its measurable effect on the peripheral circulating lymphocytes, using currently available techniques, lasts less than 24 hrs; and that these effects are more profound on the T-lymphocytes. In a number of patients, children and adults, inadequately responding to HSDAD-Pred we have demonstrated on an individual-case basis a remarkable therapeutic response to added azathioprine (3 mg/kg/d) to establish the efficacy of azathioprine (added to an unchanged dose of prednisone), a double-blind trial in otherwise refractory DM/PM patients, 15 to date, is continuing. We are also measuring the patients' lymphocyte responses, including killer T-lymphocyte responses, to azathioprine (with NCI). Our demonstration, with non-invasive cardiologic techniques, of cardiac involvement (conduction blocks, arrhythmias, systolic mitral prolapse) in the majority of 20 cases of DM/PM is being published (with NHLI, V.A. Hosp. DC, and Georgetown Univ.).

Treatment of the massive subcutaneous calcification, which can be a crippling complication of childhood DM, is often unsuccessful. However, in some severely affected patients the calcium has remarkably diminished as the muscle and skin were responding to our combined azathioprine-HSDAD-Pred program. The reasons for the calcification are being sought by our histochemical, electron-miscropscopic, and autoradiographic studies (v.i.). ^{99m}Tc-diphophonate body scanning (with NM,CC) continues to clinically detect that calcification in its very early stages (for new microscopic detection methods, v.s. and v.i.).

A new and possibly disease-characteristic histochemical finding in DM/PM patients of microscopic foci calcium accumulation in collagenous connective tissue of muscle and subcutaneous regions has been published. Its pathogenesis is being sought, as is its correlation with our positive ^{99m}Tc-diphosphonate patient-scans for calcium, our autoradiographs of patient skin and muscle biopsies for calcium, and our previously described highly-disease-characteristic histochemical finding of alkaline-phosphatase staining in the intramuscular connective tissue (also published).

Another new approach to this problem is an immunocytologic characterization, at light- and electron-microscopic levels, of the collagen in the muscle biopsies of DM/PM patients cf. normal and Duchenne muscular dystrophy, other myopathy and neuropathy disease-controls. Collagen has four immunologically-identifiable polymorphic variants (determined by the aminoacids of its three polypeptide chains) and is a component of connective tissue and cellular basement membranes, each variant having distinct mechanical properties and normal location. The type of collagen synthesized by abnormal cf. normal muscle biopsies growing in culture is also being studied by this approach.

Chronic vacuolar myopathy, seen in 14 of our patients, often considered a variant of the DM/PM complex, we have newly separated off as a distinct disease (or syndrome). It is characterized by acid-phosphatase-positive

vacuoles in muscle fibers which ultrastructurally are membrane-bound (lysosomal) 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 mμ "diameter" and 100 mμ periods. The muscle in culture reincarnates the typical vacuoles after 10 days of growth. In one case, the cultured muscle fibers showed by electron-microscopy a number of unusual structures strongly resembling reovirus virons situated near the nuclei or plasmalemma; re-scrutinizing the original biopsy revealed rare examples of identical structures.

Other Myopathies and Neuromuscular Diseases of Uncertain Classification:

Malignant hyperthermia-rigidity (MHR) is a syndrome,70% fatal, of acute rise of body temperature and muscle rigidity during general anaesthesia. A number of the patients have underlying not-well-defined neuromuscular disorders. In one MHR patient, and his father, we have reported central-core diseases (CCD), with its typical type-I muscle fiber predominance (with Children's Hospital, D.C.). Because we have two additional families with CCD and MHR and there are in the literature two more such families, we have issued a caution to all our CCD patients and their physicians regarding the possibility of MHR during general anaesthesia. The mechanism(s) of the attack of MHR is not known. It appears that there is an excess of free intracellular calcium in the muscle fiber, which we have proposed might be due to an effect of the anaesthetic or muscle-relaxant agent on the calcium-barrier function of the plasmalemma (v.s.) rather than the SR as proposed by others). We are now investigating why central core disease, which we have earlier postulated to be due to a pre-natal monophasic neuronal involvement (impaired primary formation or excessive loss in neuronothanosis) mainly affecting the type-II units, should predispose to the development of MHR. We continue to try to develop specific clinical and muscle-biopsy diagnostic tests to identify patients predisposed to develop MHR.

"Ragged-red" muscle fibers, which contain severe mitochondrial abnormalities, are the commonest histochemical manifestation in limb muscles of the heterogeneous syndrome of oculocraniosomatic neuromuscular disorder 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 (with DMN), we are reporting that most of the mitochondrial changes, including increased number and greatly increased size of mitochondria, wide distorted "twisted-ribbon" cristae, and "mushy" inclusion material, have been re-incarnated, although the mitochondrial crystal-like inclusions have not yet been (perhaps they take longer to develop). We are also reporting that the same changes were produced in normal human muscle cultures after 2 days exposure to dinitrophenol, and the ragged-red-fiber cultured 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 and crystalline inclusions lacked cytochrome oxidase staining by our EM-cytochemistry. (On normal and abnormal human muscle in culture dinitrophenol also

produced leptomeres, clustering of nuclei, dilation of Golgi, Z-disc smearing, and glycogen accumulation as membrane-bound balls, showing more diverse effects on the muscle cell.)

Clinically, some OCSNMD-RR patients with opthalmoplegia have cerebellar ataxia, mental impairment, some denervation evident in muscle biopsy, and spinal-fluid protein increase being reported is that some such patients have by CAT-scan 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.

Developmental abnormalities of the motor-unit have been classified in a new system. They can be genetically programmed or induced by the environment (fetal and/or maternal). They are classified as: Lower Motor Neuron (LMH), Schwann Cell, Muscle Fiber, Blood Supply, and Humoral Factors. They can affect one LMN, Schwann cell, or muscle fiber type or subtype selectively or non-selectively.

We continue to study the selective atrophy of the type-II (glycolytic-rich, oxidative poor) muscle fibers, especially the subtype IIB fibers, which 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 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 now actively consider 3 mechanisms, perhaps summated, in the cancer patients: (a) insidiously decreased oral fuel (caloric) intake, which we have recently documented; (b) fuel wastage due to metabolic derangement within neoplastic cells; (c) possibly a circulating small-molecule remote-effect acting on type-II fibers. We will be studying the role of insulin reception by and action upon muscle fibers to explore these mechanisms.

Basic Mechanisms and Cross-Category Aspects:

Plasmalemma of human and animal muscle have received our major attention, through a multidimensional approach.

Results of the following studies are now being published or have been presented at meetings. Pure fractions of rat plasmalemma (PL) were obtained, and the methods perfected so that adequate quantities of plasmalemmal-membrane can, from normal human muscle, be obtained from limb amputation or radical mastectomy, and even from pathologic muscle biopsies for these studies. Now, we have been able also to do these studies on normal and abnormal human and animal muscle grown in tissue culture. With the plasmalemmal fractions and subfractions, methods have been established for studying acetylcholine receptor, acetylcholinesterase, Na^+-K^+ ATPase (Na^+-stimulated phosphorylation),

divalentcation (viz., Ca^{++}) binding/transport, Ca^{++}-stimulated ATPase, adenylcyclase, guanylate cyclase and β- and α-adrenergic receptors. Some of these have been studied in sarcoplasmic reticulum (SR) and mitochondrial (mito) fractions as well.

From normal human skeletal muscle, membrane fractions (PL, SR, Mito) were prepared. With ^{125}I-hydroxypindolol binding to localize the β-adrenergic receptor (βAR), we have found that βAR-binding was predominately associated with PL, with kinetic, affinity and blocking characteristics of typical βARs of animal muscle. These results are being correlated with our previous studies of muscle adenylate cyclase and will be a standard for comparing βAR properties in various human muscle diseases.

In rat skeletal muscle, our previous studies indicated that denervation results in increased guanylate cyclase but decreased adenylate cyclase in muscle PL membranes. However, cyclic nucleotide phosphodiesterase (PDE) activities (against both cAMP and cGMP) were enhanced in the denervated muscle. Now the actual cAMP and cGMP levels in normal and denervated muscle have been estimated by radioimmunoassays to determine whether the cyclic nucleotide levels directly reflect on those altered enzyme activities. Following sciatic denervation of rat gastrocnemius, soleus and EDL, cAMP showed a 30% increase at 7 days and remained so until till 21 days (end of experiment). However, cGMP showed no significant change. Thus, one cannot necessarily infer cyclic nucleotide levels from values of enzymes associated with them.

The effect of denervation on the β-adrenergic-receptor-(β)-adenylate-adenylate-cyclase-(AC)-system was investigated in rat skeletal muscle because denervation renders skeletal muscle physiologically supersensitive to catecholamines. βARs, as determined by binding of ^{125}I-hydroxypindolol (HYP), a potent β-blocker, were markedly increased in PL from denervated muscle. The increase was evident 2 days after sciatic nerve-section, rose to 50% by one week, and was 100% greater than control by two weeks. The finding of ^{125}I-HYP to PL of normal and denervated muscle was selectively and stereospecifically inhibited by various βAR agents, but not by α-adrenergic drugs; even though βAR-agents was unchanged. Parallel studies on AC in PL showed marked decrease of basal, isoproterenol- and NaF-stimulated activities, being about 50% of control after 2 weeks of denervation. Because denervation induces differential effects on AC and βAR, they must be separate entities responding differently to denervation. Treatment of denervated rats with cycloheximide (0.1 mg/kg) a known protein synthetic inhibitor, did not block the increased βAR binding in denervated PL, suggesting that the increased βAR in denervated muscle is not due to synthesis of new sites but to unmasking of existing receptor sites.

Developmental aspects were studied, coorelating development of βAR and its coupling to AC (as measured by the sensitivity of AC to catecholamines), in 18-day PL isolated from hind leg muscles of 18-day embryonic neonatal, and

1-30-day post-natal stages of development. βARs, per binding of ^{125}I-HYP, were above the adult level in the embryonic muscle, further increased at birth, reached its peak 3 days after birth, and declined thereafter to the adult (60-day-old) level by the 20th day. However, AC of embryonic and newborn rat muscle PL was not stimulated by catecholamines. Catecholamine-sensitivity of the enzyme was noted on 3rd day after birth, reached its maximum on day 5, and decreased thereafter to the adult level by day 20. Basal activity of AC (in the absence of added stimulant) was embryonic and newborn muscle 10-15 times greater than in adult muscle and drastically decreased after birth to reach the adult level by day 20. NaF stimulated the AC at all stages of development, but its activation was very low in embryonic and newborn muscle membranes. cAMP-phosphodiesterase activity (in homogenates, PL and cytosol fractions) showed a pattern similar to that of basal AC. This, βAR and AC exist even from embryonic stages but their coupling occurs only 3 days after birth. We will next seek factors underlying the establishment of receptor-enzyme coupling in the first 3 days of life.

Autoradiography of rat muscle following in vivo administration of ^{125}I-hydroxybenzylpindolol (HYP), a potent β-adrenergic blocker, was used, with pharmacologic controls, to demonstrate the cellular locus of β-adrenergic receptors, since with biochemical assays of cell fractions from whole-muscle homogenates it is impossible to define the cell-type possessing the activity demonstrated. We found: (1) much greater amount of β-adrenergic receptors in arterial-tree vessels than in muscle fibers; (ii) post-denervation increase of both (paralleling our biochemical studies, v.s.), which could underlie the known post-denervation supersensitivity of vessels and muscle fibers to β-adrenergic agonists; (iii) the fallacy of assuming β-adrenergic binding studied biochemically in whole-tissue muscle homogenates is only in muscle cells; (iv) potential importance of arterial-vessel as well as muscle-fiber β-adrenergic receptors in human neuromuscular diseases. We will now extend this by developing a method to look at those receptors in human neuromuscular-disease muscle biopsies and cultures and at α-adrenergic receptors and insulin receptors in animal-model and human diseases.

Lectin probes for membrane-bound saccharide components, displayed at light and electron-microscopic levels were presented as a new approach to neuromuscular pathology of muscle biopsies and cultures. Normal muscle fibers and cells of blood vessels showed only plasmalemmal (± basement membrane) localization with Con A, LC, RCA 120 and WGA; while Soy stained only cells of vessels and the perineurium. Most of the pathologic muscle showed no abnormalities. The only abnormalities seen in a large variety of neuromuscular diseases were: small veins excessively stained and smudgy with Con A in dermatomyositis (not in Duchenne dystrophy); fiber-splitting and capillary-invasion delineated by ConA and WGA; in regen-degen fibers increased staining of sarcolemma and slight staining throughout the intermyofibrillar regions with Soy, and slightly with ConA; in myotonic atrophy some fibers having small circular profiles within them. Cultured human muscle fibers were stained without obvious abnormalities.

The ultrastructural cytochemistry of plasmalemma of cultured normal human and animal muscle fibers was studied in greater detail, by use of a lectin probe (Con A, for α-D-glucosides and α-D-mannosides), ruthenium red (for acid mucopolysaccharides), α-bungarotoxin (for nicotinic acetylcholine receptors), and tannic acid. The specific staining profile of myoblasts through development to multinucleated muscle fibers in culture is being presented in detail. Tannic acid was especially interesting, not binding to plasmalemma of single myoblasts or young myotubes, but to plasmalemma of mature muscle fibers as well as to T-tubules (rat and chick muscle) T-tubule-originating lace of cultured chick-embryo muscle, saccular membranes within human fibers (? T-tubule or plasmalemmal precursors, since T-tubules do not form in cultured human muscle) and to the outer membranous coat of virus C-particles in cultured "normal" chick muscle. Thus, tannic acid appears to be a very good probe for studying muscle cell maturation in culture. These data now provide standards to which abnormal muscle in culture can be compared. We will also be using peroxidase-labelled antibodies against type-IV collagen (the basement membrane collagen) (with NIDR).

Abundant leptomeres were found, and being reported, in muscle cultured from 5 patients with acid-maltase deficiency, 1 with autophagic vacuolar myopathy , and 1 with abnormal mitochondria, but not in our other cultured normal or pathologic muscle. Similar abundant leptomeres were reproduced in normal human muscle fiber cultures exposed to 0.5 mM dinitrophenol, which also provoked prolonged "tonic" contractions in them.

Phosphodiesterases (PDEs), because of their potentially important role in the breakdown of cAMP and cGMP, were studied histochemically with the α-naphthyl-thymidine-5'-phosphate for alkaline PDE (PDE-I). None was localizable in normal or abnormal human or rat muscle cells. Rat muscle showed heavy staining of all blood vessels, which was increased 3-6 days post-denervation -- this paralleled biochemical assays but demonstrated precise cellular localization not possible with biochemical assays of whole-tissue homogenates. Reacting interstitial tissue and inflammatory cells of regenerative rat muscle, but not the regenerating muscle fibers, were strongly positive. In human muscle, only mast cells stained, and the only abnormality of pathologic muscle was an increase of mast cells in dermatomyositis/polymyositis. Thus it is fallacious to presume (a) that, from biochemical studies of rat-muscle homogenates, a significant amount of PDE-I is in muscle fibers, and (b) that vessels and connective-tissue of rat muscle are like those of human muscle. (For PDEs of platelets and erythrocytes, v.i.).

Acetylcholinesterase (AChE) studies (being published) of subcellular distribution and properties showed: (a) majority present extrajunctionally, mostly in microsomes/SR but some in the soluble phase; (b) after denervation, the early AChE reduction is mainly in the microsomal/SR 4-S fractions; (c) the 16-S form is restricted to the neuromuscular junction (per others), and d the plasmalemma had only 10-S and 16-S. Synthesis, degradation and release of the different molecular forms of AChE will now be studied in normal and various pathologic circumstances.

Platinum-thymine complex was used - in combinations with Feulgen pretreatment, DNAse or RNAse - for selectively staining RNA or DNA at the electronmicroscopic level. 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 cells in culture).

Modifications of our original techniques for selection and drilling of specific fibers in plastic-embedded muscle cultures have been published.

Single-fiber electromyography (SFEMG) to measure motor-unit densification, EMG, and muscle histochemistry were correlated in 54 patients with various neuromuscular diseases (with Uppsala, Sweden), showing: (i) fiber-type and, more specifically, fiber-subtype grouping as the histochemical correlate of densification of the motor unit in neurogenous and myogenous deinnervations; (ii) SFEMG can be a more sensitive index of motor-unit densification.

Of the eye muscle fiber-types, none is histochemically like a limb-muscle fiber-type. Published was our demonstration of their normal histochemical patterns in Rhesus monkey and identification of 3 types, "fine", "granular" and "course". The first two have one endplate per fiber and probably are different types of twitch fibers; the last has multiple endplates and may be a tonic fiber. Following denervation the first two developed diffuse extra-junctional acetylcholine receptors but the coarse fibers did not; no fibers were positive beyond 13 weeks post-denervation.

Histochemistry of matched interrupted serial cross-sections, and alternate sections for electronmicroscopy along the entire length of spindle muscle fibers in rat soleus showed with the "myofibrillar" Plt 9.4 ATPase: distinctions between nuclear-chain, nuclear-bag_1 and nuclear-bag_2 fibers; regional staining differences along the bag_1 and bag_2 fibers; ultrastructural heterogenity of poler cf. equatorial portions of bag_1 fibers. Endings of both "plate" and "diffuse" type, inferred from cholinesterase stainings, occurred on bag_1 and bag_2 fibers but did not correlate with zones of ATPase heterogenity and thus probably did not determine them. Possibly sensory innervation and/or regions of passive-vs-active stretch governed the ATPase staining. Sensory-deinnervated (3-12 mos) spindle muscle fibers had altered ATPase-staining and other abnormalities.

Normal human spindle muscle fibers of intercostal muscles, studied in the same manner, had one bag_2 and 1-2 bag_1 fibers, the latter having absent M-lines and usually of smaller diameter and shorter cf. bag_2's. Nuclear chain fibers and bag_2 fibers had M-lines. Histochemical profiles of each of the 3 types were clearly established, with regional differences of histochemistry and ultrastructure along the fiber lengths; because they appear analogous to those of cat spindles, they probably have different functional roles, as is known for the cat spindle 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 "autoimmune" or "dysimmune". Untreated patients usually are seriously handicapped and many die. Palliative treatment with anticholinesterases and antipathogenic treatment consisting of thymectomy, ACTH and, most recently, prednisone has helped considerably but much disability, some fatality, and drug side-effects still occur.

We previously reported the first identification of a factor, an IgG, in the sera of MG patients which blocks binding of α-bungarotoxin (αBT) to the human junctional nicotinic acetylcholine receptor (nAChR) at the normal neuromuscular junction (41% of MG patients) and extrajunctional AChR of denervated human fibers (72% of MG patients, including all who had a thymoma), i.e., an "antireceptor antibody". We next reported that all MG patients having an IgG "antimuscle antibody" (first found by others) also had the blocking factor (although only half having the latter had the former) (the only discordant finding being in one non-myasthenic polymyositis patient with thymoma who had antimuscle antibody but no detectable blocking factor) and suggested that these may be the same antibody or, if different, virtually always co-produced. Our junctional localization of the blocking factor put it in the correct position to impair neuromuscular transmission and cause the weakness of MG. We found the nicotinic acetylcholine receptor ultrastructurally localized both post-synaptically and presynaptically and proposed the pathologic antibody acts at both sites to cause disease -- this has now been confirmed by others who, initially doubting our pre-synaptic localization, found IgG and C-3 complement localized both post- and pre-synaptically.

We have now published our tissue-culture studies of human, rat and chick skeletal muscle showing that with α-BT the cultured animal fibers contain "extrajunctional", actually non-junctional, nAChR diffusely in the plasmalemma without "hot-spots" claimed by others and that the binding of α-BT to pure non-junctional nAChR is readily blocked by the pathologic IgG of MG patients. This demonstrates a new environmentally-controlled test-object (the cultured muscle fibers) for identifying circulating pathogenic factors.

On the basis of those studies and our EAMG studies (v.i.) we have now published our hypothesis that: (a) there are normally two subsets of acetylcholine receptor (AChR), a "junctional" (J) and an "extrajunctional" (E) form; (b) both J and E AChR occur at the neuromuscular junction but extrajunctionally only E-AChR is present; (c) the IgG blocking factor in human MG is directed mainly against the E type of AChR while that of the EAMG model we worked with is mainly against the J-AChR; (d) the diffuse AChR of thymic epithelial cells, v.i., may be E-type.

From our work with the induced autoimmune model (rabbits injected with electric-fish AChR), originated by others, of experimental allergic MG (EAMG) (with U. Maryland) we have now published our demonstration of: (a) binding of that rabbit sera to human neuromuscular junctions but not to extrajunctional receptor of denervated fibers at light-microscopic resolutions; that junctional binding of EAMG sera was not blocked by blocking-factor-positive (or

negative) MG sera, only partially blocked by αBT, and not blocked by carbamylcholine, decamethonium or tubocurarine; (b) binding of it by electromicroscopic resolution to the plasmalemma diffusely in rat and chick muscle fibers in tissue culture; (c) binding of that rabbit sera to the original antigen in a radioimmunoassay we developed (with IB), but no binding of blocking-factor-positive (or negative) MG sera to that antigen; (d) similarities but also distinct differences of the model with human MG, indicating it is not a perfect model of the latter although it could still be a model-in-principle.

The rationale for the empirically-observed benefit of thymectomy (v.i.) is still being sought. Our demonstration last year that in the thymus the epithelial cells, in both "hyperplastic" and "involuted", all contain AChR demonstrable histochemically by αBT binding (with IB and Washington Univ.) fulfills a step in our earlier hypothesis that the mechanism of MG might be an alteration of thymic epithelial cells e.g., by an exogenous virus, making them "foreign", in response to which B-cells make anti-AChR antibody (perhaps programmed by intermediary T-lymphocytes) that co-reacts with junctional AChR to cause paralysis. Because the thymic epithelial cell is, by others, considered pluripotential, this same hypothetical pathogenic mechanism could apply to other dysimmune diseases in respect to thymic epithelial cell molecules altered to become other antigens. Now we have found that even in MG thymuses considered "atrophic" by existing histopathologic criteria there are evident in our fresh-frozen sections many small nests of cells that look "active" -- accordingly, we have postulated that they have a detrimental role in the pathogenesis of MG and their removal may be the basis of improvement following thymectomy of the older MG patient who typically has such thymic histopathology.

We have in press our finding of serum hemopexin 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 (with Columbia). That small amount of myoglobin leakage may be a manifestation of a heretofore minimal subclinical plasmalemmal-leaking myopathy in many MG patients. Our electronmicroscopic studies of human thymic epithelial cells from myasthenia gravis patients, directly (regular and pyroantimonate-stained tissue) and after being grown in culture, shows desmosomes and tonofibrils typical of epithelial cells and no muscle-cell-like myofibrils, contrary to what has been reported by others in cultures of normal animal thymuses (which might have been contaminated with non-thymus muscle cells during tissue removal).

The role of thymectomy in the treatment of MG and for which MG patients, has recently been questioned. Review of our last consecutive 55 thymectomies done over the past 10 years has revealed: patients with onet age 16-29, 84% improved (one Osserman class or better and on same or less medication); 4 of 6 patients with thymoma improved, all having had onset over age 29 and 4 not preoperatively diagnosed; of patients with onset over age 29, 71% improved; 83% of patients with thymic hyperplasia improved as did 70% of those

with "involuted" thymus (v.s.); zero operative mortality, low operative morbidity; severity of myasthenia itself not a contraindication, but rather an indication for surgery; 84% improvement rate in patients "thymectomized" within 10 years of onset of MG cf. 33% improvement rate if duration >10 years the transcervial surgical approach unsatisfactory, -- worsening of MG within 1/2-7 years in 7 of 9 so operated (including 4 whose transcervical operations were performed at centers favoring and experienced with that procedure) necessitated reoperation by sternal-splitting disclosed residual/recurrent thymus in all (as much as 1/2-3/4 of the presumed original thymus, typically the inferior portion of one or both poles) and resulted 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 (with SB, NIHL and Harvard).

Thus thymectomy is potentially beneficial in all patients with onset in teen-age or later, and repeat thymectomy can be remarkably beneficial in patients previously improved who subsequently exacerbate and do not respond to medical management. Serum antibody levels against nAChR before and after thymectomy are being correlated with clinical response (with Salk Inst.).

In a number of those patients as well as primary thymectomy patients, we have found positive ^{67}gallium clinical scans. ^{67}Ga, which localizes in thymus, was used in both clinical scanning of the thymus preoperatively and for in vitro counting of the thymus removed by therapeutic thymectomy. Before thymectomy (hyperplastic) thymuses were positive but 10 (one thymoma, 6 hyperplastic, 3 "involuted") were false negatives; one repeat thymectomy was positive (and hyperplastic tissue was found, and became negative after thymectomy), the other was falsely positive with no thymic tissue locatable. Of 14 MG patients scanned only post-thymectomy, all were negative. In vitro isotope counting showed ^{67}Ga concentration in all positive and false-negative thymuses, indicating need for more sensitive pre-operative ^{67}Ga scanning and intraoperative probes to localize thymic tissue.

Abnormal lymphocyte function is the pathogenic step presumably suppressed by corticosteroid treatment of MG. Confirmed and adopted by most other physicians has been the treatment we introduced to MG, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred). In our own series it continues to be extremely beneficial in the majority of cases, 60 of 64, and for as long as 12 years in a child and 8 years in an adult. Responding best are the older-onset patients, especially the older males. Our 4 non-responders were females, 3 in the menstruating age group. Importantly, though, we continue to find that none of our responding patients has become absolved of his/her requirement for prednisone even after a gradual tapering of the dose. Typically, patients exacerbate 1-4 months after stopping a 5 mg q.o.d. dose (about the time taken for resumption of synthesis of measurable levels of abnormal

IgG, per others in another dysimmune disease). Because neither anticholinesterase nor prednisone treatment is either curative or completely suppressive, nor thymectomy always satisfactorily beneficial, more details or the pathokinesis are needed (v.s.).

The effect of the HSDAD-prednisone treatment on lymphocytes was measured over a 48-hour cycle in a number of MG and other patients, and the results reported. At 6 hurs. after the 8:00 a.m. prednisone dose there is marked depression of T-lymphocyte counts and lymphocyte responses to T-lymphocyte mitogens, and a lesser effect on B-lymphocytes and response to B-lymphocyte mitogens, and there was return of these measurable effects to normal by 24 hrs. after the dose (yet clinically the prednisone has a cumulative beneficial effect over weeks and months). The effects were approximately dose-dependent, establishing that these parameters might answer the original need, but the considerable individual-patient variability means that further precision must be gained.

In the cerebrospinal fluid (CSF), 7 of 23 MG patients had oligoclonal IgG bands and 5 more had a monoclonal bands; since IgG "synthesis" (Tourtellotte formula) in the CNS was normal, the CSF pathologic bands are probably from the serum (with ID). 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 and perhaps other "soft" CNS findings.

Periodic Paralyses (PP) are hereditary or acquired disorders causing chronic weakness punctuated by attacks of paralysis. 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.

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 12 years. Since muscle does not contain carbonic anhydrase, the mechanism of acetazolamide benefit in hypokalemic PP remains unknown.

Myctonia is a crippling symptom to various degrees in myotonia congenita and paramyotonia congenita (inherited diseases of unknown cause). We have reported a controlled clinical trial showing moderate to remarkable benefit from acetazolamide in 8/10 patients who had failed to respond to, or had been intoxicated by, other anti-myotonia drugs. It remains the long-term treatment of choice in 6 of those patients.

Myotonic atrophy (myotonic "dystrophy") is an inherited multisystemic disease, with progressive wasting, of unknown pathogenesis. We have previously raised the possibility of at least a partially neurogenic aspect. With our new concept of "myogenous de-innervation", we have now extended that

hypothesis to include a possible myogenous muscle plasmalemmal non-receptivity to neural-short- and long-term trophic influences. We have found a 30-60% decrease of adenylate cyclase in 7 patients with myotonic atrophy (v.i.).

The model of 20,25 diazacholesterol-induced myotonia of animals, used to evaluate myotonic phenomena, was reported. Muscle of the markedly myotonic animals showed: (a) no histochemical changes, (b) no blockage in vivo of the myotonia by α-bungarotoxin, d-tubocurarine, succinylcholine or atropine in contrast to blockage of sciatic-neurectomy-induced fibrillations by all four drugs (and blockage of both myotonia and fibrillations by procaine, tetrodotoxin, KCl or ischemia and of neither by pyridostigmin). Our results demonstrated a major difference, and several similarities, between fibrillation and myotonic discharges -- we hypothesized that fibrillations and DAC-induced myotonia are mediated through mechanisms involving ionic channels, that both can be produced by activation of junctional/nonjunctional AChRs (or some mechanism coupled to those receptors), but that an unfettered αBT binding portion of the AChR molecule, and an unblocked atropine-binding site, are obligatory only for production of fibrillations. This, and our showing lack of αBT binding histochemically to muscle fibers in human myotonic diseases, indicate essential differences between myotonic and fibrillating plasmalemmas. Presented last year was that DAC-induced-myotonic-rat-muscle plasmalemma had 40-60% lower levels of basal and fluoride- and catecholamine-stimulated adenylcyclase activity; this change, like that in myotonic atrophy patients (v.s.), could be due to confirmational changes of the enzyme molecule or an altered sterol composition of the plasmalemma. In order to evaluate direct influence of diazacholesterol on muscle fibers which is not possible in the intact animal, tissue culture system was used. Pulses of 0.005 mM of diazacholesterol were given to contracting cultured rat muscle (after about 8-9 days of growth) for 10-15 min. on 3 consecutive days, resulting in changed contraction rhythym, which became more continuous and fibrillation-like; also some granularity of the muscle fibers was observed. Electronmicroscopic studies of those DAC-treated muscle cultures are being done. The cultured rat muscle treated with diazacholesterol, had quantitatively lower levels of adenylate cyclase (basal, and NaF and isoproterenol stimulated) compared to untreated control-cultures; however, β-adrenergic receptors assayed by ligand binding were unchanged. These results are in agreement with our previous data from muscle of intact animals with DAC-induced myotonia and also comparable to biopsies of myotonic atrophy patients.

We confirmed histochemically the finding of an increased number of intrafusal muscle fibers in muscle spindles of myotonic atrophy patients. Our new finding was that muscle spindles are normal in myotonic congenita patients and in rats rendered chronically myotonic with 20,25-diazacholesterol. Because we have found similar spindle abnormalities in rat muscle spindles experimentally denervated (neurogenously), it is possible that the spindle abnormalities in myotonic atrophy perhaps are due to fusimotor deinnervation (? neurogenous or myogenous deinnervation).

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 01039-16 MN

PERIOD COVERED
October 1, 1977 through September 30, 1978

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

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LAB/BRANCH
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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7	5	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)

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.

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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 pathophysiologic states.

Methods Employed: A variety of techniques, encompassing histochemistry, tissue-culture, 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 thereof. Conducted were double-blind, single-blind and non-blinded therapeutic trials, the efficacy of which is 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).

Patient Material: Medical Neurology Branch patients and neurology consultation patients from the various services of the Clinical Center, who had diagnostic muscle biopsy and other diagnostic procedures.

Major Findings:

Amyotrophic Lateral Sclerosis (ALS): The cause of ALS is unknown -- dysmetabolic vs. viral are the two main possibilities. We favor the former but are pursuing both.

Therapeutic trials: We have found, and presented, that CSF levels of cAMP and cGMP depressed, 40% and 50% respectively, in a group of 58 ALS patients, due to decreased production within the CNS on the basis of the i.v. probenecid test (with NNMC). Phthalazinol, a cyclic nucleotide (especially cAMP) phosphodiesterase-inhibitor, used to raise cyclic nucleotide levels caused a dose-related increase of cAMP but not cGMP. A single-blind placebo-controlled 4-month-each crossover therapeutic trial with phthalazinol (20-75, mean 43, mg/kg/d showed no alteration of the steady carefully quantitated progression of disease in 9 ALS patients; one man with only bulbar lower-motor-neuron ALS steadily progressive for 2.5 yr. prior to the drug has had no further progression for 3-1/2 years on phthalazinol 35 mg/kg/d. (with NNMC and Atherosclerosis Res. Inst. Japan). Technical factors influencing CSF cAMP and cGMP assays have been delineated, including pH, storage, freezing, and CSF-phosphodiesterase-inactivation; no ventricular-lumbar gradient exists. A blood-to-CSF barrier for cAMP in man, resistent to 40-fold increase of serum cAMP (glucagon-provoked) was reported (with NNMC). In the wobbler mouse, a mutant with motor-neuron degeneration, decreased spinal-cord cGMP is being reported (with ID).

Polyinosinic-polycytidylic-acid: (Poly-ICLC) an inducer of interferon (an autogenous intracellular antiviral substance) has been used in 3 ALS patients without therapeutic effect, even though interferon production was moderate (with NIAIDD); several parameters of leucocyte response were concurrently measured v.i. Human leucocyte interferon, was not beneficial in one ALS patient (with Stanford Univ. and Finnish Red Cross).

Adenine arabinoside (Ara-A), an antiviral agent, 10 µg/kg/d i.v. X10d. is being evaluated as a possible therapeutic agent in 10 ALS patients.

We have previously reported a neurologic abnormality with certain features like ALS in many cases of primary and secondary hyperparathyroidism; we have also studied a few cases with both frank hyperparathyroidism and frank ALS. To study the possible role of abnormality of parathyroid function and/or calcium metabolism in ordinary ALS, we have studied, now reported and are presenting various parameters of calcium homeostasis. Reduction in retention of oral $^{47}CaCl_2$ was seen in all neuromuscular diseases studied, but in ALS patients was more pronounced than in myopathy and neuropathy patients (>80 retention tests). That reduction was due in part to inactivity because it was present in paraplegia regardless of etiology, but was especially low in paraplegics with ALS. That decrease was not due to decreased 25-hydroxy vitamin D levels found to be normal, nor to resistance to dihydrotachysterol because administration of the latter mathematically corrected the decreased retention (but did not cause clinical improvement). Serum-calcium, urinary 24-hr calcium excretion, and renal cAMP clearances were normal. Serum mean parathyroid hormone concentrations, measured, were higher than normal in 40-50% of all patients with neuromuscular disease and similar in all subgroups: ALS, myopathy, neuropathy. Response to calcium infusion will now be evaluated to determine if there is an abnormal response to imposed serum calcium fluctuations.

Folate (oxidized and reduced forms) and B_{12} in CSF and serum in sporadic and familial ALS were measured and compared to values in neuropathy and myopathy (with Johns Hopkins and Bronx VAH) and no significant reductions found when age and sex-matched groups were compared (being reported). Thus the posterior column degeneration in familial forms (originally described from this Branch) is not directly due to local reduction of folate or B_{12} (however, interference with its action on cells could be occurring). Additional data suggest that probenecid does not cause rise in CSF folate in ALS patients.

In ALS patients we continue to search for evidence of a viral cause in ALS sera, CSF and tissues by profiling of viral antibodies (Ab) (with Johns Hopkins and IDB), and various tissue-culture techniques (including activation and evluation by morphologic, antigenic, and reverse-transcriptase assays). To date, no viral evidence has been found. In 54 ALS patients, including 6 with a late-post-polio progressive muscular atrophy (LPPPPMA)

syndrome compared with matched other-neuromuscular-disease controls also with muscle wasting, Ab-distribution and geometric-mean Ab-titers in sera were not stastistically different for poliovirus 1,2,3, Coxsackie B3,B4, influenza A, mumps, varicella, measles, rubella, herpesvirus 1,2, cytomegalovirus, and toxoplasmosis; nor was CSF poliovirus 1,2,3, Ab abnormal. CSF HI-Ab (or HI-Ab-like activity) $\geq$ 1:2 against rubella antigen was presnet in 10% of ALS patients (and also 10% of multiple-sclerosis patients, but none of our 52 neuromuscular-disease controls. The positive ALS patients had somewhat more rapid early atrophy but no history of overt rubella infection involving the CNS. Serum/CSF Ab ratios did not demonstrate local specific Ab synthesis in the CNS against any of the virus antigens tested, in both ALS and non-ALS patients. Thus, if ALS is caused by a virus, its detection will require techniques other than the assay employed here.

Benign Focal Amyotrophy, important because of its excellent prognosis but usual misdiagnosis as fatal ALS, was originally described by us 10 yrs. ago as a separate clinical syndrome, and in young-adult males possibly a distinct disease. We have now presented our updated experience. It is a limited form of lower-motor-neuron disease confined to the upper extremities, unilateral or markedly assymetric, gradual in onset, progressive for 1/2-4 years and then clinical stability, or only very minimal progression, for 2-10 yrs to present. We have recently found CSF oligoclonal Ig bands in some, raising a question of a viral/dysimmune pathogenesis. If related to ALS as a spontaneously arresting form, it may hold a clue to treating that disease.

Motor neurons of 15-day fetal-rat spinal cord are now being grown in tissue culture and serving as test objects of possibly toxic fluids or agents related to ALS patients.

CSF and blood cells are being analyzed biochemically seeking an abnormality specific to ALS. Total fatty-acid composition of CSF is being analyzed by quantitative computer-integrated gas-chromatography of fatty-acid methyl-esters purified by thin-layer chromatography; with this technique we have just finished showing no fatty-acid abnormality of erythrocyte membranes in ALS patients, and will also study their platelet membranes. Enolases, neuron-specific and non-neuronal, are now being studied in ALS CSF (with NIMH). ALS platelets shown a reduced rate of initial uptake of ^{3}H-serotonin and of initial release to 0.5 μ/ml thrombin, and normal cAMP- and cGMP-phosphodiesterase.

Histochemical properties of lower motor neurons (LMNs) are being explored, looking for special properties of them and disease-characteristic defects thereof. We have previously shown phosphorylase to be special for LMNs. Now, using the alkaline phosphodiesterase (PDE-I) reaction, α-naphthyl-thymidine-5'-PO_4 as substrate, we find in normal cat cord dark staining of the pia and of large and small vessels within the cord (but not capillaries), and no neuron soma or fiber or glia staining. Five fluoroscein-labelled

lectin probes, for various membrane-bound saccharide groups, in normal and ventral-root-section reacting cat cord showed: with all but Soy, gray > white staining, possibly attributable to more surface-membranes; root-myelin > tract-myelin stained with ConA; only Soy selectively stained vessels; a latero-posterior zone capping the posterior horn demonstrated only with Soy; only wheat-germ stained the nucleoplasm (not nucleolus or cytoplasm) of motor neurons unchanged in chromatolysis. In ALS cord (autopsy), no abnormal findings with any lectin was detectable.

As an induced animal-model (with LNC and Johns Hopkins), Swiss mice acutely infected with poliomyelitis, which attacks anterior horn cells, are being studied to ascertain whether the time-course of reduction of neuro-transmitters, substrates high-energy intermediates, and cyclic nucleotides shows an early drop, e.g., of cGMP, in spinal cord antecedent to paralysis, attempting to determine the sequence of the virus-induced biochemical alter-ations.

In Shy's Scientific Basis of Neurology, two extensive chapters have been published, one on numerous aspects of the biology of the lower motor neurons as a basis for understanding and investigating diseases thereof (>1000 refer-ences), and the other on various aspects of the several motor neuron diseases; as well as 3 chapters on other neuromuscular diseases: central core disease, rod disease, and muscle fiber hypotrophies. A clinical-research conference on ALS is being published (with Mayo Clinic and Johns Hopkins).

The hypothetical pathogenesis of central core disease has now been re-fined on the basis of 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 we propose it to be a paucity of lower motor neuron (LMNs), especially of the hypothetical type-II LMNs occurring during development, either impaired formation or increased normal loss as a part of "neurothanosis" (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).

To study the dyschwannian neuropathies we have developed techniques to grow in tissue-culture human schwann cells, e.g., obtained from diagnostic nerve biopsies. In primary dysschwannian neuropathies (e.g., metachromatic leucodystrophy, familial idiopathic "Charcot-Marie-Tooth" neuropathy the schwann cells in culture should have the biochemical defect, which can be elucidated and when identified can be treated in culture. They might also in other ? acquired neuropathies such as diabetes mellitus. Electron-microscopy and electronmicroscopic enzyme- and immunocytochemistry is being done on both the original biopsied nerve and the schwann cells cultured from

it for direct comparison. Surface membrane markers being localized electron-microscopically include concanavalin A (for α-d-mannoside and α-d-mannoside and α-d-glucoside groups), ruthenium red for acid mucropolysaccharides, α-bungarotoxin for nicotinic acetylcholine receptors, and tannic-acid. A variety of biochemical assays are being applied to the cultured schwann cells.

In the majority of patients we see the PN is of undiscernable cause. Those which are non-familial we may treat with LT-HSDAD-prednisone, especially if less than 5-years duration, postulating a dysimmune pathogenic mechanism. We are publishing a summary of our ongoing experience in 25 carefully-documented patients who have had good to outstanding success, most having been given-up on by others and some having come with diagnoses of non-treatable diseases (e.g., ALS or "Charcot-Marie-Tooth" disease). Long-term treatment is required -- too-rapid reduction of dosage too soon results in exacerbation. Excellent results have been sustained for as long as 13 years in an adult and 10-1/2 years in a child (who at age 22 is still regaining motor skills). Our correlative studies indicate that patients most likely to respond have the triad (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 LT-HSDAD-Pred. Prednisone has a broader scope of responsitivity in this disease than previously believed. Virtually all our corticosteroid-responsive patients are cortiosteroid-dependent requiring 5-20 mg single-dose q.o.d. to prevent exacerbation.

In the cultured non-innervated muscle fibers of chick, rat and human tissue, we have now published that α-bungarotoxin demonstrated diffuse plasmalemmal nicotinic acetylacholine receptors (nACHRs) of single myoblasts through multinucleated well-differentiated contracting fibers. They did not appear electron-microscopically to have hot-spots", as reported by others from light-microscopic autoradiography, - we suggested hot-spots" may be artifacts based on subtle plasmalemmal folds.

Correlations of histochemistry with single-fiber-EMG (SFEMG) in patients with various neuromuscular diseases have been published. Ordinary neurogenous deinnervations have significant densification of motor-units (from collateral foreign reinnervation of orphaned muscle fibers) and fiber-subtype grouping histochemically. Chronic myopathies (Duchenne dystrophy, polymyositis/dermatomyositis, limb-girdle morphologically-non-specific myopathy snydrome) had mild densification by SFEMG but no definite subtype grouping histochemically, indicating a greater sensitivity of SFEMG. From study of 8 patients with muscle fiber-type predominance without major evidence of ordinary denervation (5 central core disease, 1 congenital rod disease, 1 type-I-fiber-hypotrophy-with-central-nuclei, 1 type-I-fiber-predominant unclassifiable neuromuscular disease) it became evident that, in a field of fiber-type predominance, subtype-grouping is requisite for a histological diagnosis of neurogenous deinnervation (although its lack does not, hypothetically exclude an abnormality of LMNs).

Biochemical aspects of neurogenously de-innervated muscle (as an organ), such as decreased guanylate cyclase and adenylate cyclase activities and increased β-adrenergic receptors, are in our Myopathy project.

In patients with scoliosis, our continuing studies show a wide variety of neuromuscular diseases (by muscle biopsy histochemistry) associated with and probably causing scoliosis, most commonly some form of neurogenic muscular atrophy. Some of our scoliosis cases were previously considered "idopathic", a group we are studying in more detail (with DuPont Inst., Wilmington, DE).

Polyneuropathy (peripheral Neuropathy) (PN): The peripheral neuropathies comprise a group of disorders of various causes, more than half unknown. They always cause serious physical handicap sooner or later in the course of the disease, sometimes associated with intractable pain and ulceration and 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 peripheral nerves.

Dysschwannian neuropathies are ones in which the neuronal-axon defect is considered secondary to Schwann cell abnormality, whereas in dysneuronal neuropathies the LMN neuron soma and/or axon is the major site of abnormality.

The mechanisms of HSDAD-Pred anti-dysimmune effect on circulating blood lymphocytes (see Myasthenia Gravis and Myopathy (Dermatomyositis/Polymyositis) projects), and its effect on cerebrospinal-fluid (CSF) lymphocytes and immunoglobulins have been studied. We are publishing our method of identifying T- and B-lymphocytes in CSF, and that percentages normally are the same as in peripheral blood (72 and 16%). In 9 chronic idiopathic relapsing polyneuropathy patients, with 2-10X elevated CSF IgM and or G, studied longitudinally, HSDAD-Pred caused borderline reduction in CSF T- and B-lymphocytes and definite reduction of IgM, IgG and IgA (but not total-protein), and serum IgG but not IgM or IgA: thus the accompanying remarkable clinical improvement may have been by prednisone affecting lympocyte Ig production (with IB) directly within the CNS rather than numbers of lymphocyte subpopulations. We showed that high-single-dose alternate-day prednisone did not impair development of antibody in patients, cf. untreated persons, prophalactically immunized with influenza virus.

A new treatment, Polyinosinic-polycytidilic acid poly-L-lysine stabilized with carboxymethyl cellulose (Poly-ICLC), has been remarkably successful in a patient with prednisone-plus-azathioprine unresponsive chronic presumably-dysimmune relapsing polyneuropathy (with NIAIDD). 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 6 months. Although Poly-ICLC is an interferon-inducer, we postulate it is beneficial in this dysimmune by an action we found, marked lymphocytopenia (to 10-20% of baseline) 1-2 days after the drug with

return to baseline by 4-5 days (granulocytes actually rose 3-fold at 6-24 hr. and fall only 30% below baseline at 2-3 days and return to baseline by 3-5 days." We propose this to be a new antidysimmune treatment potentially beneficial to other dysimmune diseases (if acting through interferon it would provide a new insight into the pathogenesis of relapsing neuropathy). We are extending the trial to other dysimmune neuropathy patients, dermatomyositis/polymyositis, and myasthenia gravis patients. We are also measuring in detail the quantitative and qualitative responses of B- and T-lymphocytes, including killer-T-lymphocytes (with NCI).

Amyloid neuropathy is of particular importance in respect to pathogenic mechanisms. The "idiopathic" form, beginning in mid or later adulthood, we reported last year a male predominance (8/10) and all patients having an associated, and probably causative, plasma-cell dyscrasia, detectable in in 8/10 of our patients as multiple myloma, and/or serum and/or urine "paraprotein" immunoglobulin fragments (IgG-kappa >> IgG-lambda, IgM-lambda). Since we proposed that the neuropathy is due to a systemic metabolic abnormality, possibly related to a circulating abnormal protein fragment (i.e., a "para-Sparafucile" phenomenon, v.s.), rather than to pressure from multifocal amyloid deposits of immunoglobulin fragments or ischemia, we are seeking the topographic lodgement of that putative fragment in amyloid patients and others, especially ones with monoclonal Ig spikes in serum, urine or CSF or with oligoclonal bands.

Substance P in CSF by radioimmunoassay has been found decreased in our neuropathy patients, but in no other neurologic diseases or myopathies surveyed (with CPB and Harvard). Further neuropathy patients are now being studied to seek possible sensory-vs.-motor and/or disease specificity.

Patients with muscle cramps and pains without detectable neurologic deficit were reviewed in detail. Of 63, 47 had abnormal muscle biopsies: 19 denervation (with normal nerve conduction velocities, 15 type-II fiber atrophy, 13 phosphorylase deficiency, 1 phosphofructokinase deficiency, 2 defect in utilizing long-chain fatty acids. This subclinical denervation is the commonest cause of otherwise unexplained muscle cramps and pains.

In multifocal eosinophilic granuloma, multifocal extradural compression of cervical roots responded rapidly and completely to corticosteroid plus radiation therapy (with RRB, NICHHD).

A new principle/model for inducing an experimental allergic neuropathy (EAN) in animals (sheep) has been reported. It utilized immunization with soluble nerve protein fraction (in contrast to lipid-associated protein of myelin used in previous EAN models). This represents a new potential model of some human dysimmune-dysneuronal peripheral neuropathies such as in some patients we have seen with prednisone-responsive neuropathies without demonstrable schwann-cell involvement. It also represents a new approach to studying certain dysimmune disorders of the CNS, such as multiple sclerosis and parainfectious encephalopathies. Since our EAN animals also have a component of

blockade of neuromuscular transmission that is responsive to edrophonium, the model may have some relevance to myasthenia gravis or other disorders of the neuromuscular junction. We now have found two patients who are rather similar, having prednisone-responsive trunk and limb muscle weakness, no cranial nerve muscle weakness, atypical neuromuscular transmission defects, and responses to anticholinesterase, normal nerve conduction velocities and normal CSF protein. Thus, 2 previously therapeutically-overlooked patients have achieved remarkable clinical improvement.

We have now established combined studies of patient sural nerve biopsies in vitro -- including in vitro nerve conduction velocities of fast and slow fibers and other electrophysiologic parameters, teased fiber histochemistry, electronmicroscopy, and EM-histochemistry, as well as tissue-culture of the schwann cells, v.s., allowing more precise and direct multidimensional analyses of the afflicted nerves in polyneuropathy patients. This has shown, for example, markedly slowed conduction in small (C) fibers correlated with small-fiber pathology while clinical conduction times (which do not "see" C-fibers) were normal. As a parallel model cat saphenous nerve is being used to work out techniques such that electrophysiologic parameters combined with metabolic manipulations can be utilized to seek normal and abnormal features of human nerve biopsies (Schwann cells and ? axons) in vitro.

Central Nervous System Disorders: 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.

cAMP is thought by some to be a mediator of synaptic transmission of some systems in the cerebellum. Our newly developed histochemical technique for its synthesizing enzyme, adenylcyclase AC, being reported, shows the greatest amount to be in cerebellar blood vessels, and of the neural-associated enzyme the greatest amount was in the basket-cell basket endings at the base of the Purkinje cell, not in the Purkinje-cell soma as previously supposed from tissue-slice biochemistry - this necessitates modification of current hypotheses of Purkinje-cell function. It also shows significant AC in blood vessels, which would influence biochemical assays of homogenates of microdissected tissue samples. If some of the AC, vascular or neuronal, might be deficient (primarily or secondarily) in spinocerebellar ataxias, treatment with a cAMP phosphodiesterase inhibitor would be indicated. Chronic electrical stimulation of the presumably normal cerebellum in patients with intractable epilepsy raised norepinepherine, did not alter cAMP of cGMP, and reduced GABA levels in CSF (with SNB).

Progressive spastic paraplegia: This is a progressively crippling disorder of children and adults. The causes are not known. Now published is our identification of three unrelated patients with a syndrome of chronic

adrenal insufficiency from infancy juvenile-onset of progressive spastic paraplegia and dysschwannian peripheral neuropathy, with normal intelligence (with RRB, NINCHD). We postulated a single metabolic defect to underlie the abnormalities in the neural and adrenal tissues (? an adrenoleucodystrophy variant). This has now been found by others to be reflected by accumulation of ultra-long chain saturated-fatty-acid cholesterol esters in the CNS. We are pursuing expressions of this defect in our patients.

Opthalmo-neurology: We have shown that the various neuromuscular disorders affecting the eyes, assuming they are correctly reflected by their limb-muscle pathology, can be on a neuropathic or myopathic basis. They cause various degrees of handicaps. Our studies seek to delineate the underlying disorder, analyze the neuro-opthalmologic defect, and, if possible, seek methods of treatment. We also seek fuller understanding of the basic biology and pathologic responses of the eye neuromuscular apparatus. The commonest associated limb-muscle pathology of the progressive external opthalmoplegia syndrome (in our series of 46 patients, after myasthenia gravis and myotonic atrophy are excluded) is a syndrome characterized by "ragged-red" muscle fibers in limb muscles, whether or not the limbs themselves are weak. Those ragged-red fibers, containing several types of mitochondrial abnormalities, have been studied intensively (see our Myopathy project). The histochemical fiber-typing of normal monkey eye muscles and the different post-denervation response of extrajunctional receptor distribution of those fiber-types has been published (see Myopathies project).

Other CNS Disorders: In epileptic patients, decreased CSF homovanillic acid and 5-OH-indolacetic acid was found, with increased turnover of the latter and normal turnover of the former (with SNB).

An analysis of the astrocytic component of cerebellar astrocytoma is being presented.

An example of chorea associated with subdural hematoma in childhood leukemia has been published.

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 lower motor neuron disorders, peripheral neuropathies, and spinocerebellar degenerations, on the treatment of some, on animal-models of some of these disorders.

Proposed Course of Project: To more fully develop the interlinked basic and clincal 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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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

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

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

To more fully elaborate the clinical, tissue-cutural, histochemical, biochemical, ultrastructural, radioisotopic electrophysiologic and immunologic abnormalities of patients with the various myopathies 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 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 for immunologic means, models of human myopathies in animals and in tissue-cultured human and animal muscle.

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(Rev. 10-76)

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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 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 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 immunologic means, models of human myopathies in animals, in tissue-cultured human and animals. 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-palmatyl-transferase; while still others, such as Duchenne muscular dystrophy and other genetic disorders bearing the name "dystrophy", are of unknown pathogenesis and are untreatable.

Our tissue culture laboratory has been rejuvenated, with greatly 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. Methodologically we have achieved techniques for obtaining abundant, reproducible and mature growth of human fibers in culture, including spontaneous twitching, for precisely selecting certain fibers for our enzyme-cytochemistry and immunocytochemistry at light- and electronmicroscopic levels and for various biochemical studies of them. This year we have grown over 100 human muscle biopsies, as well as numerous rat and chick embryo cultures. Specific studies are noted below.

The Muscular Dystrophies

Biochemically-distinct genetic myopathies

Lysosomal defects: In adult-onset acid maltase deficiency caused by a defect of lysosomal acid-maltase, our earlier reported "reincarnation" in cultured muscle fibers of the biochemical, histochemical and electromicroscopic defects characteristic of the disease was a first proof of a muscular dystrophy having its myopathos endogenous in the muscle fiber. Now we have shown similar reincarnation of the severe biochemical and morphologic defects in 5 additional chronic-infantile and 4 adult cases of acid-maltase deficiency, and partial defects in 4 heterozygotes (with Columbia). These results (a) define the cell of origin of the disease and (b) provide a new test system for manipulations directed toward the treatment or prevention of muscle-fiber damage in this disease without risk to the patient.

Probably another lysosomal defect of muscle fibers is explained as morphologically characteristic "cabbage bodies" (multilaminated material in lysosomes) in muscle fibers of a patient with a chronic myopathy, which we have reproduced in his cultured muscle fibers. Although the biochemical defect is not known, both the biopsied and cultured muscle had elevated acid phosphatase, suggesting the patient has a defect of a yet-unpinpointed lysosomal hydrolytic enzyme (with Institut de Pathologie Moleculaire, Paris). In that and our other cultures of human and animal muscle we are assaying 12 different lysosomal enzymes to search for defects thereof (with Institut de Pathologie Moleculaire, Paris).

"Afuelias": We have introduced the term "afuelias" to describe the defects, known and unknown, of (i) glycogen/glucose utilization Institut de Pathologie Moleculaire. The former cause muscle-fiber breakdown during heavy exercise, especially ischemic exercise - they include phosphorylase, and phosphofructokinase deficiencies, and infantile fatal fasting rhabdomyolysis.

We have now twice reincarnated a glucolytic-enzyme defect of muscle, phosphofructokinase (PFK) deficiency, by demonstrating extremely reduced levels of that enzyme were found in the muscle fibers cultured from a patient with that defect. In the patient, forearm ischemic exercise was reported to

produce electrically-silent contracture, preferential damage to type-II muscle fibers (calcium accumulation when mild and frank necrosis when severe) and positive ^{99m}Tc-diphosphonate scan.

In phosphorylase deficiency, a glycogenolytic-enzyme defect of muscle, we have in 6 additional cases confirmed our previous finding that the enzymatic absence from the muscle fibers is "cured" with their growth in culture -- viz., a remarkable and unexpected recovery of quantitatively normal levels of phosphorylase activity, and not an excess of glycogen, is seen in fibers in the regenerative state in culture and in vivo. We have now reported, immunologically and with histochemical staining of isozyme-focused gels, that there is the normal mature "muscle"-type isozyme phosphorylase as well as the immature "brain-type (fetal-muscle-type) isozyme in the muscle cultured from the phosphorylase-deficiency patients, identifical to that of cultured control human muscle. Thus we have demonstrated a true rejuvenation of an enzyme genetically programmed ultimately to be deficient in mature fibers (with Institut de Pathologie Moleculaire, Paris). It becomes evident that a therapeutic thrust will be needed to provoke and maintain that phosphorylase in the mature fibers of the patient. As one approach, because phosphorylase is activitable via cAMP, we are seeking a clinically beneficial effect of an available phosphodiesterase-inhibitor.

An abnormality of migrating isozyme bands of phosphorylase has been found in the muscle homogenates of some patients with phosphorylase deficiency (with U. Miami).

"Alternate-pathway-therapy" is another approach to the treatment of afuelias. In two phosphorylase-deficiency patients utilizing customized treadmill exercise to achieve reproducible serum CPK rises and muscle cramps under standard conditions, we have shown that that i.v. triglycerides prevented the CPK rise but not the cramps whereas glucose-insulin prevented both. In one patient, an oral medium-chain-triglyceride ketogenic diet for two weeks also prevented the exercise-induced CPK rise. Thus acute-intravenous and chronic-oral triglyceride therapy can prevent exercise-induced rhabdomyolysis, reflecting plasmalemmal breakdown from failure of ATP supply (since it did not mitigate the cramps as did glucose-insulin, different mechanisms and/or different biological type of muscle fibers must be involved).

In two glycogen/glucose afuelias, phosphorylase deficiency and phosphofructokinase deficiency a new diagnostic test has been introduced and reported positive (and negative in many other neuromuscular disorders): ^{99m}Tc-diphosphonate scanning showed increase of calcium in forearm muscle 24 hrs after the standard diagnostic forearm ischemic-exercise test (FIET), and this uptake approximately parallels serum CPK elevations. Correlated were histochemical and our autoradiographic technique for localizing the clinically-administered gamma-emitting ^{99m}Tc-disphosphonate, which documented uptake of the tracer into injured, calciumed muscle fibers; they showed preferential injury of, and

calcium accumulation in, type-II muscle fibers after FIET. (This is part of our effort to develop techniques for doing autoradiography of patient biopsy samples following injection of some of the short-lived gamma-emitting radionuclides used for patient-scanning, establish direct scanning-histoautoradiographic correlations.)

Another way to seek which fibers are more damaged in phosphorylase deficiency was by our single-fiber EMG study of the patients during and after FIET. It established electrical failure of all motor units during FIET, and later no abnormal jitter and no motor-unit densification (i.e., no foreign reinnervation of type-I muscle fibers) -- thus although both fiber types fail during FIET, the I-fibers were not remarkably damaged (perhaps because of greater supplies of, and ability to use, endogenous non-glycolytic substrates and myoglobin-associated O_2 or possibly their axons could have "protectively" shut off. These findings provide a basis for formulating alternate-pathway -substrate therapy in MPD.

A new model of defects in muscle glycogen/glycose utilization was reported using iodoacetate (which inhibits glyceraldehyde-3-phosphate dehydrogenase) intra-aortically plus repetitive sciatic-nerve stimulation; there was an electrically-silent contracture and 24 hrs later a positive ^{99m}Tc-disphosphonate scintiscan, and the low-oxidative type-II muscle fibers were preferentially injured, evidenced hisotchemically by calcium-uptake early and later frank necrosis. This model makes possible controlled analyses of the stages of intrinsic energy-depletion muscle injury, and subsequent repair, as well as evaluation of various alternate-pathway therapies applicable to human glycogen/glucose utilization defects, e.g., phosphorylase or phosphofructokinase deficiencies.

A new syndrome of fasting-induced (presumably) fatal rhabdomyolysis is being reported. The biochemical cause is unknown -- none of the known biochemical defects of muscle is present and anaerobic glycolysis is intact. Lipid droplets were slightly increased in type-I muscle fibers and biochemically carnitine-palmityl-transferase was 2X normal -- suspected is a defect of lipid/ketone-body/glycerol utilization for ATP synthesis (with Columbia).

Tissue-culture of the muscle of a baby with familial rhabdomyolysis and lipid-droplet accumulation in muscle fibers showed abnormal transplasmalemmal (? transtubular) leakage of the MM and MB isozymes of CPK (with CP, CC).

Adenylate deaminase deficiency has recently been reported by others as a biochemical defect of human skeletal muscle present in 2% of their muscle biopsies from various neuromuscular-disease patients. We have improved the histochemical assay of that enzyme but have not found any defect in our first 75 biopsies assayed. We will continue to seek whether the enzyme deficiency is present and pathogenically significant in our neuromuscular-disease patients.

"Hypocyclasias": We have found reduced plasmalemmal adenylate cyclase but normal β-adrenergic receptors 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 (the last two are discussed in the Episodic Weakness and Myotonia Project). The first two have muscle fiber smallness with central nuclei. Muscle biopsies of affected infants from two families with X-linked recessive infantile-fatal muscle-fiber-hypotrophy-with central nuclei were flown to use from Amsterdam, The Netherlands and from Texas for tissue-culture. Both showed the same abnormalities: (i) cultured muscle cells had a marked, apparently uncontrolled, ability to proliferate, resembling that of neoplastic cells, which has presented through many passages over 8 months to date, and was not controlled by CNS extract or CNS co-culture; (ii) 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 (per ^{125}I-hydroxypindilol-binding). Thus demonstrated was an intrinsic defect of the muscle cell and apparently impaired control mechanisms related to cAMP -- that could, among other effects, have resulted in impaired response to neurogenic maturational mechanisms, i.e., one kind of "myogenous deinnervation". The identified defect now can be the basis of treating such patients with a phosphodiesterase inhibitor to increase cellular cAMP.

Our "Calcium Hypothesis" was published this year, and was detailed in our 1977 Annual Report. In it we proposed that entry of calcium into the muscle fiber following a pathologic breach of the plasmalemma from any cause (e.g., endogenous afuelia, exogenous ischemia toxin, toxic antibody, or toxic T-lymphocyte, etc.), (a) if slight and restricted, is relatively benign, and is the "spark for repair/regeneration" or hypertrophy of muscle fibers, or (b) if severe and unrestricted, begins a lethal cascade of events pushing muscle fibers past their point of no return, i.e., is the "ultimate molecular assassin" or the "messenger of molecular doom". We 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 electron-microscopic histochemistry, autoradiography, biochemistry, and clinical scanning.

A detailed study of mechanisms of muscle-cell injury in relation to Duchenne dystrophy has been published as has a survey chapter (>500 references) of the biology of the muscle cell in relation to myopathies.

Duchenne muscular dystrophy (DMD) the most prevalent of the so-called "muscular dystrophies" by old terminology, 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. For example, the defect of erythrocyte morphology reported by others we have previously reported as not present in our controlled studies. We now report a further aspect of erythrocyte normality in our finding that in 7 DMD patients there was no increase of erythrocyte turnover as measured directly by ^{51}chromium-labeled erythrocyte survival (with H,CC) or indirectly by hemoglobin, reticulcyte count, plasma-hemoglobin. The total fatty-acid composition of erythrocyte of DMD patients and carriers was normal by careful cellular preps and quantitative computer-integrated gas-chromatography of fatty-acid methyl-esters purified by thin-layer chromatography (as well as in myotonic atrophy and many other neuromuscular-disease-controls), thus contradicting the "defect" of palmitoleic acid reported by others. The defect of the muscle-fiber plasmalemma evident by peroxidase penetration reported by others we have reported not to be disease-specific. The alteration of muscle homogenate adenylate cyclase reported by others we have reservations about because: (i) it was not exclusive to DMD, and (ii) its cellular site (presumed by them to be muscle-fiber plasmalemma) could not be determined from those biochemical studies. Our own newly developed histochemical methods for adenylate cyclase (AC), utilizing AMP-PNP as the substrate and Ca++ (which doesn't inhibit AC like Pb++ does) as the capture agent, was reported to show highest muscle AC in blood vessels and almost none detectable in normal muscle at shorter incubation times; but in regenerative fiber fairly high amounts. This technique we also developed at the EM-cytochemical level and showed AC precisely localized to the plasmalemmal and t-tubule membranes. In DMD biopsies, many fibers, often appearing normal with other histochemical reactions, show subtle regenerative features with the AC histochemical technique.

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 (v.i.). Human regenerative fibers have certain fetal isozymes -- e.g., LDH-1,2 has been known, and we now confirm the recent finding of CPK-MB. We have introduced the use of elevations of CPK-MB isozyme in serum (and in muscle biopsies) for detecting DMD carriers. Our other two new methods of carrier detection are elevated serum hemopexin and serum myoglobin (v.i.). These together we use as fine-tuning for DMD-carrier detection, identifying carriers missed by all other carrier-detection methods. Our data have also been reported in the context that elevated plasma CPK-MB is not diagnostic of myocardial infarction as some cardiologists have reported -- we find it elevated in any kind of leaky skeletal-muscle disease, e.g., DMD and dermatomyositis/polymyositis (with CP, CC). Immunocytologic methods to localize the fetal, MB, form of CPK within muscle fibers has demonstrated in DMD and other myopathic biopsies regenerative fibers that are not evident with ordinary stains. Our new test of ^{99m}Tc-diphosphonate forearm scanning 24 hours after ischemic exercise (6 hrs, after isotope injection) did not defect DMD carriers (or patients) like it did phosphorylase and phosphotfructokinase deficiency patients, (with NM, CC).

Hemopexin is an inducible, liver-produced, heme-transport protein. It was first discovered elevated in DMD patients and carriers by an Assistant Neurologist a few years ago. We are reporting our confirmation of that. To support the hypothesis that the elevation is due to subtle myoglobin leakage from minimally damaged muscle fibers, we are reporting that: (a) with a sensitive radioimmunoassay, myoglobin leakage into the serum of nearly all DMD patients and half of the carriers (with Columbia); (b) hemopexin elevation in monkeys with experimentally crushed muscle or injected with myoglobin, which persisted long after CPK levels returned to normal (in the crush studies). A large number of neuromuscular-disease patients were surveyed with the immunodiffusion assay for hemopexin quantitation. Only active myopathies, especially dermatomyositis/polymyositis and DMD patients and carriers, and myasthenia gravis patients, had hemopexin elevations, and all of those groups of patients had elevated serum myoglobin. We are now studying ^{125}I-hemopexin molecular turnover in a variety of neuromuscular-disease patients, especially in DMD patients and carriers and in monkeys with experimentally altered hemopexin levels (with UCSD).

Our new and reported model involving the blockage of leg-muscle anaerobic glycolysis by intra-aortic iodoacetate, which results in the secondary loss of plasmalemmal membrane integrity of the damaged fibers (v.i.), establishes an experimental basis for at least the possibility of hypothesis "b" re. DMD pathogenesis (above, in harmony with our clinical demonstration that in muscle phosphorylase deficiency ischemic exercise precipitates acute breakdown of the plasmalemmal barrier, allowing prominent creatine-phosphokinase (CPK) egress and calcium ingress (detected by scanning with ^{99m}Tc-diphosphonate), v.s.

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 and of our experimental ischemic myopathy in animals. As yet, an ischemia mechanism, although possible in DMD patients, has not been demonstrated in them directly (that would be the next logical step). However, our now-published study demonstrates that in known ischemic human muscle, caused by occlusive vascular disease, the histochemical pattern of the regions of lesser involvement of muscle (groups of necrotic or regenerating fibers in fields of normality) is indistinguishable from that of early DMD and of our experimental ischemic myopathy in animals, thus being at least harmonious with the ischemic myopathy in animals, thus being at least harmonious with the ischemia hypothesis (with VAH, DC). To date, none of the studies attempting to detract from our ischemia hypothesis is conclusive, nor even incisive.

Platelet uptake of serotonin, which 10 yrs ago we found impaired in DMD, now remeasured with a new technique, again shows reduction of initial uptake rates in DMD patients and carriers cf. normal and neuromuscular-disease controls (only ALS patients showed similar reduction) and normal serotonin-release rates in DMD. We are also evaluating platelet serotonin packet size and dense-body counts in DMD patients (with CP,CC).

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.

Although phosphodiesterase (PDE) inhibition is considered to reduce peripheral arteriolar resistance and increase peripheral blood-flow, a PDE-inhibitor has not benefited our DMD patients.

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 "autoimmune" or "dysimmune". Before the introduction of antidysimmune therapy, all patients were seriously incapacitated and many died.

The special variation of anti-dysimmune treatment we introduced to this disease 9 1/2 years ago, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred) has continued to be, in our series (about 70 cases) and others, the single best available treatment for children and adults (without or with an associated cancer). It has the greatest benefit/side-effect ratio and is easiest to manage. However, because not all patients respond and because the side-effects of LT-HSDAD-Pred can be significant, we are: (a) defining some of the parameters of their immunologic response to prednisone and seeking predictive indicators thereof, and (b) testing other antidysimmune agents.

By use of T- and B-lymphocyte cell markers, T- and B-lymphocyte mitogens, and T-lymphocyte cytotoxicity on tissue-cultured chromium-labeled muscle fibers, we have now published that in PM/DM patients, although HSDAD-Pred is clinically cumulatively effective for months and longer, its measurable effect on the peripheral circulating lymphocytes, using currently available techniques, lasts less than 24 hrs; and that these effects are more profound on the T-lymphocytes. In a number of patients, children and adults, inadequately responding to HSDAD-Pred we have demonstrated on an individual-case basis a remarkable therapeutic response to added azathioprine (3 mg/kg/d) to establish the efficacy of azathioprine (added to an unchanged dose of prednisone), a double-blind trial in otherwise refractory DM/PM patients, 15 to date, is continuing. We are also measuring the patients' lymphocyte responses, including killer T-lymphocyte responses, to azathioprine (with NCI). Our demonstration, with non-invasive cardiologic techniques, of cardiac involvement (conduction blocks, arrhythmias, systolic mitral prolapse) in the majority of 20 cases of DM/PM is being published (with NHLI, V.A. Hosp. DC, and Georgetown Univ.). Pulmonary interstitial fibrosis we have found to be common in our DM/PM patients detailed pulmonary functions are being evaluated in all our DM/PM patients, 14 to date (with NHLI).

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 elimination/treatment of an exogenous agent could be curative (as opposed to the merely suppressive action of all current treatments). We are now seeking evidence of that exogenous agent(s), and some preliminary clues have been found (also v.i.).

Treatment of the massive subcutaneous calcification, which can be a crippling complication of childhood DM, is often unsuccessful. However, in some severely affected patients the calcium has remarkably diminished as the muscle and skin were responding to our combined azathioprine-HSDAD-Pred program. The reasons for the calcification are being sought by our histochemical, electron-miscropscopic, and autoradiographic studies (v.i.). ^{99m}Tc-diphophonate body scanning (with NM,CC) continues to clinically detect that calcification in its very early stages (for new microscopic detection methods, v.s. and v.i.).

The exact mechanism of muscle damage in PM/DM is unknown. Previously we have reported immunoglobulin complexes deposited in blood vessels in 83% of the childhood cases and 29% of adult cases, findings supporting our earlier hypothesis that an aspect of the muscle damage may be vascular. While disorders of both immunoglobulins (produced by B-lymphocytes) and direct immunocyte toxicity (T-lymphocytes) might be occurring in all cases of PM/DM, we think the former (deposited as intravascular immune complexes) may be more muscle-damaging in the childhood form and the latter are more pathogenic in the adult form. Therefore, the possible cytotoxic role of T-lymphocytes, including killer T-lymphocytes, is being evaluated in the DM/PM patients, in tissue-cultured muscle, and in animal models (with ID and NCI). It is conceivable that the immunologic abnormalities of DM/PM are ultimately provoked by an exogenous infectious agent, however, our continuing attempts to see or "rescue" an agent from DM/PM patients' muscle directly after tissue culture of it, or after animal inoculation, thus far have been negative (with ID-NINCDS and NCI), except in the chronic vacuolar myopathies (v.i.); nor has reverse transcriptase been found so far.

A new and possibly disease-characteristic histochemical finding in DM/PM patients of microscopic foci calcium accumulation in collagenous connective tissue of muscle and subcutaneous regions has been published. Its pathogenesis is being sought, as is its correlation with our positive ^{99m}Tc-diphosphonate patient-scans for calcium, our autoradiographs of patient skin and muscle biopsies for calcium, and our previously described highly-disease-characteristic histochemical finding of alkaline-phosphatase staining in the intramuscular connective tissue (also published).

Another new approach to this problem is an immunocytologic characterization, at light- and electron-microscopic levels, of the collagen in the muscle biopsies of DM/PM patients cf. normal and Duchenne muscular dystrophy, other myopathy and neuropathy disease-controls. Collagen has four immunologically-identifiable polymorphic variants (determined by the aminoacids of

its three polypeptide chains) and is a component of connective tissue and cellular basement membranes, each variant having distinct mechanical properties and normal location. The type of collagen synthesized by abnormal cf. normal muscle biopsies growing in culture is also being studied by this approach.

Chronic vacuolar myopathy, seen in 14 of our patients, often considered a variant of the DM/PM complex, we have newly separated off as a distinct disease (or syndrome). It is characterized by acid-phosphatase-positive vacuoles in muscle fibers which ultrastructurally are membrane-bound (lysosomal) 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 mμ "diameter" and 100 mμ periods. The muscle in culture reincarnates the typical vacuoles after 10 days of growth. In one case, the cultured muscle fibers showed by electron-microscopy a number of unusual structures strongly resembling reovirus virons situated near the nuclei or plasmalemma; re-scrutinizing the original biopsy revealed rare examples of identical structures.

Other Myopathies and Neuromuscular Diseases of Uncertain Classification:

Malignant hyperthermia-rigidity (MHR) is a syndrome,70% fatal, of acute rise of body temperature and muscle rigidity during general anaesthesia. A number of the patients have underlying not-well-defined neuromuscular disorders. In one MHR patient, and his father, we have reported central-core diseases (CCD), with its typical type-I muscle fiber predominance (with Children's Hospital, D.C.). Because we have two additional families with CCD and MHR and there are in the literature two more such families, we have issued a caution to all our CCD patients and their physicians regarding the possibility of MHR during general anaesthesia. The mechanism(s) of the attack of MHR is not known. It appears that there is an excess of free intracellular calcium in the muscle fiber, which we have proposed might be due to an effect of the anaesthetic or muscle-relaxant agent on the calcium-barrier function of the plasmalemma (v.s.) rather than the SR as proposed by others). We are now investigating why central core disease, which we have earlier postulated to be due to a pre-natal monophasic neuronal involvement (impaired primary formation or excessive loss in neuronothanosis) mainly affecting the type-II units, should predispose to the development of MHR. We continue to try to develop specific clinical and muscle-biopsy diagnostic tests to identify patients predisposed to develop MHR.

"Ragged-red" muscle fibers, which contain severe mitochondrial abnormalities, are the commonest histochemical manifestation in limb muscles of the heterogeneous syndrome of oculocraniosomatic neuromuscular disorder 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 are reporting that most of the mitochondrial changes, including increased number and greatly increased size of mitochondria, wide distorted "twisted-ribbon" cristae, and "mushy" inclusion material, have been re-incarnated, although the mitochondrial crystal-like inclusions have not yet been (perhaps they take longer to develop). We are also reporting that the same changes were produced in normal human muscle cultures after 2 days exposure to dinitrophenol, and the ragged-red-fiber cultured 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 and crystalline inclusions lacked cytochrome oxidase staining by our EM-cytochemistry. (On normal and abnormal human muscle in culture dinitrophenol also produced leptomeres, clustering of nuclei, dilation of Golgi, Z-disc smearing, and glycogen accumulation as membrane-bound balls, showing more diverse effects on the muscle cell.)

Clinically, some OCSNMD-RR patients with opthalmoplegia have cerebellar ataxia, mental impairment, some denervation evident in muscle biopsy, and spinal-fluid protein increase being reported is that some such patients have by CAT-scan 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).

Developmental abnormalities of the motor-unit have been classified in a new system. They can be genetically programmed or induced by the environment (fetal and/or maternal). They are classified as: Lower Motor Neuron (LMH), Schwann Cell, Muscle Fiber, Blood Supply, and Humoral Factors. They can affect one LMN, Schwann cell, or muscle fiber type or subtype selectively or non-selectively.

A model of the human fetal alcohol syndrome is being studied by producing chronic ethanolism in pregnant rats and examining its neuromuscular and central-nervous-system manifestations. We continue to study the selective atrophy of the type-II (glycolytic-rich, oxidative poor) muscle fibers, especially the subtype IIB fibers, which 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 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 now actively consider 3 mechanisms, perhaps summated, in the cancer patients: (a) insidiously

decreased oral fuel (caloric) intake, which we have recently documented; (b) fuel wastage due to metabolic derangement within neoplastic cells; (c) possibly a circulating small-molecule remote-effect acting on type-II fibers. We will be studying the role of insulin reception by and action upon muscle fibers to explore these mechanisms.

Basic Mechanisms and Cross-Category Aspects:

Plasmalemma of human and animal muscle have received our major attention, through a multidimensional approach.

Results of the following studies are now being published or have been presented at meetings. Pure fractions of rat plasmalemma (PL) were obtained, and the methods perfected so that adequate quantities of plasmalemmal-membrane can, from normal human muscle, be obtained from limb amputation or radical mastectomy, and even from pathologic muscle biopsies for these studies. Now, we have been able also to do these studies on normal and abnormal human and animal muscle grown in tissue culture. With the plasmalemmal fractions and subfractions, methods have been established for studying acetylcholine receptor, acetylcholinesterase, Na^+-K^+ ATPase (Na^+-stimulated phosphorylation), divalentcation (viz., Ca^{++}) binding/transport, Ca^{++}-stimulated ATPase, adenylcyclase, guanylate cyclase and β- and α-adrenergic receptors. Some of these have been studied in sarcoplasmic reticulum (SR) and mitochondrial (mito) fractions as well.

From normal human skeletal muscle, membrane fractions (PL, SR, Mito) were prepared. With ^{125}I-hydroxypindolol binding to localize the β-adrenergic receptor (βAR), we have found that βAR-binding was predominately associated with PL, with kinetic, affinity and blocking characteristics of typical βARs of animal muscle. These results are being correlated with our previous studies of muscle adenylate cyclase and will be a standard for comparing βAR properties in various human muscle diseases.

In rat skeletal muscle, our previous studies indicated that denervation results in increased guanylate cyclase but decreased adenylate cyclase in muscle PL membranes. However, cyclic nucleotide phosphodiesterase (PDE) activities (against both cAMP and cGMP) were enhanced in the denervated muscle. Now the actual cAMP and cGMP levels in normal and denervated muscle have been estimated by radioimmunoassays to determine whether the cyclic nucleotide levels directly reflect on those altered enzyme activities. Following sciatic denervation of rat gastrocnemius, soleus and EDL, cAMP showed a 30% increase at 7 days and remained so until 21 days (end of experiment). However, cGMP showed no significant change. Thus, one cannot necessarily infer cyclic nucleotide levels from values of enzymes associated with them.

The effect of denervation on the β-adrenergic-receptor-(β)-adenylate-adenylate-cyclase-(AC)-system was investigated in rat skeletal muscle because denervation renders skeletal muscle physiologically supersensitive to catecholamines. βARs, as determined by binding of ^{125}I-hydroxypindolol (HYP), a potent β-blocker, were markedly increased in PL from denervated muscle. The increase was evident 2 days after sciatic nerve-section, rose to 50% by one week, and was 100% greater than control by two weeks. The finding of ^{125}I-HYP to PL of normal and denervated muscle was selectively and stereospecifically inhibited by various βAR agents, but not by α-adrenergic drugs; even though βAR-agents was unchanged. Parallel studies on AC in PL showed marked decrease of basal, isoproterenol- and NaF-stimulated activities, being about 50% of control after 2 weeks of denervation. Because denervation induces differential effects on AC and βAR, they must be separate entities responding differently to denervation. Treatment of denervated rats with cycloheximide (0.1 mg/kg) a known protein synthetic inhibitor, did not block the increased βAR binding in denervated PL, suggesting that the increased βAR in denervated muscle is not due to synthesis of new sites but to unmasking of existing receptor sites.

Developmental aspects were studied, coorelating development of βAR and its coupling to AC (as measured by the sensitivity of AC to catecholamines), in 18-day PL isolated from hind leg muscles of 18-day embryonic neonatal, and 1-30-day post-natal stages of development. βARs, per binding of ^{125}I-HYP, were above the adult level in the embryonic muscle, further increased at birth, reached its peak 3 days after birth, and declined thereafter to the adult (60-day-old) level by the 20th day. However, AC of embryonic and newborn rat muscle PL was not stimulated by catecholamines. Catecholamine-sensitivity of the enzyme was noted on 3rd day after birth, reached its maximum on day 5, and decreased thereafter to the adult level by day 20. Basal activity of AC (in the absence of added stimulant) was embryonic and newborn muscle 10-15 times greater than in adult muscle and drastically decreased after birth to reach the adult level by day 20. NaF stimulated the AC at all stages of development, but its activation was very low in embryonic and newborn muscle membranes. cAMP-phosphodiesterase activity (in homogenates, PL and cytosol fractions) showed a pattern similar to that of basal AC. This, βAR and AC exist even from embryonic stages but their coupling occurs only 3 days after birth. We will next seek factors underlying the establishment of receptor-enzyme coupling in the first 3 days of life.

Autoradiography of rat muscle following in vivo administration of ^{125}I-hydroxybenzylpindolol (HYP), a potent β-adrenergic blocker, was used, with pharmacologic controls, to demonstrate the cellular locus of β-adrenergic receptors, since with biochemical assays of cell fractions from whole-muscle homogenates it is impossible to define the cell-type possessing the activity demonstrated. We found: (1) much greater amount of β-adrenergic receptors in arterial-tree vessels than in muscle fibers; (ii) post-denervation increase of both (paralleling our biochemical studies, v.s.), which could underlie the known post-denervation supersensitivity of vessels and muscle fibers to

β-adrenergic agonists; (iii) the fallacy of assuming β-adrenergic binding studied biochemically in whole-tissue muscle homogenates is only in muscle cells; (iv) potential importance of arterial-vessel as well as muscle-fiber β-adrenergic receptors in human neuromuscular diseases. We will now extend this by developing a method to look at those receptors in human neuromuscular-disease muscle biopsies and cultures and at α-adrenergic receptors and insulin receptors in animal-model and human diseases.

Lectin probes for membrane-bound saccharide components, displayed at light and electron-microscopic levels were presented as a new approach to neuromuscular pathology of muscle biopsies and cultures. Normal muscle fibers and cells of blood vessels showed only plasmalemmal (± basement membrane) localization with Con A, LC, RCA 120 and WGA; while Soy stained only cells of vessels and the perineurium. Most of the pathologic muscle showed no abnormalities. The only abnormalities seen in a large variety of neuromuscular diseases were: small veins excessively stained and smudgy with Con A in dermatomyositis (not in Duchenne dystrophy); fiber-splitting and capillary-invasion delineated by ConA and WGA; in regen-degen fibers increased staining of sarcolemma and slight staining throughout the intermyofibrillar regions with Soy, and slightly with ConA; in myotonic atrophy some fibers having small circular profiles within them. Cultured human muscle fibers were stained without obvious abnormalities.

The ultrastructural cytochemistry of plasmalemma of cultured normal human and animal muscle fibers was studied in greater detail, by use of a lectin probe (Con A, for α-D-glucosides and α-D-mannosides), ruthenium red (for acid mucopolysaccharides), α-bungarotoxin (for nicotinic acetylcholine receptors), and tannic acid. The specific staining profile of myoblasts through development to multinucleated muscle fibers in culture is being presented in detail. Tannic acid was especially interesting, not binding to plasmalemma of single myoblasts or young myotubes, but to plasmalemma of mature muscle fibers as well as to T-tubules (rat and chick muscle) T-tubule-originating lace of cultured chick-embryo muscle, saccular membranes within human fibers (? T-tubule or plasmalemmal precursors, since T-tubules do not form in cultured human muscle) and to the outer membranous coat of virus C-particles in cultured "normal" chick muscle. Thus, tannic acid appears to be a very good probe for studying muscle cell maturation in culture. These data now provide standards to which abnormal muscle in culture can be compared. We will also be using peroxidase-labelled antibodies against type-IV collagen (the basement membrane collagen) (with NIDR).

Pyruvate kinase assayed in cultured human muscle by electrofocusing and histochemical staining was shown for the first time to have fetal isozymes (with Institut de Pathologie Moleculaire, Paris).

Abundant leptomeres were found, and being reported, in muscle cultured from 5 patients with acid-maltase deficiency, 1 with autophagic vacuolar myopathy , and 1 with abnormal mitochondria, but not in our other cultured

normal or pathologic muscle. Similar abundant leptomeres were reproduced in normal human muscle fiber cultures exposed to 0.5 mM dinitrophenol, which also provoked prolonged "tonic" contractions in them.

Phosphodiesterases (PDEs), because of their potentially important role in the breakdown of cAMP and cGMP, were studied histochemically with the α-naphthyl-thymidine-5'-phosphate for alkaline PDE (PDE-I). None was localizable in normal or abnormal human or rat muscle cells. Rat muscle showed heavy staining of all blood vessels, which was increased 3-6 days post-denervation -- this paralleled biochemical assays but demonstrated precise cellular localization not possible with biochemical assays of whole-tissue homogenates. Reacting interstitial tissue and inflammatory cells of regenerative rat muscle, but not the regenerating muscle fibers, were strongly positive. In human muscle, only mast cells stained, and the only abnormality of pathologic muscle was an increase of mast cells in dermatomyositis/polymyositis. Thus it is fallacious to presume (a) that, from biochemical studies of rat-muscle homogenates, a significant amount of PDE-I is in muscle fibers, and (b) that vessels and connective-tissue of rat muscle are like those of human muscle. (For PDEs of platelets and erythrocytes, v.i.).

Acetylcholinesterase (AChE) studies (being published) of subcellular distribution and properties showed: (a) majority present extrajunctionally, mostly in microsomes/SR but some in the soluble phase; (b) after denervation, the early AChE reduction is mainly in the microsomal/SR 4-S fractions; (c) the 16-S form is restricted to the neuromuscular junction (per others), and d the plasmalemma had only 10-S and 16-S. Synthesis, degradation and release of the different molecular forms of AChE will now be studied in normal and various pathologic circumstances.

Platinum-thymine complex was used -- in combinations with Feulgen pretreatment, DNAse or RNAse -- for selectively staining RNA or DNA at the electronmicroscopic level. 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 cells in culture).

Modifications of our original techniques for selection and drilling of specific fibers in plastic-embedded muscle cultures have been published.

Single-fiber electromyography (SFEMG) to measure motor-unit densification, EMG, and muscle histochemistry were correlated in 54 patients with various neuromuscular diseases (with Uppsala, Sweden), showing: (i) fiber-type and, more specifically, fiber-subtype grouping as the histochemical correlate of densification of the motor unit in neurogenous and myogenous deinnervations; (ii) SFEMG can be a more sensitive index of motor-unit densification.

Of the eye muscle fiber-types, none is histochemically like a limb-muscle fiber-type. Published was our demonstration of their normal histochemical patterns in Rhesus monkey and identification of 3 types, "fine", "granular" and "course". The first two have one endplate per fiber and probably are different types of twitch fibers; the last has multiple endplates and may be a tonic fiber. Following denervation the first two developed diffuse extra-junctional acetylcholine receptors but the coarse fibers did not; no fibers were positive beyond 13 weeks post-denervation.

Histochemistry of matched interrupted serial cross-sections, and alternate sections for electronmicroscopy along the entire length of spindle muscle fibers in rat soleus showed with the "myofibrillar" Plt 9.4 ATPase: distinctions between nuclear-chain, nuclear-bag$_1$ and nuclear-bag$_2$ fibers; regional staining differences along the bag$_1$ and bag$_2$ fibers; ultrastructural heterogenity of poler cf. equatorial portions of bag$_1$ fibers. Endings of both "plate" and "diffuse" type, inferred from cholinesterase stainings, occurred on bag$_1$ and bag$_2$ fibers but did not correlate with zones of ATPase heterogenity and thus probably did not determine them. Possibly sensory innervation and/or regions of passive-vs-active stretch governed the ATPase staining. Sensory-deinnervated (3-12 mos) spindle muscle fibers had altered ATPase-staining and other abnormalities.

Normal human spindle muscle fibers of intercostal muscles, studied in the same manner, had one bag$_2$ and 1-2 bag$_1$ fibers, the latter having absent M-lines and usually of smaller diameter and shorter cf. bag$_2$'s. Nuclear chain fibers and bag$_2$ fibers had M-lines. Histochemical profiles of each of the 3 types were clearly established, with regional differences of histochemistry and ultrastructure along the fiber lengths; because they appear analogous to those of cat spindles, they probably have different functional roles, as is known for the cat spindle 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 studies 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 01190-14 MN

PERIOD COVERED
October 1, 1977 through September 30, 1978

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

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Medical Neurology Branch

SECTION
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☒ (a1) MINORS ☐ (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 myasthenia gravis. Especially, to seek new or improved methods of treatment.

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PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To apply clinical, immunologic, tissue culture, histochemical, pharmacologic, electrophysiologic, autoradiographic, radionuclide-scanning, and electronmicroscopic 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 include 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, thymus and other tissue were obtained during diagnostic or therapeutic trials. Sera, muscle, thymus and other tissue were obtained during diagnostic or therapeutic procedures from these Medical Neurology Branch patients.

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 "autoimmune" or "dysimmune". Untreated patients usually are seriously handicapped and many die. Palliative treatment with anticholinesterases and antipathogenic treatment consisting of thymectomy, ACTH and, most recently, prednisone has helped considerably but much disability, some fatality, and drug side-effects still occur.

We previously reported the first identification of a factor, an IgG, in the sera of MG patients which blocks binding of α-bungarotoxin (αBT) to the human junctional nicotinic acetylcholine receptor (nAChR) at the normal neuromuscular junction (41% of MG patients) and extrajunctional AChR of denervated human fibers (72% of MG patients, including all who had a thymoma),i.e., an "antireceptor antibody". We next reported that all MG patients having an

IgG antimuscle antibody" (first found by others) also had the blocking factor (although only half having the latter had the former) (the only discordant finding being in one non-myasthenic polymyositis patient with thymoma who had antimuscle antibody but no detectable blocking factor) and suggested that these may be the same antibody or, if different, virtually always co-produced. Our junctional localization of the blocking factor put it in the correct position to impair neuromuscular transmission and cause the weakness of MG. We found the nicotinic acetylcholine receptor ultrastructurally localized both post-synaptically and presynaptically and proposed the pathologic antibody acts at both sites to cause disease -- this has now been confirmed by others who, initially doubting our pre-synaptic localization, found IgG and C-3 complement localized both post- and pre-synaptically.

We have now published our tissue-culture studies of human, rat and chick skeletal muscle showing that with α-BT the cultured animal fibers contain "extrajunctional", actually non-junctional, nAChR diffusely in the plasmalemma without "hot-spots" claimed by others and that the binding of α-BT to pure non-junctional nAChR is readily blocked by the pathologic IgG of MG patients. This demonstrates a new environmentally-controlled test-object (the cultured muscle fibers) for identifying circulating pathogenic factors.

On the basis of those studies and our EAMG studies (v.i.) we have now published our hypothesis that: (a) there are normally two subsets of acetylcholine receptor (AChR), a "junctional" (J) and an "extrajunctional" (E) form; (b) both J and E AChR occur at the neuromuscular junction but extrajunctionally only E-AChR is present; (c) the IgG blocking factor in human MG is directed mainly against the E type of AChR while that of the EAMG model we worked with is mainly against the J-AChR; (d) the diffuse AChR of thymic epithelial cells, v.i., may be E-type.

From our work with the induced autoimmune model (rabbits injected with electric-fish AChR), originated by others, of experimental allergic MG (EAMG) (with U. Maryland) we have now published our demonstration of: (a) binding of that rabbit sera to human neuromuscular junctions but not to extrajunctional receptor of denervated fibers at light-microscopic resolutions; that junctional binding of EAMG sera was not blocked by blocking-factor-positive (or negative) MG sera, only partially blocked by αBT, and not blocked by carbamylcholine, decamethonium or tubocurarine; (b) binding of it by electromicroscopic resolution to the plasmalemma diffusely in rat and chick muscle fibers in tissue culture; (c) binding of that rabbit sera to the original antigen in a radioimmunoassay we developed (with IB), but no binding of blocking-factor-positive (or negative) MG sera to that antigen; (d) similarities but also distinct differences of the model with human MG, indicating it is not a perfect model of the latter although it could still be a model-in-principle.

The rationale for the empirically-observed benefit of thymectomy (v.i.) is still being sought. Our demonstration last year that in the thymus the epithelial cells, in both "hyperplastic" and "involuted", all contain AChR

demonstrable histochemically by αBT binding (with IB and Washington Univ.) fulfills a step in our earlier hypothesis that the mechanism of MG might be an alteration of thymic epithelial cells e.g., by an exogenous virus, making them "foreign", in response to which B-cells make anti-AChR antibody (perhaps programmed by intermediary T-lymphocytes) that co-reacts with junctional AChR to cause paralysis. Because the thymic epithelial cell is, by others, considered pluripotential, this same hypothetical pathogenic mechanism could apply to other dysimmune diseases in respect to thymic epithelial cell molecules altered to become other antigens. Now we have found that even in MG thymuses considered "atrophic" by existing histopathologic criteria there are evident in our fresh-frozen sections many small nests of cells that look "active" -- accordingly, we have postulated that they have a detrimental role in the pathogenesis of MG and their removal may be the basis of improvement following thymectomy of the older MG patient who typically has such thymic histopathology.

We have in press our finding of serum hemopexin 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 heretofore minimal subclinical plasmalemmal-leaking myopathy in many MG patients. Our electronmicroscopic studies of human thymic epithelial cells from myasthenia gravis patients, directly (regular and pyroantimonate-stained tissue) and after being grown in culture, shows desmosomes and tonofibrils typical of epithelial cells and no muscle-cell like myofibrils, contrary to what has been reported by others in cultures of normal animal thymuses (which might have been contaminated with non-thymus muscle cells during tissue removal).

The role of thymectomy in the treatment of MG and for which MG patients, has recently been questioned. Review of our last consecutive 55 thymectomies done over the past 10 years has revealed: patients with onset age 16-29, 84% improved (one Osserman class or better and on same or less medication); 4 of 6 patients with thymoma improved, all having had onset over age 29 and 4 not preoperatively diagnosed; of patients with onset over age 29, 71% improved; 83% of patients with thymic hyperplasia improved as did 70% of those with "involuted" thymus (v.s.); zero operative mortality, low operative morbidity; severity of myasthenia itself not a contraindication, but rather an indication for surgery; 84% improvement rate in patients "thymectomized" within 10 years of onset of MG cf. 33% improvement rate if duration >10 years; the transcervial surgical approach unsatisfactory, -- worsening of MG within 1/2-7 years in 7 of 9 so operated (including 4 whose transcervical operations were performed at centers favoring and experienced with that procedure) necessitated reoperation by sternal-splitting disclosed residual/recurrent thymus in all (as much as 1/2-3/4 of the presumed original thymus, typically the inferior portion of one or both poles) and resulted in clinical improvement in all 7; our modified transverse sternal-splitting upper-steronotomy

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 5B, NIHL and Harvard).

Thus thymectomy is potentially beneficial in all patients with onset in teen-age or later, and repeat thymectomy can be remarkably beneficial in patients previously improved who subsequently exacerbate and do not respond to medical management. Serum antibody levels against nAChR before and after thymectomy are being correlated with clinical response (with Salk Inst.).

In a number of those patients as well as primary thymectomy patients, we have found positive ^{67}gallium clinical scans. ^{67}Ga, which localizes in thymus, was used in both clinical scanning of the thymus preoperatively and for in vitro counting of the thymus removed by therapeutic thymectomy. Before thymectomy (hyperplastic) thymuses were positive but 10 (one thymoma, 6 hyperplastic, 3 "involuted") were false negatives; one repeat thymectomy was positive (and hyperplastic tissue was found, and became negative after thymectomy), the other was falsely positive with no thymic tissue locatable. Of 14 MG patients scanned only post-thymectomy, all were negative. In vitro isotope counting showed ^{67}Ga concentration in all positive and false-negative thymuses, indicating need for more sensitive pre-operative ^{67}Ga scanning and intraoperative probes to localize thymic tissue.

Abnormal lymphocyte function is the pathogenic step presumably suppressed by corticosteroid treatment of MG. Confirmed and adopted by most other physicians has been the treatment we introduced to MG, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred). In our own series it continues to be extremely beneficial in the majority of cases, 60 of 64, and for as long as 12 years in a child and 8 years in an adult. Responding best are the older-onset patients, especially the older males. Our 4 non-responders were females, 3 in the menstruating age group. Importantly, though, we continue to find that none of our responding patients has become absolved of his/her requirement for prednisone even after a gradual tapering of the dose. Typically, patients exacerbate 1-4 months after stopping a 5 mg q.o.d. dose (about the time taken for resumption of synthesis of measurable levels of abnormal IgG, per others in another dysimmune disease). Currently we give to patients not simultaneously taking anticholinesterase drugs, a single-dose 100 mg prednisone daily for the initial 2-4 weeks before converting to the alternate-day schedule, apparently resulting in more rapid improvement. Fragile patients are not taken off their anticholinesterases but to them prednisone is given in a gradually incrementing single-dose-daily schedule beginning with 10-20 mg. They are usually taking both an anticholinesterase and prednisone, often have a more "brittle" myasthenia, and must be watched carefully for ingravescent overdosage by the anticholinesterase. Because neither anticholinesterase nor prednisone treatment is either curative or completely suppressive, nor thymectomy always satisfactorily beneficial, more details of the pathokinesis are needed (v.s.).

Some of our patients have required much higher prednisone doses than 100 mg q.o.d., or even q.d., dosages arrived at empirically in each patient after a period of time. We would like to be able to give a single test-dose and measure parameters of immunosuppression to immediately establish the minimum effective dosage needed, since overdosage, and even therapeutic dosage, produces side-effects of various degrees. Accordingly, the effect of the HSDAD-prednisone treatment on lymphocytes was measured over a 48-hour cycle in a number of MG and other patients, and the results reported. At 6 hrs. after the 8:00 a.m. prednisone dose there is marked depression of T-lymphocyte counts and lymphocyte responses to T-lymphocyte mitogens, and a lesser effect on B-lymphocytes and response to B-lymphocyte mitogens, and there was return of these measurable effects to normal by 24 hrs. after the dose (yet clinically the prednisone has a cumulative benefitical effect over weeks and months). The effects were approximately dose-dependent, establishing that these parameters might answer the original need, but the considerable individual-patient variability means that further precision must be gained (citation in our Myopathy project).

In the cerebrospinal fluid (CSF), 7 of 23 MG patients had oligoclonal IgG bands and 5 more had a monoclonal bands; since IgG "synthesis" (Tourtellotte formula) in the CNS was normal, the CSF pathologic bands are probably from the serum (with ID). 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 and perhaps other "soft" CNS findings.

The remarkable ancillary benefit that broad-aspect nursing can provide to an MG patient is repeatedly evidenced in our patients -- our multidimensional nursing care approach for myasthenics is available on videotape (with Nursing, CC) and available for general distribution -- this undoubtedly will help improve the care and perhaps save the lives of some myasthenic patients with serious disease, especially in hospitals not frequently caring for such patients.

A new experimental model of neuromuscular junction transmission defect found after immunizing animals with peripheral nerve axoplasmic "soluble" protein has been reported (see our ALS Project).

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.

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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 01189-10 MN

PERIOD COVERED
October 1, 1977 through September 30, 1978

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, Medical Neurology Branch, NINCDS

OTHER: N. Bojji Reddy, Ph.D., Guest Worker, MN, NINCDS
Roger A. Brumback, M.D., Clinical Associate, MN, NINCDS
Valerie Askanas, M.D., Ph.D., Associate Neurologist, Medical Neurology Branch, NINCDS
Jan Kucera, M.D., Clinical Associate, MN, NINCDS

COOPERATING UNITS (if any)
Robert C. Griggs, M.D., The University of Rochester, Rochester, New York

LAB/BRANCH
Medical Neurology Branch

SECTION
--

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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. Attention is directed toward those conditions in which evidence suggests that the main site of intermittent dysfunction is somewhere within the following portions of the muscle fiber: plasmalemma, T-system, sarcoplasmic reticulum, 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.

PHS-6040
(Rev. 10-76)

Summary (Continued)

Tissue culture of the human abnormal muscle is used for purposes of reincarnating disease in culture and thence its treatment, and for induction of models of disease by chemical agents, which are also used to induce models in cultured human or animal muscle.

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. Attention is directed toward those conditions in which evidence suggests that the main site of intermittent dysfunction is somewhere within the following portions of the muscle fiber: plasmalemma, T-system, sarcoplasmic reticulum, 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 for purposes of reincarnating the disease in culture and thence its treatment in vitro, and for induction of models of disease by chemical agents, which are also used to induce models in cultured human or animal muscle.

Methods Employed: Various techniques of clinical investigation (including electromyography and clinical biochemistry), muscle biopsy with samples for histochemical analysis, electronmicroscopy, tissue culture and biochemical assays of tissue were utilized. 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 acetazolamide given to treat myotonia. Diazacholesterol, a myotonogenic agent, was adminstered 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:

Periodic Paralyses (PP) are hereditary or acquired disorders causing chronic weakness punctuated by attacks of paralysis. 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.

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 12 years. Since muscle does not contain carbonic anhydrase, the mechanism of acetazolamide benefit in hypokalemic PP remains unknown.

Myotonia is a crippling symptom to various degrees in myotonia congenita and paramyotonia congenita (inherited diseases of unknown cause). We have reported a controlled clinical trial showing moderate to remarkable benefit from acetazolamide in 8/10 patients who had failed to respond to, or had been intoxicated by, other anti-myotonia drugs. It remains the long-term treatment of choice in 6 of those patients.

Myotonic atrophy (myotonic "dystrophy") is an inherited multisystemic disease, with progressive wasting, of unknown pathogenesis. We have previously raised the possibility of at least a partially neurogenic aspect. With our new concept of "myogenous de-innervation", we have now extended that hypothesis to include a possible myogenous muscle plasmalemmal non-receptivity to neural short- and long-term trophic influences. We have found a 30-60% decrease of adenylate cyclase in 7 patients with myotonic atrophy (v.i.).

The model of 20,25 diazacholesterol-induced myotonia of animals, used to evaluate myotonic phenomena, was reported. Muscle of the markedly myotonic animals showed: (a) no histochemical changes, (b) no blockage in vivo of the myotonia by α-bungarotoxin, d-tubocurarine, succinylcholine or atropine in contrast to blockage of sciatic-neurectomy-induced fibrillations by all four drugs (and blockage of both myotonia and fibrillations by procaine, tetrodotoxin, KCl or ischemia and of neither by pyridostigmin). Our results demonstrated a major difference, and several similarities, between fibrillation and myotonic discharges -- we hypothesized that fibrillations and DAC-induced myotonia are mediated through mechanisms involving ionic channels, that both can be produced by activation of junctional/nonjunctional AChRs (or some mechanism coupled to those receptors), but that an unfettered αBT binding portion of the AChR molecule, and an unblocked atropine-binding site, are obligatory only for production of fibrillations. This, and our showing lack of αBT binding histochemically to muscle fibers in human myotonic diseases, indicate essential differences between myotonic and fibrillating plasmalemmas. Presented last year was that DAC-induced-myotonic-rat-muscle plasmalemma had 40-60% lower levels of basal and fluoride- and catecholamine-stimulated adenylcyclase activity; this change, like that in myotonic atrophy patients (v.s.), could be due to confirmational changes of the enzyme molecule or an

altered sterol composition of the plasmalemma. The subcellular localization of ^{3}H-diazacholesterol in those muscles was to evaluate direct influence of diazacholesterol on muscle cells, which is not possible in the intact animal, pulses of 0.005 mM of diazacholesterol were given to contracting cultured rat muscle (after about 8-9 days of growth) for 10-15 min. on 3 consecutive days, resulting in changed contraction rhythym, which became more continuous and fi rillation-like; also some granularity of the muscle fibers was observed. Electronmicroscopic studies of those DAC-treated muscle cultures are being done. The cultured rat muscle treated with diazacholesterol, had quantitatively lower levels of adenylate cyclase (basal, and NaF and isoproterenol stimulated) compared to untreated control-cultures; however, β-adrenergic receptors assayed by ligand binding were unchanged. These results are in agreement with our previous data from muscle of intact animals with DAC-induced myotonia and also comparable to biopsies of myotonic atrophy patients.

We confirmed histochemically the finding of an increased number of intrafusal muscle fibers in muscle spindles of myotonic atrophy patients. Our new finding was that muscle spindles are normal in myotonic congenita patients and in rats rendered chronically myotonic with 20,25-diazacholesterol. Because we have found similar spindle abnormalities in rat muscle spindles experimentally denervated (neurogenously), it is possible that the spindle abnormalities in myotonic atrophy perhaps are due to fusimotor deinnervation (? neurogenous or myogenous deinnervation).

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 period paralysis and the pathogenesis of the disease itself. To seek even better therapeutic agents. To explore the underlying nature of myotonia and seek improved methods of treating it and the underlying disorders.

Publications:

Griggs, R. C., Moxley, R. T., Riggs, J. E. and Engel, W. K.: Effect of acetazolamide on myotonia. Ann. Neurol. 3:531-537, 1978.

Brumback, R. A., Bertorini, T. E., Engel, W. K., Trotter, J. L., Oliver, K. L. and Zirzow, G. C.: The effect of pharmacologic acetylcholine receptor blockade on fibrillation and myotonia in rat skeletal muscle. Arch. Neurol. 35: 810, 1978.

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ANNUAL REPORT
October 1, 1977 through September 30, 1978
Surgical Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke

A. K. Ommaya, M.D., F.R.C.S., Acting Chief

Summary of Studies in Surgical Neurology Branch

This annual report completes the third anniversary of the incumbent's role as Acting Chief of this Branch. As in the Branch Chief's summary for 1977, this report concerns our progress in three major program areas as follows:

1. Reintegration and regeneration in the nervous system after injury.
2. Immunologic and oncogenetic factors in the management of malignant gliomas.
3. Diagnostic techniques for neurology and neurosurgery.

The five individual research projects under the first major area of reintegration and regeneration reported last year have been reorganized into two current research projects. First, on regeneration of the spinal cord and second, on neural reintegrative mechanisms. The projects on stroke and headache management have been discontinued because of difficulties in patient accrual.

1. REINTEGRATION AND REGENERATION IN THE NERVOUS SYSTEM AFTER INJURY

A. Regeneration of the Spinal Cord After Trauma

Over the course of the past year we have concentrated our efforts in solving the difficult problems raised by creating a suitable model of paraplegia for long-term evaluation of recovery after spinal cord injury in the rhesus monkey. To date this had never been successfully achieved before because of nursing problems, and we are pleased to report that we have solved all of the major problems and now have a viable monkey model available for regeneration studies. Peripheral nerve grafting procedures to the crushed spinal cord as described in the previous report have been successfully achieved in two monkeys which are surviving and doing well 3 months and 2 months, respectively, after the nerve grafting. No measurable change in their paraplegia has been noted. In the course of this part of our research we have evolved from padded cage and primate chair techniques for paraplegic primate management to a playpen concept for nursing the animals. This has proved to be very successful in preventing decubiti and will be very useful for other investigators in this field.

Preliminary review of our histological studies in earlier control monkeys subjected to cord crushing have revealed that the sequence of

neuropathologic changes with regard to neuroglial scar and cavity formation follows the same course and are of the same nature as that described in other mammals by Guttman, Kao and other workers. We have also initiated a study of the vascular alterations in the spinal cord using our technique of selective angiography of the spinal cord in collaboration with the Department of Radiology in the Clinical Center with angiography carried out before and after experimental paraplegia and treatment by nerve grafting. Our current correlations of the clinico-pathologic changes in the paraplegic monkey subjected to peripheral nerve grafting over the long term will be the first such observations in the subhuman primate. The pathologic changes noted in the spinal cord lesions without nerve grafting to date appear to be very similar to those described in man.

We have also evaluated the claim that pulsed radiofrequency energy used as a treatment will diminish neuroglial scar formation and encourage neural regeneration in nervous tissues. This study has been completed in 35 guinea pigs. No significant effect of pulsed radiofrequency as compared to control animals was discovered in our treated animals. This study involved both spinal cord crushing and sciatic nerve section and resuture. A current study on the effect of hematoma healing in the ears of rabbits, as influenced by pulsed radiofrequency, is currently in progress.

We are also planning to test an elegant hypothesis for its potential as a unified collaborative approach to certain basic aspects of spinal cord regeneration as well as to our other major clinical interest, i.e. malignant gliomas. This hypothesis states that the rate of proliferative activity in somatic cell populations can be modulated by regulation of the transmembrane potential level (E_m). The Chief of Biophysics at the V.A. Research Center, Hampton, Virginia has shown that such modulation is accomplished through changes in DNA synthesis, presumably by variations in the intracellular ionic conditions which accompany transmembrane potential changes. Induction of mitogenesis in adult mature neurones by ouabain and inhibition of cell proliferation by FUdR has been demonstrated in tissue culture preparations. We are currently planning in vivo studies to validate such in vitro results using our technique of local perfusion of the CNS in primates to test this hypothesis in our spinal cord regeneration model.

The clinical arm of the project on spinal cord regeneration has completed a study on two patients with traumatic paraplegia in whom selective angiography has been completed. These observations are being correlated with observations in our primate model in whom pre- and post-crush and post-nerve graft selective angiograms were carried out. Our preliminary data would suggest that the major blood supply to the traumatized cord appears to be intact, although it is very likely that the perforated vessels are abnormal in the area of the spinal cord lesion. Our plan to develop a pool of documented patients with paraplegia who could serve as future candidates for spinal cord grafting is proceeding in the hope that our experiments on grafting in the primate model will prove successful. We should have definitive data with regard to this question within the next 12 to 24 months.

B. Neural Reintegrative Mechanisms

Our observation that recovery of neural functions after head injury recapitulates ontogenetic development of those functions has been supported by further observations. Such studies offer the potential for improved management of neural trauma as well as clues to neural integrative mechanisms. To facilitate the study of such mechanisms the Acting Branch Chief has formulated a new hypothesis for cerebral asymmetry and cognitive functions which was formally presented and critiqued at a Workshop on Cognitive Sciences organized by Prof. Naom Chomsky at M.I.T. on June 10, 1978. The hypothesis was received with great interest and is generating useful experiments. Starting in July 1978, two Research Fellows are joining our staff to carry out experiments testing our hypothesis in both animal and clinical research. It should be emphasized that our studies of reintegrative phenomena are closely related to work in spinal cord regeneration, both of which seek to improve methods for acute treatment as well as for rehabilitation of patients with neural traumatic impairments. Our approach is aimed at "cure" of the deficit rather than the conventional palliative approach.

2. IMMUNOLOGIC AND ONCOGENETIC FACTORS IN THE MANAGEMENT OF MALIGNANT GLIOMAS

Our current clinical trial of local chemotherapy is a Phase 3 study evaluating the specific effects of local 8-Azaguanine given after surgery and radiotherapy. To date we have shown that local chemotherapy with 8-Azaguanine adds significantly to median survival of patients with malignant gliomas (64 weeks in our series as compared to 48 weeks in others). We are awaiting the release of the first batch of synthesized α_1 fraction 5 of thymosin for clinical trial in our patients with malignant gliomas. In collaboration with the National Cancer Institute we have studied the changes in peripheral blood leucocytes, lymphocyte counts, T-lymphocyte levels and change in percentage of T-lymphocyte levels after incubation with thymosin correlated with a battery of skin tests and measurements of humoral immunologic factors in our patients with non-neoplastic neural disease, primary metastatic intracranial tumors and primary glial brain tumors. The effects of surgery and radiation on these indices were also evaluated. Our results supported those previously reported by Mahaley et al. in finding depression of some immune parameters in patients preoperatively compared to control patients but differed in that they showed a trend for these parameters in both the glioma and control patients to return to pretreatment levels. We were encouraged in finding positive values for the changes in the percentage of T-lymphocytes after incubation with thymosin at all time periods, which was found with T-lymphocytes from the glioma patients. This suggests a rational basis for a trial of thymosin in brain tumor patients. Roche Laboratories will be supplying the Acting Branch Chief with the α_1 fraction of thymosin for a Phase 1 study, the protocol for which is now in preparation.

In our Neural Oncogenesis and Immunology Laboratory we are developing two

techniques; 2-dimensional gel electrophoresis for examining changes in proteins and polypeptides of normal and neoplastic glia and testing an impedance measuring device which enables the rapid testing of neoplastic cells in suspension against a variety of cytotoxic and cytostatic agents including T-lymphocytes.

3. DIAGNOSTIC TECHNIQUES

The work in transmission computerized tomography of the Section on Neuroradiology and Computed Tomography in our Branch has continued to progress. Workers in this section have developed a method to discriminate between transependymal resorption of the CSF in hydrocephalus and periventricular decreased attenuation connected with certain demyelinating processes in leukomyelacia. This section has also developed a dual energy scanning technique which is serving to lay the groundwork for what we have previously described as "in vivo neuropathology" or "tomochemistry" The major finding is consistent reproduction of the same numbers for attenuation of values with CSF using the dual energy technique. This quantitative signature for the CT CSF is now available for routine use. It has also been shown that protons can be used to detect differences in the physical properties in material at the 0.1% level. A new journal, The Journal of Computer Assisted Tomography, originates from this section. An exciting new development in computer assisted tomography is that of emission (as opposed to currently used transmission techniques) computed tomography, which is currently in development in collaboration with members of the staff in the Department of Radiology, Division of Nuclear Medicine in the Clinical Center. This technique will allow correlation of morphological data on the CNS with dynamic functional data such as regional cerebral glucose consumption rate and measurements of the storage, degredation and turnover of metabolites using radio labeled agents.

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 00907-17 SN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Regeneration of the Spinal Cord after Trauma (Previous Title: Reintegration 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	SNB	NINCDS
	Carrie Walters	Medical Officer	SNB	NINCDS
Other:	James Reed	Clinical Associate	SNB	NINCDS
	Lawrence Mononen	Staff Fellow	SNB	NINCDS
	John Doppman	Chief	DR	CC

COOPERATING UNITS (if any)

LAB/BRANCH
Surgical Neurology Branch

SECTION
Section on Applied Research

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (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:

I. 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.

II. 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 2. 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 micro suction. 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. Gomoris 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^{o} 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.

III. MAJOR FINDINGS:

Originally 6 monkeys were operated upon. Three of the six underwent sciatic nerve grafting, two at 1 week and one at 2 weeks. This series was carried out before the Diapulse arrived. They were nursed in the unmodified, padded primate chair. The longest survivor was only 23 days. The cords are now undergoing histologic examination.

The second series consisted of only one monkey which was to serve the dual purpose of being a control in our series (i.e. crush only) and a "test" monkey for the modified primate chair. He survived only 28 days.

The third series, which we began 2 months ago, to date consists of 2 monkeys, both alive and well, one after 3 months and the second after 8 weeks. Monkey #1 has had control angiography, crush injury, post-crush angiography, nerve grafting at 1 week, post-grafting angiography and is being treated with the Diapulse BID as described above. Monkey #2 has had control angiography, crush injury, post-crush angiography and nerve grafting at 2 weeks. He also is being treated with the Diapulse. To date no clinical recovery of lower limb function has been noted.

IV. PROPOSED COURSE:

This project as it is now being carried out attacks the problems of spinal cord regeneration primarily at the "macro" level. The introduction of treatment with electromagnetic energy is carrying us over into the "micro" sphere but its mechanism of action is not known. Several reports have been published demonstrating enhanced healing of peripheral nerves in rats and microscopic evidence of spinal cord regneration in cats

treated with the Diapulse. There have been suggestions that it works by altering the membrane potential of injured cells. It is also possible that the electromagnetic field generated by the Diapulse increases the rate of axonal flow down the injured nerve fiber in much the same way that the "current of injury" is thought to aid axonal flow. Preliminary plans have been made to collaborate with Dr. C. L. Li to measure the membrane potential of injured spinal cord axons before, during and after treatment with the Diapulse. These studies will initially be carried out as acute experiments in rats.

Dr. Clarence Cone has suggested that mitotic activity within neurones is a function controlled by the membrane potential: a hyperpolarization leading to decreased mitotic activity, while depolarization leads to increased mitotic activity. His work to date has been limited to cells in tissue culture. Our monkey model with a modification of the Ommaya "ventricular reservoir" to a "subarachnoid spinal" reservoir affords an ideal mechanism whereby drugs such as ouabain, which is known to depolarize nerve cells, can be instilled into the site of the spinal cord injury in an attempt to enhance spinal cord regeneration. Other substances such as nerve growth factor, which is to be made available to us by Dr. Perez-Polo, can be introduced into the injured area in the same manner and their effects monitored using the same anatomical, histological, vascular and physiological criteria as outlined above. Once the original premise (i.e. that depolarization of nerve cells at the site of spinal cord injury enhances regeneration) is found valid, the combination of various drugs and electrolyte solutions to find the optimal combination will be explored.

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 01025-16 SN

PERIOD COVERED
October 1, 1977 to September 30, 1978

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	SNB NINCDS
OTHER:	C. Walters	Medical Officer	SNB NINCDS
	J. Reed	Medical Officer	SNB NINCDS
	P. Kochie	Neuropathologist	SNB NINCDS
	P. Chretien	Senior Investigator	SURG NCI
	C. H. Kent	Radiation Oncologist	DCT NCI
	D. Poplack	Pediatric Oncologist	PO NCI
	D. Sadowsky	Statistician	OBE NINCDS

COOPERATING UNITS (if any)
Surgery Branch, NCI; Radiation Oncology and Pediatric Oncology, NCI

LAB/BRANCH
Surgical Neurology Branch

SECTION
Section on Applied Research

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

A controlled clinical study of the effect of intratumoral chemotherapy using 8-Azaguanine compares this method given in combination with systemic CCNU after surgery and radiotherapy to the effect of adjuvant chemotherapy 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, given after completion of surgery and radiotherapy. A Phase II study will follow closely after. These studies on the first clinical use of Thymosin in brain tumors are based on in-vitro studies with Thymosin in our gliomas patients.

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 qulity 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 (Grades 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 propective, 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 utilizes 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 is being developed in collaboration with Dr. David Houchens of Battelle Institute.

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.

Major Findings

We have treated 106 cases of brain glioma in the past 10 years and have completed detailed follow-up in 74 cases of malignant glioma. In the latter series, patients with Grade IV lesions receiving surgery plus radiotherapy plus combination local and systemic chemotherapy (8-Azaguanine + CCNU) had a median survival of 1.25 years in good clinical condition as compared to 0.83 years for patients not receiving any chemotherapy. The 2-3 year survival of Grade IV (glioblastoma) patients receiving our adjuvant combination therapy was 45.3% as compared to the less than 10% survival in the historical data and 27.3% 2-3 year survival in our patients receiving radiotherapy only after surgery. The results for Grade III astrocytoma cases were, however, the reverse, i.e., median survial of 5 years for surgery + radiotherapy versus only 1.3 years for patients receiving surgery + radiotherapy + combination chemotherapy.

When the data for Grade III and IV cases were lumped, there was no significant effect of the chemotherapy in either direction. The biological significance of these results may be related to data on the cellular immune response in a subgroup of these patients, as tested by DNCB. Compared to an anergic response rate of 25% in an equivalent group of non-neoplastic patients, anergic response to DNCB was found in 100% of patients with Grade IV tumors, but in only 30% of patients with Grade III tumors.

This finding that patients with lesser degrees of glioma malignancy had a lesser degree of immunological incompetence is also supported by similar data in patients with Grade I and II gliomas. It is hypothesized that chemotherapy as an adjuvant for glioma therapy may, indeed, provide short-term tumor reduction, but that prolonged chemotherapy, particularly with systemic agents which depress cellular immune responsiveness, may serve to prevent immune surveillance mechanisms from "curing" the patient. When such data is considered, along with the quite unimpressive results of chemotherapy with nitosoureas as reported by the NCI-BTSG, we are led to the hypothesis that systemic chemotherapy may indeed be deleterious

for any long-term survival. Accordingly, we are dropping the use of CCNU and other systemic chemotherapeutic agents and will focus our future work on the use of local chemotherapy with 8-Azaguanine alone, supplemented by immunotherapy with Thymosin.

Proposed Course

1. To pursue a Phase III trial of 8-Azaguanine given after surgery and starting with radiotherapy or added at completion of radiotherapy.

2. To develop a practical intravital staining method in humans in order to facilitate near total surgical removal of gliomas.

3. To conduct a Phase I trial of Thymosin given after completion of surgery, radiotherapy and chemotherapy.

4. To study the oncogenesis of gliomas in the nude mouse model.

5. To pursue our intrathecal assay of chemotherapeutic drugs in our monkey model.

Publications:

Poplack, D.G., Bleyer, W.A., Wood, J.H., Kostolich, M., Savitch, J.L., and Ommaya, A.K.: A primate model for study of methotrexate pharmacokinetics in the central nervous system. Cancer Res. 37: 1982-1985, 1977.

Wood, J.H., Poplack, D.G., Flor, W.J., Gunby, E. N., and Ommaya, A.K.: Chronic ventricular cerebrospinal fluid sampling, drug injections, and pressure monitoring using subcutaneous reservoirs in monkeys. Neurosurgery 1: 132-135, 1977.

Meeker, W.R., Jr., Baskies, A.M., Chretien, P.B., Weiss, J.F., and Ommaya, A.K.: Lymphocyte, T-cell, glycoprotein, and skin test responses to therapy of brain tumors. Surgical Forum, 1978. In press.

Ommaya, A.K., Baskies, A.M., Meeker, W.R., Jr., and Chretien, P.B.: A model for combination immunochemotherapy of malignant gliomas in man. Neurologia en Colombia, 1978. In press.

Ommaya, A.K., Baskies, A.M., Meeker, W.R., Jr., and Chretien, P.B.: Hematologic indices in the design of immunotherapy for gliomas. Neurologia en Colombia, 1978. In press.

C.T. No. 76-N-343

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 02349-01 SN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Reintegration of Cognitive Functions after Neural Trauma -- A Study of Cognitive Coding

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	SNB	NINCDS
OTHER:	J. Payne	Postdoctoral Fellow	SNB	NINCDS
	L. Mononen	Staff Fellow	SNB	NINCDS
	C. Walters	Medical Officer	SNB	NINCDS
	J. Reed	Clinical Associate	SNB	NINCDS
	P. Kochie	Neuropathologist	SNB	NINCDS
	J. Moore	Exec. Sec.	CDRC	NINCDS
	E. Elkins	Audiologist	CDP	NINCDS

COOPERATING UNITS (if any)
Dr. H. Rizzoli, Dept. of Neurosurgery and Dr. D. O. Davis, Dept. of Neuroradiol., George Washington Univ. Med. Ctr.; Med. Exam. Off., D.C. Government

LAB/BRANCH
Surgical Neurology Branch

SECTION
Section on Applied Research

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER and sub-human primates

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This is an interdisciplinary study of how the central nervous system recovers after focal or diffuse trauma. The classical view of neurological functions based on focal lesion effects will be supplemented by our analysis of the diffuse lesion effects produced by closed head injury where the centripetal theory of cerebral concussion is used to predict lesion locations. Methods used include neuropsychologic testing, averaged evoked potential recording, transmission and emission computerized tomography and neurophysiologic recording in humans and sub-human primates.

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

To proceed and develop above and discover the basis of cognitive coding.

Publications

Ommaya, A.K.: Indices of neural trauma: An overview of the present status. In Popp, A.J., Bourke, R.S., Nelson, L.R. and Kimelberg, H.K. (Eds.): Seminars in Neurological Surgery: Neural Trauma. New York, Raven Press. In press.

Ommaya, A.K.: Reintegrative action of the nervous system after trauma. In Popp, A.J., Bourke, R.S., Nelson, L.R. and Kimelberg, H.K. (Eds.): Seminars in Neurological Surgery: Neural Trauma. New York, Raven Press. In press.

Ommaya, A.K.: Frontiers of functional neurosurgery in biomedical research. In Rasmussen, T. (Ed.): Functional Neurosurgery. New York, Raven Press. In press.

Ommaya, A.K.: Cerebral asymmetry and human consciousness. A unified hypothesis for cognitive functions. In Chomsky, N. (Ed.); M.I.T. Workshops in Cognitive Science. M.I.T. 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 01047-16 SN

PERIOD COVERED
October 1, 1977 to September 30, 1978

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

PI:	G. Di Chiro	Head, Section on Neuroradiology and Computed Tomography	SN NINCDS
OTHER:	G.S. Johnston	Chief, Nuclear Medicine Dept.	NM CC
	A.E. Jones	Assistant Chief	NM CC
	R.M. Kessler	Staff Physician	NM CC

COOPERATING UNITS (if any)

LAB/BRANCH
Surgical Neurology Branch

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.01	.01	.0

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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)

Isotope ventriculography and isotope cisternography are diagnostic tools permitting the morphologic and dynamic study of the cerebrospinal fluid pathways more accurately than has ever been possible with any other diagnostic test.

The adjunction of emission computed tomography 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. Experiments have been carried out in the rhesus monkey after injection of radiopharmaceuticals within the ventricular system. The scintiphotographic data are appraised with the assistance of a computer. Digital analysis is performed using a small dedicated computer (HP 5407A Hewlett Packard Scintigraphic Data Analyzer), mated to the Anger camera. For this purpose the pin-hole collimator is positioned so that the entire lateral length of the cerebrospinal space is within the field of view of the Anger camera detector. Nine regions of interest cursors are drawn: one over the cerebral convexity, and one to include the entire spinal subarachnoid space. Time-activity curves are obtained from each region of interest, simultaneously without moving the animal for the next three and one-half hours.

Preliminary experience has been gained in clinical material on the descent to the spinal subarachnoid space of the CSF. Many patients, all newborn infants, and all with abnormalities of the CSF circulation--the majority was made up of cases of myelomeningocele--have been subjected to the following procedure. A radiopharmaceutical has been injected into the lateral ventricles of the brain and the descent of the cerebrospinal fluid into the spinal canal has been studied with an Anger camera, and in selected cases with the help of a computer. When the computer has been used, total gathering of the data has been attained above the entire spine for a period of at least one hour following the intraventricular injection. A number of patients affected by meningeal leukemia, and in whom radionuclide ventriculography was carried out, were also followed up with spine scanning for the purpose of gaining experience with the spinal CSF descent.

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.

Last year's investigations should have practical implication for the diagnosis and followup of such conditions as porencephaly and hemispheric

glioma.

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 01195-14 SN

PERIOD COVERED
October 1, 1977 to September 30, 1978

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	Head, Section on Neuroradiology and Computed Tomography	SN NINCDS
OTHER:	J.L. Doppman	Chief	DR CC
	J.R. Herdt	Deputy Chief	DR CC
	A.K. Ommaya	Acting Chief	SN NINCDS
	G.S. Johnston	Chief	NM CC
	A.E. Jones	Assistant Chief	NM CC

COOPERATING UNITS (if any) L. Wener, Chairman, Dept. of Radiology, Greater Southeast Community Hospital, Wash., DC; M.M. Mishkin, Professor of Radiology, Hospital of the Univ. of Pennsylvania, Philadelphia, PA; Medical Examiner's Office, City of Philadelphia, Dept. of Public Health, Philadelphia, PA.

LAB/BRANCH
Surgical Neurology Branch

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Section on Neuroradiology and Computed Tomography

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.01	.01	.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)

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 indicates that this new methodology may be useful in the evaluation of certain vascular lesions of the spinal cord.

Project Discription:

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, cinescintiphotographic 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 introduction 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 new development in the current fiscal year has been the introduction of computed tomography (CT) in the analysis of vascular diseases of the spinal cord. We have gathered preliminary experience in six patients with arteriovenous malformation of the spinal cord using a CT scanner. We have been able to recognize the pathological vessels of two 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 therapeutic attempt.

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 polimeric, ion-balanced and non-ionic iodinated x-rays contrast media.

We will dedicate much of our attention to technical improvements in the newly introduced radioisotope angiography of the spinal cord. This method which we are extensively using as a screening and followup procedure, could become a more definitive and informative diagnostic examination. By increasing our resolution through a computer-assisted reconstruction and enhancement of the images, we should be able to extract a lot of diagnostic information from this simple and innocuous technique.

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

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 01654-11 SN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Experimental Spinal Cord Angiography

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

PI:	G. Di Chiro	Head, Section on Neuroradiology and Computed Tomography	SN NINCDS

COOPERATING UNITS (if any) J. Fein, formerly of Armed Forces Radiobiology Research Institute, Bethesda, MD; K. Earle, Chairman, Dept. of Pathology, Uniformed Services University of the Health Sciences, Washington, DC.

LAB/BRANCH
Surgical Neurology Branch

SECTION
Section on Neuroradiology and Computed Tomography

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.01	.01	.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)

Experimental spinal cord angiography in the rhesus monkey is increasing our understanding of the blood supply of the spinal cord both in physiological and pathological conditions.

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. In the past fiscal year much of our experimental investigation has delt with an iatrogenic pathological condition, postradiation myelomalacia (myelitis).

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 the LINAC accelerator (A.F.R.R.I.) 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 microangiography) 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 01866-08 SN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on Cerebral Blood Flow by Radiographic and Radioisotopic Methods

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

PI:	G. Di Chiro	Head, Section on Neuroradiology and Computed Tomography	SN NINCDS
	M.K. Hammock	Senior Staff Fellow	SN NINCDS
	P.C. Williams	Staff Fellow	SN NINCDS
OTHER:	M.V. Green	Chief, Applied Physics Section	NM CC
	H. Agress, Jr.	Medical Research Analyst	LAS DCRT
	G.S. Johnston	Chief, Nuclear Medicine Dept.	NM CC
	A.E. Jones	Assistant Chief	NM CC
	S.L. Bacharach	Physicist, Applied Physics Section	NM CC

COOPERATING UNITS (if any) K. Earle, Uniformed Services University of the Health Sciences, Washington, DC

LAB/BRANCH
Surgical Neurology Branch

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Section on Neuroradiology and Computed Tomography

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.0	.0	.0

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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)

An experimental stroke model in the rhesus monkey has been developed. The "stroked" animals are being evaluated by x-ray angiography, radionuclide scanning, radionuclide cerebral blood flow determination, radionuclide particles (macroaggregates) transit, Fluorescein angiography, Laser-Doppler velocimetry, microangiography, and autoradiography. The CBF in the experimental infarcted brain is being modified by various physiological, chemical, pharmacological and surgical (revascularization) means. In a collateral experiment various parameters of the cerebral circulation are being studied after production of arterial spasm in the circle of Willis in monkeys. The spasm is caused by total blood - or fractions thereof - introduction within the subarachnoidal space (chiasmatic cistern). Publication: Williams, P.C., Stern, M., Bowen, P.D., Brooks, R.A., Hammock, M.K., Bowman, R.L. and Di Chiro, G.: Mapping of cerebral cortical strokes in rhesus monkeys by Laser Doppler spectroscopy. Medical Research Engineering (In press)

Project 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 02073-05 SN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
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

PI:	G. Di Chiro	Head, Section on Neuroradiology and Computed Tomography	SN NINCDS
	R.A. Brooks	Senior Staff Fellow	SN NINCDS
	T. Arimitsu	Visiting Scientist	SN NINCDS
	L. Dubal	Visiting Scientist	SN NINCDS
OTHER:	T.N. Chase	Director	IRP NINCDS
	D.B. Calne	Clinical Director	NINCDS
	P.F. Teychenne	Clinical Associate	IRP NINCDS
	A. Neophytides	Clinical Associate	IRP NINCDS
	R.M. Eiben	Acting Chief, Clinical Investigations Service	DMN NINCDS
	W.K. Engel	Chief	MN NINCDS
	T.E. Bertorini	Clinical Associate	MN NINCDS

COOPERATING UNITS (if any) D. Schellinger, Dept. of Radiology, GTU Med. School, Wash., DC; A.L. Martins, Neurosurg. Svc., B. Jabbari, Dept. of Psychi. and Neurol., WRAMC, Wash., DC; J.M. Pellock, Dept. of Neurosurg., NNMC, Beth., MD; A.M.Cormack, Physics Dept., Tufts Univ., Medford, MA; A. Koehler, Cyclotron Lab., Harvard Univ., Cambridge, MA

LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.08	2.08	.0

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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)

Computed tomography (CT) represents the main research area of the Section on Neuroradiology and Computed Tomography. Ongoing research projects include the following pathological processes: degenerative, demyelinating and atrophic processes of the brain; postradiation cerebral necrosis; surgically correctable lesions in young patients affected by chronic epilepsy; schizophrenia; diseases of the spine and the spinal cord; oculo-orbital lesions; hydrocephalus; attempts at tissue characterization of normal and abnormal (e.g., tumoral) cerebral tissue.

A number of mathematical and physical projects have been completed or are being carried out: simultaneous dual-energy CT using an originally designed split detector; studies of slice geometry in CT; mathematical and physical experimental analysis of a variety of CT artifacts; mathematical improvements of interpolation procedure in CT reconstruction.

Preliminary feasibility tests to build a new type of CT device which will use protons rather than x-rays are under way.

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

OTHER:	M.C. Dalakas	Visiting Fellow	MN NINCDS
	C.R. Kollarits	Staff Fellow	CB NEI
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	J.R. Herdt	Deputy Chief	DR CC
	M. Vermess	Assistant Deputy Chief	DR CC
	G.S. Johnston	Chief	NM CC
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	D.M. Conca	Senior Staff Fellow	NM CC
	D.G. Poplack	Senior Investigator	POB DCT NCI
	N.P. Ramu	Visiting Fellow	POB DCT NCI
	R.J. Wyatt	Director	DSMR M
	D.R. Weinberger	Staff Psychiatrist	DSMR M
	G.H. Weiss	Chief	PSL CR

Project Description:

Objectives: To advance the clinical applications of CT. Particular attention is being devoted to trying to improve the resolution of the CT devices. Differentiation of the various tissues' chemical components through dual-energy scanning or, possibly in the future, by means of proton CT, represent the most promising line of our research. "Tomochemistry" of the CNS is our ultimate goal.

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) Studied a large number of voluntary normal control patients.

2) Accumulated a large number of cases of demyelinating degenerative processes (leukodystrophies and - in the broadest sense - leukoencephalopathies).

3) Studied a large number of Parkinsonians. Many patients affected by Huntington's disease and their next of kin (subjects at risk) have been examined.

4) 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.

5) Analyzed the brain CT findings (demyelination in particular) after intrathecal methotrexate (administered for controlling meningeal leukemia and metastatic lesions).

6) Analyzed a large group of young patients affected by chronic epilepsy to determine how frequently previously unrecognized, surgically correctable epileptogenic lesions can be detected by CT.

7) Observed interesting findings in a large group of schizophrenic patients. Specifically, we have noted that patients affected by schizophrenia show essentially normal scans in the early stages (group of patients in the third decade of life), significant atrophic changes (ventricular and subarachnoid space dilatation) are noted. In the latter group of schizophrenics (fourth decade) quite frequently marked dilatation of the periventricular subarachnoid spaces is demonstrated. We are investigating if these atrophic changes (particularly cerebellar ones) are disease or medication related.

8) Continued our work on the spinal cord. We have accumulated further experience on the techniques of computer assisted myelography (CAM) and on computed tomographic angiography of the spinal cord (see Project Number Z01 NS 01195-14 SN) which we introduced two years ago.

9) Initiated a research project comparing CT with radionuclide scanning of the spine in metastatic processes.

10) Developed a method to discriminate between two pathological conditions which have a very similar appearance in cranial CT. The two processes are: 1) the transependymal resorption of the CSF in hydrocephalus, and 2) periventricular decreased attenuation connected with certain demyelinating processes and leukomalacias. Both types of processes create a characteristic halo of hypodensity surrounding the lateral ventricles. An analysis of the numerical attenuation values from the ventricles toward the cortex of the brain demonstrates that different types of gradients are recognizable in the two conditions. We are evaluating whether or not the differentiation of these gradients is typical enough to be used for diagnostic purposes.

11) Our attempts at tissue characterization (tomochemistry) using, in particular, the dual energy scanning (see below) have been concentrated on a possible discrimination of the various fluid containing cerebral cavities (normal and dilated CSF cavities, tumoral and nontumoral cysts, abscesses, resorbing hematomas, infarction with tissue liquidation as well as on the differentiation of nonliquid tissues (normal white and gray matter, solid glial tumors (particularly the various types of astrocytomas), intra- and extra-cerebral blood collections, fat containing tumors). A major finding is that we have consistently been able to reproduce the same attenuation values for CSF using a dual energy technique. Thus, a CT-CSF "signature" is now available.

In the physics area the most important findings are:

1) Demonstration of the feasibility of simultaneous dual-energy scanning using an original split detector.

2) Demonstration that protons can be used to detect differences in the physical properties of material at the 0.1% level. This is a significant advance when compared with the ½% resolution limit of the present commercial scanners.

3) Emphasis has been placed on understanding the origin of artifacts which are produced during CT studies of certain areas of the head and the spine.

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 Section on Neuroradiology and Computed Tomography, 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, 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:

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Fusner, J.E., Poplack, D.G., Pizzo, P.A. and Di Chiro, G.: Leukoencephalopathy following chemotherapy for rhabdomyosarcoma: reversibility of cerebral changes demonstrated by computed tomography. J. Pediat. 91: 77-79, 1977

Eiben, R.E. and Di Chiro, G.: Computer assisted tomography in adrenoleukodystrophy. J. Comput. Assist. Tomogr. 1: 308-314, 1977

Martins, A.N., Johnston, J.S., Henry, J.M., Stoffel, T.J. and Di Chiro, G.: Delayed radiation necrosis of the brain. J. Neurosurg. 47: 336-345, 1977

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Peylan-Ramu, N., Poplack, D.G., Pizzo, P.A., Adornato, B.T. and Di Chiro, G.: Abnormal CT scans of the brain in asymptomatic children with acute lymphocytic leukemia after prophylactic treatment of the central nervous system with radiation and intrathecal chemotherapy. New Eng. J. Med. 298: 815-818, 1978

Di Chiro, G.: Technical progress and prospects of computed tomography in neuroradiology. Neuroradiology 15: 45-46, 1978

Arimitsu, T., Jabbari, B., Buckler, R.E. and Di Chiro, G.: Computed tomography in a verified case of tuberculous meningitis. Neurology (In press)

Bertorini, T., Engel, W.K., Di Chiro, G. and Dalakas, M.: Leukoencephalopathy in oculocraniosomatic neuromuscular disease with mitochondrial abnormalities (ragged-red fibers): demonstration by computed tomography. Arch. Neurol. (In press)

Chew, E., Weiss, G.H., Brooks, R.A. and Di Chiro, G.: Effect of CT noise on detectability of test objects. Am. J. Roentgenol. (In press)

Di Chiro, G., Arimitsu, T., Pellock, J.M. and Landes, R.D.: Periventricular leukomalacia related to neonatal anoxia: recognition by computed tomography. J. Comput. Assist. Tomogr. (In press)

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. (In press)

Authors: Di Chiro, G. and Brooks, R.A.: Technical Aspects of Computed Tomography. New York, Elsevier North-Holland, Inc., (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 02315-01 SN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

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

PI:	G. Di Chiro	Head, Section on Neuroradiology and Computed Tomography	SN NINCDS
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	G.S. Johnston	Chief	NM CC
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	R.M. Kessler	Staff Physician	NM CC
OTHER:	L. Sokoloff	Chief	LCM M

COOPERATING UNITS (if any) M.M. Ter-Pogossian, Washington Univ., St. Louis, MO; D.E. Kuhl and M.E. Phelps, Div. of Nuclear Medicine, Dept. of Radiological Sciences, UCLA, Los Angeles, CA; Naval Research Laboratory, Washington, DC; A.P. Wolf, Brookhaven National Laboratory, Upton, NY

LAB/BRANCH
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SECTION
Section on Neuroradiology and Computed Tomography

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.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)

Emission computed tomography (ECT) 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/100gm 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). Two groups of radiopharmaceuticals may be used for ECT; 1) single photon, and 2) positron emitters. The unique property of ECT is that it may provide physiologic information not available with any other imaging procedures.

Project Description

Objectives: Emission computed tomography (ECT) predated (see Kuhl's extensive work in the 60's) the transmission computed tomography (TCT) (Hounsfield's EMI-Scanner, 1972). However, due to its relatively poor resolution capability, ECT has yet to encounter a degree of acceptance comparable to that of TCT.

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

Methods Employed: The instrumentation design and application strategies in ECT are diverse and in a state of rapid development. The approaches are divided into two major categories of single-photon counting (SPC), with a variety of radiopharmaceuticals, and annihilation coincidence detection (ACD) of positron emitting radionuclides. In both approaches, reconstruction of tomographic sections of the body is obtained: the radioactivity originating from the 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. For the single-photon ECT, the radionuclides most frequently used are tagged with ^{99m}Tc. For the positron ECT, probably 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/100gm of brain substance) of the cerebral glucose metabolism. Other positron emitting radiopharmaceuticals of interest are $^{68}GaDTPA$ or $^{68}GaEDTA$ (^{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 (ECT 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: None

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, those using relatively long-lived radionuclides and those employing short-lived radionuclides.

Group 1

A) Regional cerebral glucose consumption using ^{18}FDG in the various stages of stroke, as well as TIA). There are reasons to believe that ^{18}FDG ECT should be more sensitive than conventional CT, radionuclide brain scans (RNS) and cerebral angiography (CAn) in the evaluation of the stroke patient.

B) Regional cerebral glucose consumption using ^{18}FDG in epilepsy. Of particular interest are studies of epileptic patients presenting with EEG and ECoG foci and negative neuroradiological tests (CT, RNS, CAn, 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}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}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}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}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}FDG ECT may prove useful here.

G) ECT 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 ECT assessment. The anticipated improvement of CSF dynamics appraisal by ECT has far-reaching implications for the study of such common pathological entities as hydrocephalus, dementia and other psychiatric

disorders. As a tracer for CSF ECT, ^{68}GaDTPA appears to be an optimal choice (see FDA-approved-for-cisternography InDTPA).

Group 2

A) For ^{11}C, ^{13}N and ^{15}O tagged compounds see above comments.

B) ^{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.

C) 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.

Proposed Course of Project: We are in the organizational stage of this project. An ECT device (ORTEC-ECAT) should be delivered to the NIH by the end of 1978. A prototype single-photon counting ECT scanner (produced by Union Carbide Corp.) may also soon be available to us. Advanced prototypes of fast, multislice cerebral positron ECT scanners are being designed by industrial companies with some input by NIH investigators. Installation of a cyclotron on the NIH premises is being contemplated as a possibility. 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: Di Chiro, G.: Technical progress and prospects of computed tomography in neuroradiology. Neuroradiology 15: 45-46, 1978

ANNUAL REPORT

October 1, 1977 through September 30, 1978

Laboratory of Central Nervous System Studies

National Institute of Neurological and Communicative Disorders and Stroke

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.

In order to better assess the current state of investigative findings we have held a series of international workshops on the "Subacute Spongiform Virus Encephalopathies and the Structure of the Unconventional Viruses Which Cause Them" in July of this year, which was highly innovative and experimental in structure, in that far more time was left for informal discussion and exchange between workers than for scheduled meetings. Even the workshop meetings were without formal programs or agendas and there were no scheduled lectures or reports or formal presentations. The whole lack of structure, at the opening of the session, left most of the 150 participants in the eight simultaneously run workshops very skeptical of possible success and, yet, already by the end of the first day it was obvious that the meetings were going to accomplish their purpose. By the end of the second day most participants were ecstatic about the way free exchange and discussion was proceeding. We purposely excluded recording of the sessions, publication of transcripts, or requests for prepared papers in order to encourage free exchange of ideas and preliminary data and most participants pointed out how well this had worked and how much it had encouraged them to attend and to report openly and informally their work and plans and ideas. The list of suggested problems for discussion in each workshop was accepted in part, rejected in part, and considerably modified and augmented by most workshops. Many workshop groups scheduled further informal meetings of their groups after evening dinner and on the weekend, and at private homes. The discussions at private homes and restaurants and over coffee around laboratory benches and on the lawn at Fort Detrick rivaled or exceeded in importance those in conference rooms.

This was the first such exceedingly informal meeting of this size attempted at the NIH and we believe that it was immensely successful and that it will influence the content and form and reference citations of most papers to be written in the field of these unconventional viruses and their structure in the future by all participants, and that it will determine many new collaborations between participants--goals we had in mind.

All participants in the workshop agree with our earlier prediction that 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 microorganism. Unusual resistance to ultraviolet and ionizing radiation, to formaldehyde, β-propiolactone, and 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 humoral or delayed hypersensitivity reactions in the diseases, as well as an absence of response to 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 dilution, and 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 which may facilitate the further work in this direction. Zonal banding and filtration studies have resulted in considerable purification of the scrapie virus and these techniques coupled with polyacylamide gel electrophoresis and electrophoretic focusing techniques and enzyme degradation techniques are being used for further purification.

Our discovery that the scrapie virus and the CJD virus may cause cell fusion in vitro has led to a rapid assay for both viruses. Such fusion occurs between cell lines with two different genetic blocks in purine and pyriimidine scavenger pathways to nucleic acid synthesis, respectively, such that only heterokaryons survive in a medium containing aminopterin to block such synthesis from amino acids. The specificity of the fusion property is

not great, and the technique unfortunately fails to yield much promise as a diagnostic aid in detecting CJD virus in brain biopsies or autopsy specimens. However, as a quick assay for fractions obtained in scrapie virus purification procedures it holds considerable promise, A test using growth of cell colonies of heterokaryons on the selection medium requires three weeks and we have developed a shorter test identifying heterokaryons morphologically by staining and microscopy which requires only 2 to 3 days, as compared to the 6 to 8 months required for mouse titrations of the scrapie virus.

Resistance to high concentration of formaldehyde, to heat, up to 85°, 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 emphasizes 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}) that 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 anamolous 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 physicochemical 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 and 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 2 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.

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.

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 which 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, goat or several strains of mice known to be susceptible to scrapie. 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. 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.

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. 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. With this in mind, we are covering all of 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 on Guam and in collaborative pathological, biochemical and pharmacological studies.

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.

The further workshop on ALS-PD complexes in high incidence in the Western Pacific area sponsored by us on Guam on August 8-9, 1978, with visitors from Japan and Indonesia (the USSR visitors, who were to discuss the focus of Vilyuisk encephalitis in the Iakutsk region of Siberia, and its ALS-PD-like features, and had agreed to attend, had their visits canceled by the Soviet government in reprisal for the American cancellation of the exchange visit of cancer investigators to the Soviet Union, in protest of the political trial of Soviet scientists) has furthered this international collaboration and, most importantly, emphasized the need of more original and innovative research concepts and more imaginative and cautious study of the various Western Pacific foci. Those studies which are underway in our collaborative project and a bibliography of recent publications (1975-1978 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 disease 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, H-LA typing, and mixed leucocyte agglutinins, dermatoglyphics, anthropometry and other gene markers. These studies have already resulted in a series of papers that are either published or are in press, and these are noted in a special bibliography by year, appended to this report.

In other areas of Micronesia, human biological field and laboratory studies continue. Studies of asthma and other chronic respiratory diseases indicate that 75% of the children under 5 years of age were found to have asthma, while over 50% of the adults over 40 years 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.

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 somehow 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 of 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 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, with the larvae of Taenia solium, the pig tapeworm, which has been 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. First, 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 unexpected seizures which occur during the sleeping hours, throwing the patient into the house fire. The unclothed people, living at 2000 meter evelation, 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 which 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.

Dr. Gajdusek had been invited by Soviet investigators as consultant to their study of the huge focus of a chronic degenerative basal ganglia extrapyramidal disorder called Vilyuisk encephalitis, which is restricted to the Yakut people of eastern Siberia. For this visit many investigators, including the Minister of Health of the Yakut Republic, came to Moscow with four patients who were suffering from the advanced stages of the disease. There were three days of presentations of field epidemiological, clinical and laboratory data which had been accumulated, and several hours of discussions on the direction that future studies should take. The disease is a syndrome which has not been previously encountered elsewhere; there is the high possibility that it is a slow virus infection. Our Soviet colleagues have indicated that they will invite us to participate in future meetings devoted to this disease.

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 inquiries they phrase 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 phrased by 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 01282-14 CNSS

PERIOD COVERED

October 1, 1977 through September 30, 1978

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., and Ralph Garruto, Ph.D.

Michael Alpers, M.D.; Richard Benfante, M.A.; Peter Fetchko, M.A.; Paul M. Hoffman, M.D.; Chev Kidson, M.D.; Klaus Mannweiler, M.D.; Colin L. Masters, M.D.; Ivan Mbagintao; Judith Meyer; Steven Ono; Robert Rohwer, Ph.D.; Donald Rubinstein; Vincent Zigas, M.D.

Jacques Bert, M.D.; Francoise Cathala, M.D.; Louis Court, M.D.; Arwin R. Diwan, Ph.D.; Richard Feinberg, Ph.D.; Father David Galles; Undapmaika Kalagune; Robert MacLennan, M.D.; Jesus Raglmar; Wulf Schiefenhovel, M.D.; Koiye Tasa

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 20014

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 employement 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, schizophrenia, neoplasms, goiter, cretinism, rheumatoid diseases, diabetes, goit, 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.

ENGLAND: Mrs. Elisabeth Beck, Dr. P.M. Daniel, Department of Neuropathology, Maudsley Hospital, Institute of Psychiatry, London; Dr. A.J. Duggan, Wellcome Museum, London; Dr. G. Edsall, Dr. David Lang, Dr. Chris Plato, London.

FIJI: Dr. R. Crocombe, University of South Pacific, Suva.

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

FRANCE: Dr. Francoise Cathala, Salpetriere Hospital, Paris and INSERM, Lyon; Dr. M. Godelier, Laboratory of Social Anthropology, l'Ecole Pratique des Hautes Etudes, Paris; Dr. J. Guiart, Musee de l'Homme, Paris; Dr. R. MacLennan, International Agency for Research on Cancer, Lyon; Dr. J. Bert, University of Marseille, Marseille.

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, Kepi; Dr. K. Dresser, T. Benoit, Associated Aviation, Sentani; Dr. W. Schweifenhoeval, Public Health Deparmtent, Sentani; Bishop A. Sowada, Fathers J. Donkers, D. Gallus, B. Mischke, F. Trenkenshuh, B. van Oers, Catholic Mission Center; Dr. C. Hoogeland, Aboahe.

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 Provine; Rev. H. Gericke Evangelical Lutheran Mission, Okapa, Eastern Highlands, Provine; Rev. W.R. Wiesner, Reb. Stuart Merriam, Koiye, Waneri, Anua and Tosetnam, Okapa, Eastern Highlands; Undopmaina, Martin, Marawaka, Eastern Highlands, Provine.

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 Tacheliol, 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 Encephalitides, Moscow; Dr. L. Fadeeva, Institute of Virology, Moscow; Dr. V. Zhdanov, Dr. A.A. Smorodentsev, 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. Rodrique, 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, NIHM, 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; Dr. Margaret Mead, American Museum of Natural History, New York; E.L. 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. Kunstadter, 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 1w through 8w.

Publications: Listed on pages 23w through 29w.

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 00969-14 CNSS

PERIOD COVERED
October 1, 1977 through September 30, 1978

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, D.V.M.; David M. Asher, M.D.; Richard Benfante, M.A.; Paul W. Brown, M.D.; C. Pagis de Micco, M.D.; Phillipe de Micco, M.D.; Sergio Galvez, M.D.; Ralph Garruto, Ph.D.; David T. Kingsbury, Ph.D.; Colin L. Masters, M.D.; Seiho Nagafuchi, M.D.; George J. Nemo, Ph.D.; Hiroshi Oda, M.D.; Robert Rohwer, Ph.D.; Lon R. White, M.D.

Michael Alpers, M.D.; Jacques Bert, M.D.; Francoise Cathala, M.D.; Huyn J. Cho, D.V.M., Ph.D.; Louis Court, M.D.; Arwin R. Diwan, Ph.D.; Chev Kidson, M.D.; Klaus Mannweiler, M.D.; Marie-Claude Moreau, Ph.D.; Ryoichi Mori, M.D., Ph.D.; Maria-Teresa Borras Puga, Ph.D.

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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
24	14	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 Universitat 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 Alegrers.

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: Francoise Cathala, M.D., Departmetn of Neurology, Hopital de la Salpetriere, Paris; Contamin, M.D., Hopital St. Antoine, Paris; Lhermitte, M.D., Hopital de la Salpetriere, Paris; F. Pertuiset, Hopital de la Salpetriere, Paris; Warot, M.D., Department of Neurology, Universitat de Lille Nord; Raymond Latarjet, Foundation Curie-Institut du Radium, Paris.

GERMANY: Klaus Mannweiler, M.D., Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie, Hamburg; A. Struppler (A. Zumsky, USA), Neurology Institute of Technology, University of Munich, Munich; Volker ter Meulen, M.D., Institut fur Virologie der Universitat, Wurzberg.

GUAM: Kwang-Ming Chem, M.D., Olivia Cruz, M.D., NINCDS Guam Memorial Hospital, Tamuning.

ICELAND: P.A. Palsson, D.V.M., Institute for Experimental Pathology, Keldur

COOPERATING UNITS: continued

INDIA: M.S. Bhat, Department of Neurology, J.J. Group of Hospital, Byculla, Bombay.

ITALY: C. Fieschi, Clinica Delle Malattie, Nervosa E. Mentali, Universita di Siena, Siena.

PAPUA NEW GUINEA: R.W. Hornabrook, M.D., Institute of Human Biology; Vincent Zigas, M.D., Public Health Department, Rabaul, New Britain.

POLAND: Eaw Osatowska, M.D., Institute of Neuropathology, Polish Academy of Science, Warsaw.

PUERTO RICO: V. Mojica, M.D., V.A. Hospital, San Juan.

SCOTLAND: Louis Herzberg, Department of Neurology, Royal Infirmary, Dundee; E.H. Jellinek, Neurological Unit, Northern General Hospital, 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. Ol'yenko, M.D., All-Union Research Institute of Influenza, Leningrad.

UNITED STATES: California--J.R. Baringer, M.D., V.A. Hospital, San Francisco; C. Espana, Ph.D., National Center for Primate Biology, University of California, Davis; F. Dixon, M.D., M. Oldstone, M.D., David Kohne, Ph.D., Scripps Clinic and Research Foundation, La Jolla; Peter W. Lampert, M.D., Department of Pathology, University of California, La Jolla; Leslie P. Weiner, M.D., Department of Neurology, University of Southern California, Los Angeles; L.J. Rubinstein, M.D., Stanford Medical School, Stanford; R. Silton, M.D., Department of Anatomical Pathology, City of Hope Hospital, Duarte; W. Tourtellotte, M.D., V.A. Hospital, Los Angeles; Roy L. Walford, M.D., Department of Pathology, University of California, Los Angeles; S. Wiesenfeld, M.D., University of California, Davis, Sacramento Medical Center, Davis; Connecticut--R. Gilbert, M.D., R. Bobowick, M.D., Waterbury Hospital, Waterbury; Florida--L. Prockop, M.D., V.A. Hospital, Tampa; Georgia--D. Camenga, M.D., R. Franco, M.D., Emory University, Atlanta; Dr. Friedman, Department of Neurology, Medical College of Georgia, Augusta; Hawaii--Arwin R. Diwan, Ph.D., Department of Tropical Medicine and Medical Microbiology, University of Hawaii, Honolulu; Nicholas E. Palumbo, D.V.M., Director, Research Animal Facility, University of Hawaii, Honolulu; Illinois--Freidrich Deinhardt, M.D., Loren Wolf, M.D., Department of Microbiology, Presbyterian-St. Luke's Hospital, Chicago; Philip Y. Paterson, M.D., Northwestern University Medical School, Chicago; M.G. Reyes, M.D., Department of Pathology, Mt. Sinai Hospital, Chicago; Donald Harter, M.D., Department of Neurology, University of Chicago, Chicago; Indiana--Donald Gustafson, D.V.M.,

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Ph.D., Purdue University, Department of Virology, West Lafayette; Wolfgang Zeman, M.D., A.N. Siakotos, Ph.D., Department of Neuropathology, Indiana University Medical Center, Indianapolis; Kansas--D. Ziegler, M.D., Kansas University Medical Center, Department of Neurology, Kansas City; Louisiana--William Greer, D.V.M., Gulf South Research Center, New Iberia; W.J. Mogabgab, M.D., Department of Medicine, Infectious Disease Section, Tulane University, New Orleans; Maine--E. David, M.D., Bangor; Maryland--R. Burks, M.D., Neil Goldstein, M.D., Robert Herndon, M.D., Richard T. Johnson M.D., Guy McKhann, M.D., William Narayan, M.D., Neil Nathanson, M.D., R. Roos, M.D., Keerti Shah, Ph.D., Johns Hopkins University Hospital, Baltimore; Theodore Diener, Ph.D., Department of Plant Biology, U.S. Department of Agriculture, Beltsville; Paul Albrecht, M.D., Lewellys F. Barker, M.D., Hope E. Hopps, Bureau of Biologics, FDA, Bethesda; Jacob A. Brody, M.D., NIAAA, NIH, Bethesda; Stuart Aaronson, M.D., Robert Martin, M.D., George J. Todaro, M.D., NCI, NIH, Bethesda; Samuel Baron, M.D., Hilton Levy, M.D., NIAID, NIH, Bethesda; Paul MacLean, M.D., NIMH, NIH, Bethesda; King Engel, M.D., David A. Fuccillo, M.D., Monique Dubois-Dalcq, M.D., Sid Houff, M.D., NINCDS, NIH, Bethesda; A. Dekaban, NIH Clinical Center, Pediatric Neurology, Bethesda; Drs. Kroot and Leshner, Department of Neurology, Bethesda Naval Hospital, Bethesda; Massachusetts--L.S. Adelman, New England Medical Center, Boston; M. Fleming, Department of Neurology, St. Elizabeth's Hospital, Brighton; Dr. Frei and William Schoene, M.D., Peter Bent Brigham Hospital, Boston; M. Oxman, M.D., Children's Medical Center, Boston; David Poskanzer, M.D., Massachusetts General Hospital, Boston; K. Scott, M.D., New England Medical Center, Boston; M. Shelansky, M.D., Boston Children's Hospital, Boston; Michigan--F. Eugene Payne, M.D., School of Public Health, Department of Epidemiology, University of Michigan, Ann Arbor; M. Jones, M.D., MSV Department of Pathology, St. Lawrence Hospital, Lansing; I. Selah, M.D., William Beaumont Hospital, Department of Neurology, Royal Oak; Minnesota--Y. Franck, M.D., Department of Neurology, V.A. Hospital, Minneapolis; Missouri--R. Torack, M.D., Department of Neurology, Washington University School of Medicine, St. Louis; Montana--W. Hadlow, D.V.M., Rocky Mountain Laboratory, NIAID, NIH, Hamilton; J.A. Newman, M.D., St. James Community Hospital, Butte; New Jersey--N. Haidri, M.D., Martland Hospital, Newark; New York--G. Budzilovich, M.D., New York University School of Medicine, New York; A.J. Lapovsky, M.D., P.N. Sawyer, M.D., Downstate Medical Center Hospital, Brooklyn; Asao Hirano, M.D., Division of Neuropathology, Montefiore Hospital and Medical Center, Bronx; Robert Ledeen, M.D., Department of Neurology, Albert Einstein College of Medicine, Bronx; Richard L. Masland, M.D., Department of Neurology, Columbia University, College of Physicians and Surgeons, New York; R. Katzman, M.D., Yeshiva University; Gerald Ward, D.V.M., Public Health Research Institute of the City of New York, Inc., Otisville; R. Carp, Ph.D., Halldor Thormar, Ph.D., Institute for Basic Research in Mental Retardation, Staten Island; N. Peress, M.D., Department of Neurology, State University, Stony Brook; P. Phillips, M.D., Hospital for Special Surgery, New York; North Carolina--John Griffith, M.D., David Lang, M.D., Department of

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YUGOSLAVIA: J. Vesenjak-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.

Project Description: Chronic Central Nervous System Disease Studies
(described fully on pages 1w through 8w)

Publications: Listed on pages 23w through 29w.

The projects (I through XXVII) 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 1w through 8w. Contractural phases of this work are being conducted at:

Gulf South Research Institute, New Iberia, Louisiana

Public Health Research Institute of New York, Otisville

PUBLICATIONS:

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Gajdusek, D.C. and Gibbs, C.J. Jr. Subacute spongiform encephalopathies and the unconventional viruses that cause them. Presented at a Workshop at the National Institutes of Health, Bethesda, Maryland, July 28-31, 1978.

I. Clinical, electroencephalographic, and neurophysiological aspects of spongiform encephalopathies. Dr. Francoise Cathala and Dr. Guy McKhann.

II. Morphological correlates of the spongiform encephalopathies.

Part I. Ultrastructure and general light microscopy. Dr. Richard Baringer and Dr. Peter Lampert.

Part II. Amyloid plaques and deposits. Dr. Sam Chou and Professor Byron Kakulas.

III. Epidemiology of the spongiform encephalopathies. Dr. Leonard Kurland and Professor W.B. Matthews.

IV. History of the spongiform encephalopathies and the unconventional viruses that cause them. Jack Baker, Dr. Walter Kirschbaum, and Sir John Eccles.

V. Immunological aspects of spongiform encephalopathies. Dr. Dale McFarlin and Dr. Abner Notkins.

VI. Physical properties in purification of the causal agents of spongiform encephalopathies. Dr. Chev Kidson and Dr. Robert Rohwer.

ANNUAL REPORT

October 1, 1977 through September 30, 1978
Clinical Neurosciences Branch
National Institute of Neurological and Communicative Disorders and Stroke

Cosimo Ajmone Marsan, M.D., Chief

Summary of Program Activity

The activity of this Branch can be divided into research and clinical diagnostic service, the latter involving a total of 3.9 man/years (1 professional and 2.9 technical-clerical).

1. Clinical-Diagnostic Service

From the time of preparation of the last Report (May 31, 1977) to that of the current Report (May 31, 1978) a total of 1175 electroencephalograms were obtained and interpreted in patients referred to our Branch as part of their routine clinical investigation or for specific research projects originating from other Branches of our Institute or from other Institutes. The distribution of these referrals according to the Institute of origin has been:

INSTITUTE	NO.	%
NINCDS	797	68.0
(OPD)	(414)	(35.0)
NIMH	155	13.0
NICHD	62	5.3
NCI	59	5.0
NHLBI	40	3.4
NIAID	27	2.3
NIAMDD	24	2.1
NEI	7	0.6
Misc	4	0.3
TOTAL	1175	100.0

The slightly lower total in comparison with the previous year is due to the one month inactivation of the laboratory during construction and relocation works and to the fact that the previous Report covered a 13 month period. One third of the referrals have been from Institutes other than NINCDS. Of these examinations, a fair number had to be performed on the ward, at the patient's bed or in the Intensive Care Unit. An additional twelve recording procedures were carried out directly from the exposed cortex in the operating room on epileptic patients. The Service continues to

provide useful material for several investigation projects in our Branch which also collaborates closely with other units, especially the section on Clinical Epilepsy of the Experimental Therapeutics Branch. It also provides material for the training in Clinical Electroencephalography so that each year one or two Clinical Associates become eligible for the Boards of qualification in such a specialty.

2. Research Activity

Of the fifteen research projects mentioned in the previous Report six had been completed or terminated and papers which were in preparation or had been submitted for publication at the time of the Report have now been, or are about to be published. The current Report includes a total of eleven projects, nine of which represent the continuation of projects initiated in the past.

a) Clinical

The study of the various electrical manifestations of epileptiform activity in the human cortex has continued. Beside the direct pre-and post-excision monitoring in twelve epileptic patients in the course of surgery for the treatment of refractory seizures, it has been possible to record, by means of a new type of floating microelectrodes, the activity of neocortical neurons in three subjects. The scarcity of available information about the behavior of "epileptic" neurons in human enhances the significance of this technically difficult study which should provide an insight into the cellular phenomena which are at the basis of human epilepsy. Suitable cases for this investigation are not readily available and furthermore the time allowed for it in each individual patient is of necessity limited. Because of these factors, completion of this project should require another year.

The analysis of clinical seizure patterns in different forms of epilepsy continues to be a main field of interest for this Branch. After having observed and analyzed a large number of ictal episodes in patients with scalp EEG evidence for the existence of an epileptogenic process within the temporal region and in various suprasylvian areas, characteristic patterns have emerged which do not always fit the classical text-book descriptions. 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 which showing typical epileptiform EEG discharges discreetly localized to the central vertex region and 30 with pathologically documented (expanding or vascular) lesions in the same area. The various clinical features and the seizures patterns of these two subgroups of patients are currently being analyzed and correlated. The results of this study should be ready for publication in the near future. The recently initiated use of a 16 channel tele-

metering EEG for monitoring clinical and electrographic ictal pattern should broaden the scope of this investigation.

Another project utilizes visual evoked potentials (VEP) and deals with patients affected by demyelinating diseases and other neurophthalmological disorders. Of particular interest has been the availability of 36 subjects belonging to a series of 16 pairs of twins referred to our Laboratory as part of a project dealing with M.S. in twins (Neuro-immunology Branch). In this series, 16 of the 17 cases which were considered clinically affected by M.S. had definitely abnormal VEP's but 20% of the 15 subjects considered to be clinically unaffected also showed an abnormal VEP.

The cortical representation of speech functions has been re-examined in another project which utilizes electrical stimulation of the cortex in patients undergoing neurosurgical treatment of their seizure disorder. In this study, cortical stimulation of the supramarginal and angular gyri back to the occipital cortex produced dysphasia qualitatively similar to that obtained from stimulation of the anterior speech regions in the temporal lobe. Speech arrest was also induced when stimulation was applied to the occipital cortex and to the cortex medial to the supramarginal gyrus. It would seem that such stimulation might interfere with a search mechanism in which the non-verbal concept of the visual stimulus is linked to specific word in memory that is then withdrawn for use. On the basis of autopsy specimens it would appear that cortex bearing indispensable speech representation extends to within a few cm of both the occipital pole and the parietal midline. This interesting study is planned to continue with a battery of more sophisticated tests and be extended to further analyze short and long-term memory impairments in patients with various types of brain lesions. Parallel studies of interhemispheric relations are also planned. Somewhat related to this project is a neuropathological investigation, by means of serial sections in the brain, of a patient affected by chronic dyslexia, dyscalculia and dysgraphia. Examination of the left parieto-occipital region showed degeneration in the central portions of the external and internal sagittal strata. In the pulvinar the degeneration involved the posteroinferior pole. Of interest is the comparison of this case with a previously reported case of chronic receptive aphasia in which the pulvinar degeneration lay in its anterosuperior lateral portion. No new findings are reported in relation to two projects, one dealing with brain mechanisms which regulate perception and the storage and retrieval of information, the other on the personality profiles and sensory disturbances of epileptic patients affected by partial complex seizures.

b) Experimental Research

One project deals with the correlation of the characteristic action potentials and the fiber size histograms in the normal and

resutured peripheral nerves. Since there has been no such correlative analysis of unmyelinated fibers and after regeneration, this study presents a particular interest. Another project investigates the late (18 months) changes in the pia-arachnoid membrane related to air exposure and low energy ultraviolet irradiation (355 nM). The objectives of a third project are to compare the ultrastructural features of retrograde responses to nerve lesions, in young adult (21 days postnatal) and immature rat (7-10 days postnatal). Specifically, the study a) investigates the sequence of events of the progressive changes in the perikaryon capacity to respond to axonal injury; b) investigates the ultrastructural mechanisms by which neurons are switched to a different metabolic program from that operating during their maturation period; c) compares the glial reaction to axon injury and correlates the cell body responses with axonal regeneration in immature and mature animals, and d) investigates the membrane events of the axon and cell body of injured neurons. Preliminary results obtained in the hypoglossal nuclei of rats 7, 10 and 21 days old subjected to hypoglossal nerve crush, ligation and transection indicate a progressive change in the character of the cell body and glia response with neuronal maturity. In the mature neuron the microglia proliferate 1-3 days post-operatively, invade the nucleus to surround the reacting cell soma while presynaptic nerve terminals are lifted off the neuronal membrane. In immature neurons there is a delay in proliferation of perineuronal glia and in the early post-operative period the neurons send out cytoplasmic protrusions which surround dendrites and presynaptic terminals in contact with its membrane. This project will continue with freeze-etch studies of these reactive changes.

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 utilize 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 previous, recently completed similar 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-examine this aspect of the problem and utilize the multibarrel micropipette technique for extra cellular recording from cortical and hippocampal neurons during and following microiontophoretic application of various aminoacids and of the epileptogenic agent in question. Preliminary findings indicate that, in the hippocampus, pyramidal neurons increase their firing during low current iontophoresis of D-l-homocysteic acid and this activity is steadily suppressed by GABA. On the other hand, when GABA was tested on the increased neuronal firing induced by topical application of penicillin, the inhibitory effect was greatly decreased. Duration of post-discharge

inhibition produced by electrical stimulation of the pyramidal neuron would also tend to decrease during penicillin iontophoresis. Future experiments in this project include the comparison of these results with those obtained with systemic administration of penicillin and the possible inter-effect of this agent with GABA in structures such as the cerebellum and olfactory bulb which are generally refractory to epileptiform activity. The iontophoresis of penicillin (-100nA) in somatosensory cortex generally produces somewhat different effects which include a transitory decrease in unit firing rate and eventually the development of grouped unit spikes. These effects are quite variable from one cell to another but the response pattern does not seem to be related to any obvious technical factor (such as electrode configuration, tip size, ejecting current, pH etc). Experiments are now in course to evaluate the reliability of ejection and distribution of penicillin in vivo. Preliminary in vitro investigations with 14 C-penicillin would indicate consistent intra-electrode ejection rates but significant interelectrode variability.

Other Activities, Honors etc.

The head of the section of Functional Neurosurgery has been visiting professor at the University of Miami and the Arkansas University. He has also served on the faculty of the Cook County Post-Graduate course, review course in Neurological Surgery, and was invited to Seizure Symposia to cover the up-to-date surgical treatment of seizures at Allen Neurosurgical Association Inc., in Allentown, PA, and at the Mexican Neurosurgical Congress in Mexico City. The chief of the Branch has continued his active involvement in editorial duties of various specialized journals (Electroenceph. clin. Neurophysiol.; Epilepsia; Arch. ital. Sci. Biol.; J. Neurophysiol) and has completed his co-editorship of the Handbook of EEG and Clinical Neurophysiology. He has been recently invited to be member of the executive committee of the International Brain Research Organization as Director of IBRO Symposia. He has been actively involved in the organization of the Scientific Program of the IX International Congress of EEG and Clinical Neurophysiology held in Amsterdam and has participated in a Symposium at that Congress. He has been invited to, and will participate in the Workshop on Adult EEG at the coming annual meeting of the American EEG Society in San Francisco. He will also chair a Symposium on generalized Epilepsies at the next meeting of Neurosciences in St. Louis.

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 00100-25 CN

PERIOD COVERED
October 1, 1978 thru September 30, 1978

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

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)

Division of Endocrinology, National Naval Medical Center

LAB/BRANCH
Clinical Neuroscience

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.8	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)

In support of the noted protocols, it was possible to record with the floating microelectrode 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.

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:

The projects in this program have considerable potential, but with the present clinical restrictions the program has doubtful viability.

Publications:

Wood, J.H.; Glaeser, B.S.; Hare, T.A.; Sode, J.; Brooks, B.R.; and Van Buren, J.M. (1977) Cerebrospinal fluid GABA reductions in seizure patients evoked by cerebellar surface stimulation. J. Neurosurg. 47: 582-589

Wood, J.M.; Ziegler, M.G.; Lake, C.R.; Sode, J.; Brooks, B.R.; and Van Buren, J.M. (1977) Elevations in cerebrospinal fluid norepinephrine during unilateral and bilateral cerebellar stimulation in man. Neurosurgery 1: 260-265

Lewis, D.V.; Mutsuga, N.; Schuette, W.H.; and Van Buren, J.M. (1977) Potassium clearance and reactive gliosis in the alumina gel lesion Epilepsia 18: 499-506

Van Buren, J.M.; Wood, J.H.; Oakley, J.; and Hambrecht, F. (1978) Preliminary evaluations of cerebellar stimulation by double-blind stimulation and biological criteria in the treatment of epilepsy. J. Neurosurgery 48: 407-416

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 00200-24-CN

PERIOD COVERED
October 1, 1977 to September 30, 1978

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:	A. Neophytides	Clinical Associate	ETB NINCDS
	T. Chase	Neurologist	ETB NINCDS
	C. Cox	Psychologist	CN NINCDS
	G. R. Frederick	Psychologist	CN NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.6	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☒ (a2) INTERVIEWS

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

An emotional and cognitive profile of individuals 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 attempts to identify the intellectual and emotional traits of at-risk individuals and with additional study, 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 two-fold purpose: 1) to provide an objective assessment of cognitive, emotional and behavioral dimensions for individuals who are classified as risk candidates for Huntington's Disease; 2) 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:

To date approximately 45 'at-risk' individuals have been evaluated. The analysis was deferred until normative data may be collated from control subjects who will be matched with 'at-risk' individuals on relevant parameters.

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 00304-23 CN

PERIOD COVERED
October 1, 1977 thru September 30, 1978

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 für Hirnforschung, Zurich Switzerland

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	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)

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 uW/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:

1. Further studies of the glial-neuronal interactions in injury are planned.

2. Further reconstruction studies of human material are planned.

Publications:

Van Buren, J.M.; Akert, K.; and Sandri, C. (1977) Neuritic growth cone and ependymal gap junctions in the feline subfornical organ during early development. Cell Tiss. Res. 181: 27-36

Streit, P.; Van Buren, J.M.; Sandri, C.; Akert, K.; and Bennett, M.V.L. (1978) Differential HRP labeling of motoneurons and electromotor neurons in the spinal cord of the gymnotid Sternarchus albifrons. Brain Research 142: 559-565

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 ZO1 NS 01245-13-CN

PERIOD COVERED
October 1, 1977 to September 30, 1978

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

PI:	P. Fedio	Psychologist	CN NINCDS
	W. Sheriff	Computer Programmer	TD NINCDS
OTHER:	M. Buchsbaum	Research Medical Officer	AP NIMH
	J. Van Buren	Associate Chief	CN NINCDS
	A. K. Ommaya	Acting Chief	SN NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☒ (a2) INTERVIEWS

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

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.

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 removals 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 PDP-12 computer has been acquired, and will be programmed to provide A-D, off line analysis of data that has been acquired. 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 01424-12-CN

PERIOD COVERED
October 1, 1977 to September 30, 1978

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

PI:	P. Fedio	Psychologist	CN NINCDS
OTHER:	J. Van Buren	Associate Chief	CN NINCDS
	G. R. Frederick	Psychologist	CN NINCDS
	C. Cox	Psychologist	CN NINCDS

COOPERATING UNITS (if any)
Department of Psychiatry, Massachusetts General Hospital, Boston, Massachusetts

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.6	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☒ (a2) INTERVIEWS

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

Emotional and sensory characteristics are studied in patients with temporal lobe epileptic foci. 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 emotions displayed by facial expressions, and learn words with different emotional connotations. Temporal 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 temporal lobe in establishing limbic associations and differences between the left and right hemispheres in regulating emotions and sensory experiences in man.

Project Description:

Objectives:

1. To identify personality profiles of epileptics with confirmed temporal lobe seizures. Although the relationship between limbic lesions and emotional disorders has been clinically suggested, most psychometric tests have been found to be 'insensitive' to the unique personality or behavioral disturbances displayed by individuals with temporal lobe disorders.

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:

1. Based on several reliable 'clinical signs', a 100-item personality questionnaire was designed: one form was completed by the patient while an alternate form of the same scale was filled out by a relative or reliable informant.

The questionnaire was administered to four groups, matched for age, educational and socioeconomic level. The temporal groups consisted of epileptic patients with unilateral EEG foci located in the left or right temporal lobe; none of the patients were being considered for surgery and all were being maintained on a standard regimen of anticonvulsant medication. Two control groups were selected, one group was composed of patients with peripheral neuropathologic disorders; another group contained normal individuals.

2. Two 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:

1. The personality profile of the epileptic group was abnormal in comparison with the self-rater inventory of both control groups. Moreover, the left and right temporal patients were also judged by their raters to demonstrate a higher degree of unusual or abnormal behavior. Although seizure frequency was not related to degree of emotional disturbance, duration (years) of seizure disorder affected abnormal personality profiles, that is, the 'longer' the subject had epilepsy, the more likely he demonstrated aberrant behavioral traits.

These data complement the classic asymmetry of cognitive functions assigned to the left and right brain, and extend these observations to an asymmetry of emotional processing by the left and right cerebral hemisphere.

2. The test materials and techniques for evaluating the effects of emotions on perception and memory were recently developed and are being standardized with normal subjects in preparation for testing temporal epileptic 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:

Bear, D. M. and Fedio, P.: Quantitative analysis of interictal behavior in temporal lobe epilepsy. Arch. of Neurology, 34: 454-467, 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 01658-11-CN

PERIOD COVERED
October 1, 1977 to September 30, 1978

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

PI:	P. Fedio	Psychologist	CN NINCDS
	J. Van Buren	Associate Chief	CN NINCDS
OTHER:	G. R. Frederick	Psychologist	CN NINCDS

COOPERATING UNITS (if any)

National Technical School for the Deaf, Rochester, New York

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	0.6	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☒ (a2) INTERVIEWS

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

The disabling effects of brain damage in man were evaluated on a broad range of perceptual, learning and memory functions. Changes in the intellectual behavior of neurologically handicapped individuals were evaluated before and after brain surgery and during electrical stimulation of the surface and depths of the brain. In contrast to these cases with confirmed brain injury, the effects of peripheral, sensory deficits were assessed in terms of possible neuropsychological dysfunctioning, and communicative disorders.

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 wherein there are sensory restrictions to language, as with congenital deafness.

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.

2. The cerebral organization and lateral representation of language in the brain of deaf individuals will be evaluated by non-invasive, behavioral techniques. The testing procedure uses a tachistoscope, an apparatus which projects visual material at high speed (msec) onto the left or right visual fields, which in turn transmit the information directly to the left or right brain for processing. Accuracy of recognition for stimuli appearing in the left or right field reflects the efficiency of processing by left or right hemisphere. A variety of stimuli will be used to assess cerebral processing (printed words, designs, manual alphabet and sign language).

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. Stimulation of the left frontal cortex (Broca's area) also interfered with object naming, albeit to a lesser degree. Stimulation of Broca's area did not prevent the patient from

recognizing or remembering objects but from stating the name until after stimulation was terminated, taking the appearance of blocked phonation or articulation. The patients experienced a similar blockade or interference with speech production during stimulation of the supplementary motor area.

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 the impact of 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.

The study of the operational efficiency of the 'language brain' in individuals who have been deprived of auditory experiences by congenital deafness, is in the pilot phase. Individuals who became deaf before language skills were acquired (pre-lingual) present unusual linguistic characteristics that are frequently seen in neurologically impaired individuals. Whether the loss is due to the absence of auditory input into the language brain or to the absence of cross modal integration, that is, auditory-visual, requires further neuropsychological investigation.

A preliminary review of certain developmental and behavioral features of a select population of deaf individuals revealed atypical distribution with regard to handedness. Whereas the estimate for left-handed individuals in the general population runs between 5-12%, the initial survey of a deaf population (college students) yielded 16% left-handed subjects. This is interesting along several lines, that is, whether the high rate of deaf sinistrals is due to some underlying brain injury or whether the reliance by deaf individuals to rely heavily on visuo-spatial communication (lip reading, finger spelling, signing) encourages development of the right brain.

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 kindred communicative disorders.

Proposed Course of the Project: A battery of tests is being designed to examine adaptive strategies used by neurologic or sensory handicapped 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.

Parallel studies of interhemispheric relations will be made during deep brain stimulation and during cortical stimulation of patients in the neurosurgical operating suite.

Publications:

Fedio, P.: Perception and immediate memory during electrical stimulation of the human brain. Sixth Annual International Neuropsychology Society Meeting, Minneapolis, Minnesota, 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 02213-03 CN

PERIOD COVERED
October 1, 1977 thru September 30, 1978

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)

None

LAB/BRANCH
Clinical Neurosciences

SECTION
Functional Neurosurgery

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.1	0.8

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 results indicate distinct differences with maturation upon the neuronal glial reaction of the hypoglossal nucleus to axonal injury in rats. Up to some ten days postnatal, the neuronal membrane engulfs the presynaptic terminals in contact with its membrane and the proliferative perineuronal glial phase is delayed to 1-3 weeks post-operative. In the mature neuron (21 days postnatal) the microglia proliferate earlier and lift the presynaptic terminals 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 the cell body and glia 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:

Freeze-etch studies of these reactive changes are 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 02269-02 CN

PERIOD COVERED
October 1, 1977 thru September 30, 1978

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.: 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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

An analysis of the morphology, amplitude and latency of the visual evoked potentials in 79 cases of various neurological and neuro-ophthalmologic problems was done.

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 widespread. The practical value of this diagnostic test is confirmed in this study.

Methods Employed and Major Findings: Photic flash VEP tests were performed in 79 cases of various neurological and neuro-ophthalmological problems. The largest group comprised 54 cases with suspected multiple sclerosis (M.S.), 6 cases of spinocerebellar syndromes, and 13 cases with various pathologies that included optic nerves, chiasm and optic radiations. Of the entire group, 43 or 54% were abnormal. Of the cases studied, 32 belong to a series of 16 pairs of twins that were referred as part of a project dealing with M.S. in twins by the Neuro-Immunology Branch. This series was a major part of the VEP study. Of the 16 pairs studied, all had VEP's, EEG's were only performed in 28 subjects. Fifteen cases were considered clinically unaffected: of these, only 3 (20%) had an abnormal VEP and only one had an abnormal EEG. Of the 17 cases that were considered clinically affected by M.S. 16 (94%) showed abnormal VEP's. There were 10 normal and 5 abnormal EEG's. These results will be correlated with the CSF immunological and HLA typing performed by another branch. VEP's appear to be valuable in separating the group of normal cases from those affected with M.S. where the incidence of abnormality was very high (94%). The relatively large percentage (20%) of abnormal VEP in clinically normal subjects was rather unexpected. Further tests might demonstrate a possible CSF abnormality/VEP abnormality correlation.

Significance to Biomedical Research and the Program of the Institute: This study will provide needed information with regards to the true incidence of M.S. in twins. This is an ideal situation for VEP evaluation where a perfect age matched normal control is available where only one twin is affected.

Proposed Course of the Project: This project is provisionally completed and the material is 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 02270-02 CN

PERIOD COVERED
October 1, 1977 thru September 30, 1978

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 THIS 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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

A correlative study of the clinical, electrographic and seizure patterns of 20 pathologically proven midline (parasagittal) lesions has been carried out. A similar group of 30 cases with electrographic interictal and ictal epileptiform discharges originating from the vertex region (CZ electrode) has been selected for comparison of their seizure pattern and behavior to differentiate this as a group from the tumor cases.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To analize 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 will be terminated in the early part of next fiscal period and publication will follow.

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 02320-01 CN

PERIOD COVERED
October 1, 1977 thru September 30, 1978

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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1	1.2

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)

Feline hippocampal pyramidal cells were studied with compound multibarrel micropipettes using the technique of combined penicillin (PCN) and amino acid iontophoresis. PCN iontophoresis increased the unit firing rate in most cases, and decreased the duration of electrically induced post-discharge inhibition. 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). GABA suppressed D-l-homocysteic acid 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-l-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: DLH (-lnA to -20nA) usually excited the neuronal pyramidal cells and increased the firing rate, whereas large current application of DLH (over -20nA) easily blocked unit firing (depolarization block). GABA (10nA to 50nA) suppressed the unit firing. (a) Surface interictal discharges occurred within a few minutes after PCN topical application and were accompanied by pyramidal cell unit firing. After the interictal discharge, unit firing was inhibited for a longer duration (2-10sec) than following electrically induced post-discharge inhibition (50-370msec). GABA failed to completely suppress the unit firing during the occurrence of a PCN-induced interictal event. (b) Duration of post-discharge inhibition produced by electrical stimulation of the pyramidal cell was shortened by iontophoresis of PCN. The pyramidal cell firing rate was usually increased during the prolonged period of PCN iontophoresis (5-20 min). These findings suggest that topical PCN-induced surface interictal discharges are associated with diffuse hippocampal population synaptic effects, whereas PCN iontophoresis may be related to direct excitation/inhibition of the pyramidal cell/basket cell circuitry, respectively.

Proposed Course of the Project: Some supplemental experiments are necessary to confirm these findings. Results will be compared in similar experiments by varying the route of PCN administration (e.g. intravenous). A similar type of investigation will be undertaken in the cerebellum and also the olfactory bulb regions, since it is generally difficult to produce epileptiform activity in these neuronal structures. From these investigations, the mechanisms which relate to the regional difference of seizure thresholds may be clarified.

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 02321-01 CN

PERIOD COVERED
October 1, 1977 thru September 30, 1978

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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1	1.2

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)

The response of cells from the feline somatosensory cortex was investigated with compound multibarrel micropipettes filled with amino acids (D-l-homocysteic acid (DLH), GABA) and penicillin (PCN). Drugs were applied by iontophoresis. 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 micropipettes 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: Excitatory responses were noted to DLH in most cells (no=107); and, inhibition of unit activity to GABA application was characteristic of the cells investigated (no=82). Onset of PCN cationic current (usually 100nA; range 30nA to 500nA) was frequently associated with a transient decrease in unit firing rates. There was slow increase in firing rate to, or exceeding, pre-PCN control levels within 3-10 min. Also the pattern of unit firing changed from single short repetitive spike bursts to grouped complexes of spikes, which were intermittently associated with surface interictal discharges. GABA suppression appeared most effective during return of the firing rate to the pre-PCN level, and least effective following onset of grouped spikes (approximately 7-13 min after PCN onset). The transient change in unit firing rates after termination of PCN was unrelated to initial changes in firing rate. There was large intercellular variability but the cause was obscure.

Proposed Course of the Project: The reliability of ejection and distribution of PCN *in vivo* must be evaluated. Determination of the PCN transport number is required to assess the rate of drug efflux. Preliminary *in vitro* investigations with 14C-PCN indicate significant interelectrode variability and consistent intraelectrode ejection rates. The effects on drug release of the following will be ascertained: 1) Filling technique, 2) electrode tip size, form, length, 3) drug characteristics (pH, pka, molarity), 4) carrier ions, and 5) current flux. After defining the most effective ejection mode for PCN, *in vivo* immediate and surround tissue sections will be analyzed autoradiographically. The neurophysiological responses and single-cell variability to PCN iontophoresis can then be more systematically correlated with the local drug concentration. Cell population activity resulting in summated surface discharges may also be identified and related to drug distribution and concentration.

Publications: None

ANNUAL REPORT
October 1, 1977 through September 30, 1978
Developmental and Metabolic Neurology Branch
National Institute of Neurological and Communicative Disorders and Stro
Roscoe O. Brady, Chief

The Branch has continued its multi-level approach to the understanding, control and therapy of heritable neurological disorders. Major efforts of the Branch focus on (1) basic and clinical investigations of complex lipid metabolism in normal and pathological states; (2) the organization and function of membrane lipids and their role as biotransducers of extrinsic regulatory signals; (3) identification and characterization of myelin and oligodendrocyte-specific glycoproteins and their role in brain development and demyelinating diseases; (4) investigations of the function of enzymes on the external surface of glial cells in relation to the pathogenesis of convulsive disorders; and (5) elucidation of clinical disorders of the nervous system of unknown etiology. The report is accordingly divided into these five categories.

I. Lipid Storage Diseases

A. *Enzyme Replacement Therapy for Inherited Disorders*

Human placental glucocerebrosidase obtained by our newly developed large-scale high yield purification procedure causes the complete catabolism of all of the accumulated glucocerebroside in liver tissue biopsy specimens derived from patients with Gaucher's disease [Pentchev, P. G., et al., in press]. However in vivo this enzyme is not as efficient as glucocerebrosidase prepared by our previously used laboratory scale procedure for its isolation. This relative inefficiency is probably due to removal of an associated lipid component in the course of its isolation by the newer method. Because simple addition of extracted lipids did not restore its effectiveness in vivo, we have begun several lines of investigation to provide the purified enzyme with a critical homing address so that it will be directed to and effectively incorporated into the cells where the offending lipid is stored. At this moment, one promising approach appears to be modification of the carbohydrate portion of the enzyme. We have carried out a series of reactions to remove specific sugar residues from the glucocerebrosidase. We have obtained a 40-fold augmentation of enzyme uptake by reticuloendothelial cells in experimental animals with this approach. This effect is most gratifying since most of the accumulating lipid is stored in these cells in affected individuals. As soon as feasible, clinical trials with appropriately modified enzyme will be carried out.

Other strategies that provide the appropriate label for the enzyme under investigation include entrapment of the enzyme within liposomes or the patient's own erythrocyte ghosts and by linking the enzyme with molecules such as heat-denatured human serum albumin which are known to be specifically endocytosed by cells of the reticuloendothelial system. It is expected that these diversified approaches will ultimately yield a clinically useful enzyme preparation for effective replacement therapy.

B. *Delivery of Enzymes to the Central Nervous System*

We have been particularly encouraged by results obtained during the past year regarding critical experiments for enzyme replacement therapy in metabolic disorders that involve the brain. In FY 77, we reported the discovery of a procedure for temporarily altering the blood-brain barrier so that enzymes injected into the circulation would penetrate into the substance of the brain. After a period of approximately four hours the barrier recloses. A critical unanswered question at that time was whether neuronal cells had membrane receptor sites for the glycoprotein enzymes that are involved in metabolic storage diseases and if they did, would the enzymes be effectively endocytosed by these cells. Both of these questions have been answered affirmatively during FY 78 using horseradish peroxidase, a glycoprotein enzyme, as a model. This enzyme will enter the CNS only after appropriate osmotic alteration of the blood-brain barrier. The enzyme then becomes selectively endocytosed by neurons, and its subcellular compartmentalization has been determined. Since neural cells have the necessary apparatus for intracellular localization of exogenous enzymes, enzyme replacement for disorders such as Tay-Sachs disease and mucopolysaccharidoses appears to be on a much more rational basis.

II. Complex Lipids as Membrane Receptors for Environmental Signals

Previous investigations revealed some similarities between the mode of action of cholera toxin and glycoprotein hormones such as thyrotropin and chorionic gonadotropin. Although a ganglioside (G_{M1}) is the specific membrane receptor for cholera toxin, studies carried out this year indicate that these lipids do not appear to be directly involved in the binding of gonadotropin to its target cell. However, similarities concerning the mechanism of action of cholera toxin as related to ADP-ribosylation of a component of adenylate cyclase and the molecular effects of trophic hormones have been demonstrated. Further, we have found that certain phospholipids are potent inhibitors of thyrotropin (TSH) binding. Hydrolysis of these endogenous lipids in thyroid cell membranes with phospholipase A increases the amount of TSH that becomes bound and incorporation of these lipids into the membranes decreases TSH binding. These observations indicate that the composition and quantity of phospholipids in target cell membranes influence the physiological state of membrane receptors and can modulate the interaction of trophic hormones.

A related study was carried out on membrane lipids in myogenesis. It was found that receptors for hormones such as insulin and epinephrine increase sharply during cell fusion whereas those for cholera toxin (ganglioside G_{M1}) diminish. This effect is accompanied by a distinct change in ganglioside pattern in embryonic muscle during the short time span of four hours when these alterations occur. These findings suggest that gangliosides may function as biotransducers for hormones other than thyrotropin and gonadotropin.

III. Myelination and Demyelinating Diseases.

Work on the major myelin glycoprotein in the central nervous system has accelerated in several relevant directions. The glycoprotein appears to be selectively concentrated in membranes that are transitional between the

oligodendroglial surface membrane and compact myelin. As myelination progresses, the molecular weight of the myelin-associated glycoprotein (MAG) decreases by 10,000 daltons. Probes of myelin with proteolytic enzymes indicate that an 80,000 dalton fragment of both larger (immature) and smaller (mature) myelin glycoprotein is resistant to hydrolysis *in situ*. This observation indicates that this portion of these molecules is embedded in the myelin sheath. Further, there was little difference in the oligosaccharides isolated from mature and immature myelin implying that the conversion of the immature (larger) form to the mature molecule occurs by limited proteolysis of the polypeptide chain during myelinogenesis. This alteration may be necessary for proper compaction of myelin since it is impaired in dysmyelinating animal models such as the Quaking mouse.

MAG has also been shown to be a surface component of myelin by lectin binding experiments. Furthermore, this glycoprotein is highly antigenic when injected into heterologous animals. Utilization of the antiserum to MAG for immunohistochemical studies in developing rat brain indicated specific localization of MAG in oligodendrocytes and myelin. Significant anti-MAG antibody titers are produced in rabbits when experimental allergic encephalomyelitis is induced by injecting whole rat myelin preparations. These findings suggest that MAG is most likely involved in demyelinating diseases in humans either as the site of viral attachment to myelin or oligodendroglial membranes or by participating in an autoimmune response to viral modification of MAG. Humoral and cell-mediated sensitization to this myelin-associated glycoprotein will be examined in human disorders such as multiple sclerosis.

IV. Studies on Epilepsy

Prior work with DBA mice indicated that the activity of Ca^{++}-stimulated ATPase was diminished in the brains of this seizure-prone animal analogue of human convulsive disorders. This experiment has been confirmed and extended in an important useful direction in tissue culture. Ecto-ATPase (enzyme on the external surface of the glial cell membrane) was greatly reduced in activity in the cells from seizure-prone animals compared with control preparations. A further important observation made in the course of this investigation was the eventual rise to 85% of control level as the cells reached the stationary stage of growth. This biological model shows extraordinary promise for examining the biochemistry and temporal organizational basis of seizure activity at the molecular level.

Another potentially important finding concerning intercellular communication was made in the course of these investigations. A number of cell lines apparently release surface (ecto) enzymes into the tissue culture medium in the form of synaptosome-like particles whose average diameter is 1 μm. Within these particles are small vesicles with a diameter of 0.05 μm. These packets have been termed exosomes. We propose that a major function of exosomes is the directed transport of growth factors or similar substances from one type of cell to another, such as from glia to neuron. If this concept can be substantiated, this discovery has important potential for neurobiology. It has long been considered likely that "factors" or

or "activators" pass between cells in the developing nervous system that facilitate the formation of neural nets and induce myelination. Because these exfoliating vesicles carry enzymes on their outer membranous surfaces, they seem likely candidates for an effective intercellular transfer system.

V. Clinical Studies on Disorders of the Nervous System

Dr. John A. Barranger was appointed Acting Head of the Section on Clinical Investigation and Therapeutics subsequent to the retirement of Dr. A. S. Dekaban. Dr. Barranger has carried out his duties in an exemplary manner, operating at several levels of competence. He has continued his highly productive investigation on the alteration of the blood-brain barrier (cited in Item I). He has supervised patient care, directed clinical research projects and engaged in a series of 26 collaborative investigations with various groups at NIH as well as at outside medical centers. These associations habe greatly improved our clinical investigative capabilities and at the same time expanded our basic research productivity. Two examples of accomplishments in the clinical area are the following: We have demonstrated a consistently significant reduction angiotensin converting enzyme in sera from patients with Gaucher's disease following injection of placental glucocerebrosidase. Collaboration with investigators in NEI has revealed previously unnoticed ocular abnormalities in Gaucher's disease. These findings may become useful parameters for assessing the effectiveness of enzyme replacement therapy in this disorder. At the basic level, studies with investigators in NIMH have demonstrated the feasibility of altering the blood-brain barrier for consideration of enzyme replacement where the brain is involved (cited previously). Studies with investigators in NIAMDD have led to the acquisition of important data on the differential cellular uptake of exogenous lysosomal enzymes and the ability to selectively control this distribution. Another example of collaborative investigation that has great potential significance for enzyme replacement therapy for metabolic disorders evolved from a project undertaken with members of the ID Branch of NINCDS. Here it was conclusively demonstrated that injections of placental glucocerebrosidase did not cause antibody production in patients with Gaucher's disease. This finding indicates that we can proceed with exploration of this form of therapy without the pervasive concern that patients would become sensitized to the enzyme they require.

Barranger has amply demonstrated his capability for directing a highly productive clinical investigation unit and his performance in this capacity has been completely satisfactory.

VI. Symposium

The Branch organized and conducted a two-day international symposium on autosomal dominant neurological disorders in collaboration with the Department of Neurology of the University of Texas Southwestern Medical School. It was the unanimous feeling of the participants that this forum provided an important and timely impetus for research related to this area of neurological disorders.

VII. Honor

A member of the staff of the Branch received the NIH Director's Award, 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 00706-19 DMN

PERIOD COVERED
October 1, 1977 - September 30, 1978

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
	Daniel E. Britton, M.D.	Clinical Associate	DMN	NINCDS
	Jan K. Steusing	Research Assistant	DMN	NINCDS
	Robert N. Nishimura, M.D.	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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.7	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)

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 genetical apprasial, brain pathology through cerebral biopsies when indicated, post mortem obtained material, tissue culture as well as biochemical determination of content and composition of basic compounds and estimation of respective enzyme activities.

Project Description:

Objectives: The majority of chronic neurological disorders make their first manifestation during childhood. Because of the chronicity and long duration of the handicaps these diseases constitute a formidable medical and social problem of our times. Pooled 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 course of the respective disorders, 4) explore preventive measures such as eugenics and prenatal diagnosis.

Patient Material: Total of 22 inpatients and 36 outpatients were studied. Patients with the following disease categories were admitted for investigation in this order of frequency: mucopolysaccharidoses, sphingolipidoses, ceroid lipofuscinosis, various somatic hereditary syndromes and familial mental retardation.

Methods Employed:

1) Extended neurological, developmental and genetic assessment of the patients studied, including family study when appropriate.
2) Determination of profiles of lipids, amino acids, proteins, mucopolysaccharides and carbohydrates in blood, and when appropriate, in urine and cerebrospinal fluid.
3) Assay of enzyme activities in the peripheral blood leukocytes of genetic diseases studied; also, preparation of the karyotypes using banding technique.
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 histochemcial 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. This has rendered several substantial contributions. The most important are in two areas: one - determination of the content and composition of mucopolysaccharides in the body fluids and tissues of patients with different types of this disorder; two - demonstration of diverse ultrastructures in the brains and certain organs of these patients. 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 mucopolysacchridosis (MPS) of one of the following types: Type V, Scheie disease (MPS-V); type I, Hurler disease (MPS-I); and type II, Hunter disease (MPS-II). The principal new finding in the brain of the patient with MPS-V 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-V 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-V 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-V; 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-V, thus suggesting a similar enzymatic defect.

2) Histochemical and electron microscopic studies of the brains inclusive leptomeninges with large blood vessels from 7 patients with MPS I, II, IIIA and V 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 inclusive large blood vessels was 10.8, 6.5, 4.5 and 2.2 times greater in patients with MPS I, II, V 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) Therapeutic modification studies were conducted in selected patients with MPS type III, V and VI, using long-range administration of corticosteroids (alternate day dosages). The corticosteroid administration was associated with improvement of joint mobility and temporary slight amelioration of mental performance and behavior. The metabolic changes in mucopolysaccharides was evaluated by determination of polymeric and break-down products of urinary mucopolysaccharides accompanied administration of corticosteroids. In general, most of these patients showed a short lasting initial increase in the urinary mucopolysaccharides which was then followed by a sustained reduction. Also, variable changes in ratios of dermatan sulfate to heparan sulfate took place in the patients with MPS type II and V. These results are now ready for submission to a medical journal.

4) Concentrated efforts were made to introduce therapeutic modifications of selected inborn errors of metabolism. In an attempt to deliniate 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 phogocytic 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.

5) 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.

6) 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.

7) 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 causes of hereditofamilial ataxia currently being actively tested are pyruvate dehydrogenase deficiency, hexosaminidase variants, and other variants of the sphingolipidoses.

8) Development of an in vitro model of enzyme replacement is being studied. Currently being investigated are the usefulness of pulmonary macrophages and Kupffer cells isolated from Gaucher patients and sustained in culture.

9) 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: This is a broad and long range project of our Section. 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.

Publications:

1. Constantopoulos, G., Dekaban, A. S., Steusing, J. K., and Eldridge, R.: Content and composition of urinary glycosaminoglycans in the patients with myoclonus epilepsy with and without Lafora bodies. Acta Neurol. Scandivav. 56: 291-298, 1977.

2. Sakuragawa, N., Constantopoulos, G., and Dekaban, A. S.: The effects of corticosteroids on urinary excretion of glycosaminoglycans in the mucopolysaccharidoses. Cong. Anom. (Japan) 17: 513-524, 1977.

3. Sakuragawa, N., Constantopoulos, G., and Dekaban, A. S.: The effects of corticosteroids on plasma and leucocyte glycosaminoglycans in the mucopolysaccharidoses. Cong. Anom. (Japan) 17: 525-534, 1977.

4. Nishimura, R. N.: Atrial myoma with a potent foramen ovale. Lancet, 2: 1138, Nov. 26, 1977.

5. Furbish, F. S., Steer, C. J., Barranger, J. A., Jones, A. E., and Brady, R. O.: Uptake of native and desialylated glucocerebrosidase by rat hepatocytes and Kupffer cells. Biochem. Biophys. Res. Commun. 81: 1047-1053, 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 00815-18 DMN

PERIOD COVERED

October 1, 1977 through September 30, 1978

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

PI:	R. O. Brady	Chief	DMN	NINCDS
OTHER:	P. G. Pentchev	Biochemist	DMN	NINCDS
	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)

Weizmann Institute of Science, Rehovot, Israel
Tufts University Medical School, Boston, Massachusetts

LAB/BRANCH

Developmental & Metabolic Neurology Branch

SECTION

Enzymology and Genetics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.6	5.6	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, cerebrosides, gangliosides, 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 galactopychosine have been similarly prepared. Ceramide-trihexoside and ceramide tetra hexoside (globoside) specifically labeled with radioactive hydrogen-^{3}H 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) We have reported that 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 observed in two of three patients infused with purified human placental glucocerebrosidase. Patients with Gaucher's disease have subnormal activity of this enzyme in their tissues. The lack of change in blood glucocerebroside in the third recipient is most likely due to the fact that only 8% of the accumulated lipid was cleared from the liver in this patient whereas there was a 26% reduction in accumulated in liver glucocerebroside in the first two patients. The discrepancy in the percentage cleared was due to the fact that the third patient had 24 times more glucocerebroside in her liver than the first and 11 times more than the second. We have devised a method for the purification of glucocerebrosidase on a large scale in a form that is suitable for administration to humans. Enzyme replacement trials in Gaucher's disease are underway at this time with the preparation. (2). Sedation and lowering the body temperature of animals injected with human placental glucocerebrosidase dramatically prolongs the presence of the exogenous enzyme in the animal tissues. This observation has potential importance for enzyme replacement in humans if it can be shown that the exogenous enzyme is catalytically active over the extended period of time. We are currently devising experiments to provide this information. (3) We have found that altering the terminal carbohydrate moiety of glucocerebrosidase, a glycoprotein, dramatically alters its fate in the circulation. Thus, by removing the terminal molecule of N-acetylneuraminic acid (sialic acid) the half-time in the blood stream after intravenous injection is reduced from 22 to 1.2 minutes. Subsequent removal of the exposed

terminal molecule of galactose does not affect the half-life but directs a larger portion of the enzyme to reticuloendothetial cells, the cite of glucocerebroside storage. We propose to examine the effect of these modified enzyme preparations in patients with Gaucher's disease.

(4) 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, we discovered that the administration of the compound known as AY-9944 to rats causes a dramatic reduction in the amount of sphingomyelinase in the liver and other organs in the recipients. The reduction in enzymatic activity is accompanied by an increase in tissue sphingomyelin, and Niemann-Pick like inclusion bodies appear in retinal neurons, an important pathological correlate of the disease in humans. AY-9944 does not affect the catalytic activity of the enzyme. The reduction of sphingomyelinase in the animal model is specific and it is caused by a decrease in the production of this enzyme. Thus, the pharmacologically induced animal model has all of the requirements for an examination of the effect of enzyme replacement on the biochemical and pathological consequences of sphingomyelinase deficiency. We propose to use this animal model to explore the effect of enzyme replacement therapy in this model experimental Niemann-Pick disease.

(5) We have improved our procedure for the purification of ceramide trihexosidase, the enzyme lacking in patients with Fabry's disease, also from placental tissue. However, we have yet to overcome difficulties with pyrogenicity of this enzyme and have not undertaken further replacement trials in Fabry's disease because of this impediment.

(6) 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. Moreover, we have shown that exogenous enzyme is readily taken up by neuronal cells in vivo a critical prerequisite for any serious consideration of enzyme replacement therapy in metabolic disorders that cause nerve cell damage.

(7) We continue to serve as a center for the diagnosis of patients and detection of carriers for all of the lipid storage diseases. Much effort is devoted to the monitoring of pregnancies at risk for heritable metabolic disorders. During the past year, we performed more than 200 diagnostic assays for physicians and genetic counselors from all over the world.

Significance: Enzyme replacement appears to offer considerable promise for the treatment of Gaucher's disease and potentially Fabry's disease. It is expected that the deleterious clinical course in these patients will be ameliorated by this form of therapy. The newly developed 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, when it becomes feasible in Fabry's disease, and mannosidosis. 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 delivery exogenous enzymes in a clinically useful fashion.

Publications:

1. Barranger, J.A., Pentchev, P.G., Rapoport, S.I.and Brady,R.O.: Augmentation of brain lysosomal enzyme activity following enzyme infusion with concomitant alteration of the blood-brain barrier. Trans. Am. Neurol. Assn. 102: 1-3, 1977.
2. Brady, R. O.: Disorders of glucocerebrosides. In Goldensohn, E. S. and Appel, S. H. (Eds.): Scientific Approaches to Clinical Neurology Philadelphia, Lea and Febiger, 1977, pp. 140-143.
3. Brady, R. O.: Disorders of sphingomyelin. In Goldensohn, E. S. and Appel, S. H. (Eds.): Scientific Approaches to Clinical Neurology. Philadelphia, Lea and Febiger, 1977, pp. 144-147.
4. Brady, R. O.: Hematological aspects of lipid storage diseases. Acta Haemat. Pol., VIII: 103-112, 1977.
5. Brady, R. O.: Perspectives therapeutiques actuelles dans les molodies par deficit enzymatique dumetabolism lipidique. Cahiers Medicanx Tome 3, No. 21; 1207-1208, 1978.
6. Brady, R. O.: Glucosyl ceramide lipidoses: Gaucher's disease. In Stanbury, J. B., Wyngaarden, J.B. and Fredrickson, D. S. (Eds.): The Metabolic Basis of Inherited Disease, 4th edition, New York, McGraw-Hill, 1978, pp. 731-746.

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10. Pentchev, P. G.: Enzyme replacement therapy in Gaucher's disease and Fabry's disease. Ann. Clin. Lab. Sci. 7: 251-253, 1977.

11. Pentchev, P. G., Brady, R. O., Gal, A. E., and Hibbert, S. R.: The isolation and characterization of sphingomyelinase from human placental tissue. Biochim. Biophys. Acta 488: 312-321, 1977.

12. Pentchev, P. G., and Barranger, J. A.: Sphingolipidoses: molecular manifestations and biochemical strategies. J. Lipid. Res. 19: 401-409, 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 01026-16 DMN

PERIOD COVERED
October 1, 1977-September 30, 1978

TITLE OF PROJECT (80 characters or less)

Treatment of Epilepsy--Clinical and Biochemical Study

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

PI:	Anatole S. Dekaban, M.D., Ph.D.	Associate Chief	DMNB NINCDS
Other:	Michael P. Whyte, M.D.	Clinical Associate	DMNB NINCDS
	Daniel E. Britton, M.D.	Clinical Associate	DMNB NINCDS
	Jan K. Steusing	Research Assistant	DMNB NINCDS

COOPERATING UNITS (if any) Department of Psychology, University of Washington, (in respect to performance testing only).

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This project has been terminated and no work has been done on it.

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 01309-13 DMN

PERIOD COVERED October 1, 1977 through September 30, 1978

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
	T. Pacuszka, Ph.D., Visiting Fellow	DMN	NINCDS
	M. Omeda-Sale, Ph.D., Visiting Fellow	DMN	NINCDS
	J. Hagmann, M.D., Visiting Fellow	DMN	NINCDS
	J. B. Parent, Ph.D., Guest Worker	DMN	NINCDS

COOPERATING UNITS (if any)

Department of Immunology, Walter Reed Army Institute of Research
Muscular Dystrophy Association of America

LAB/BRANCH

Enzymology and Genetics

SECTION

Developmental & Metabolic Neurology Branch

INSTITUTE AND LOCATION

NINCDS, IRP, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.1	3.1	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)

The cell surface receptor for cholera toxin is the ganglioside G_{M1}. This has been shown in all vertebrate cells examined to date including fat cells which are very sensitive to the toxin but have only trace amounts of G_{M1}. Cholera toxin is multivalent and binding to more than one G_{M1} receptor on the cell surface is a necessary step for the subsequent activation of adenylate cyclase by the toxin. Although there are similarities between cholera toxin and the hormones thyrotropin and gonadotropin, which also activate adenylate cyclase, the specific determinants for hormone binding appear to reside on membrane proteins in the respective target cells. The interaction of these hormones with their receptors may be influenced by membrane phospholipids and the internalization of the hormonal signal may be mediated by membrane gangliosides. When avian myoblasts fuse in culture to form myotubes, the cell membranes undergo coordinate series of complex changes. These include the appearance of certain hormone receptors and a dramatic alteration in membrane gangliosides. Thus, gangliosides in addition to their demonstrated function as toxin receptors may potentially be involved in hormonal regulation and cell differentiation.

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 [^{14}C]-N-acetylneuraminyl-lactosyl-ceramide ([^{14}C]-G_{M3}) is synthesized from lactosylceramide and CMP-[^{14}C] sialic acid with the specific sialyltransferase activity.

Binding of toxins and hormones to cells, cell membranes and liposomes is determined with [^{125}I]-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 exchange and gel filtration chromatography. Purity and composition is assessed by thin layer and gas liquid chromatography.

Major Findings:

A. Interaction of Cholera Toxin with Gangliosides

1) Continuing our studies on the role of ganglioside G_{M1} as the cholera toxin receptor on cell membranes, we were able to demonstrate the presence of G_{M1} in rat adipocytes. Although these fat cells are extremely sensitive to cholera toxin which causes lipolysis, other laboratories were not able to detect G_{M1} in these cells and thus questioned the concept that G_{M1} is the natural receptor for the toxin. Using a variety of sensitive techniques, we were able to detect the ganglioside. These included: i) a bioassay using G_{M1}-deficient cells that are unresponsive to cholera toxin; ii) labeling the fat cell membranes with galactose oxidase and sodium borotritide and showing that cholera toxin protected the G_{M1} from being labeled;

iii) converting the labeled gangliosides to oligosaccharides and precipitating the cholera toxin-oligosaccharide complex with antibodies to the toxin; and iv) identifying the labeled oligosaccharide by paper chromatography. The results will be published in the Journal of Biological Chemistry and we received a letter of appreciation from the editors for clarifying the nature of the toxin receptor.

2) We have recently demonstrated that cholera toxin has lectin-like properties and can agglutinate liposomes and erythrocytes containing G_{M1}. These observations are consistent with our previous studies indicating that the toxin is multivalent.

3) Ongoing work is now being directed at establishing that multivalent attachment of cholera toxin to several G_{M1} molecules on the cell surface is essential for its subsequent activation of adenylate cyclase. Cells (HeLa, human lymphocytes) with a low number of toxin receptors per cell bind cholera toxin and respond as measured by increased adenylate cyclase activity. When lateral mobility of membrane components is prevented by incubating the cells with the toxin on ice, addition of the oligosaccharide moiety of G_{M1} displaces the bound toxin and blocks the activation of adenylate cyclase after the cells are warmed up to 37°C. This inhibitory effect of oligosaccharide is not observed with neuroblastoma N-18 or Friend erythroleukemic cells which have a high number of toxin receptors. Toxin bound to these cells at 4°C is not displaced by the oligosaccharide and adenylate cyclase activation is not blocked. The number of toxin receptors can be increased in HeLa cells by incubating the cells in medium containing G_{M1} or sodium butyrate which induces G_{M1} synthesis in HeLa cells. In these treated cells, binding of cholera toxin can not be displaced and activation of adenylate cyclase prevented by the oligosaccharide. These observations are consistent with multivalent binding of the toxin to several G_{M1} receptors as an essential step in its mechanism of action.

B. Mechanism of Action of Glycoprotein Trophic Hormones

1) Previous studies indicated certain similarities between the action of cholera toxin and certain hormones such as thyrotropin (TSH) and gonadotropins (hCG and LH). We have utilized the phenomenon of down regulation to explore the mechanism of action of hCG. When male rats are administered a high dose of hCG, their testes rapidly lose the ability to bind hCG. Although rat testes contain an unusually large amount of complex gangliosides, there is no apparent change in ganglioside content following down regulation. These and other observations indicate that gangliosides are not directly involved in the binding of gonadotropins to their target cells. We are examining the down regulation of TSH receptors in thyroid cells and are attempting to develop model systems to elucidate the role of gangliosides in the transmembrane signalling of hormonal messages.

2) We have recently observed that certain phospholipids isolated from bovine thyroid glands are potent inhibitors of TSH binding to thyroid membranes. Preincubating the membranes with these phospholipids reduces the amount of TSH that binds whereas preincubating the membranes with phospholipase A increase the amount of TSH that binds to the membrane. These results indicate that membrane phospholipids influence the state of the TSH receptor and its ability to interact with the hormone.

C. Membrane Biochemistry of Avian Muscle Cells During Myogenesis.

We are investigating the biochemistry of the plasma membranes of quail embryo myoblasts as they fuse to form myotubes in culture. Preliminary experiments indicate that the number of receptors for certain hormones (insulin and epinephrine) increase during cell fusion whereas those for cholera toxin decrease. In addition, during this same time period which lasts only a few hours, there are striking changes in the ganglioside pattern of the muscle.

Significance: These studies are providing information on the function of membrane glycosphingolipids. Gangliosides serve as receptors for cholera toxin activates adenylate cyclase. Gangliosides potentially may function as biotransducers for other hormones.

Proposed Course: The project will be continued with emphasis placed on the influence of membrane phospho- and glycolipids on the state of hormone receptors. Further work will be done on the relationship between biochemical changes in the muscle cell membrane and the myogenic process.

Publications:

1. Fishman, P. H., Bradley, R. M., Brown, M. S., Faust, J. R., and Goldstein, J. L.: Similar content of phospholipids and gangliosides in normal and homozygous familial hypocholesterolemia fibroblasts. J. Lipid Res. 19: 304-308, 1978.

2. Fishman, P. H., Bradley, R. M., Moss, J., and Manganiello, V. C.: Effect of serum on ganglioside uptake and choleragen responsiveness of transformed mouse fibroblasts. J. Lipid Res. 19: 77-81, 1978.

3. Fishman, P. H., Moss, J., and Osborne, J. C.,Jr: Interaction of choleragen with the oligosaccharide of ganglioside G_{M1}: evidence for multiple oligosaccharide binding sites. Biochemistry 17: 711-716, 1978.

4. Pacuszka, T., Osborne, J. C., Jr., Brady, R. O., and Fishman, P. H. Interaction of human chorionic gonadotropin with membrane components of rat testes. Proc. Natl. Acad. Sci. USA. 75: 764-768, 1978.

5. 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 choleragen. J. Biol. Chem., in press.

6. Pacuszka, T., Duffard, R. O., Nishimura, R., Brady, R. O. and Fishman, P. H.: Biosynthesis of bovine thyroid gangliosides. 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 01457-12 DMN

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
The Chemical Synthesis of Radioactive Sphingolipids

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, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

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)

Radioactive isotopes were synthesized and used for metabolic studies and as diagnostic tools in sphingolipidoses. The labelings were made by ^{14}C and tritium by gas exposure, synthetic and semi-synthetic techniques and a new approach: Functional group exchange.

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 predetermined 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 are complex. Also this type of compound require more elaborate enzyme assays.

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 therpeutic 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.

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 of functional group exchange.

Publications:

See Project Nos. ZO1 NS 00815-18 DMN and ZO1 NS 00816-18 DMN.

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 01480-11 DMN

PERIOD COVERED
October 1, 1977 through September 30, 1978

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 & Metabolism Section, DMN NINCDS
OTHER: A. A. Benson, Assoc.Director,Scripps Inst. Oceanography
S. Patton, Borland Prof., Div. Food Science, Penn. State Univ.
P. Gilbert, Director, Mote Marine Lab.

COOPERATING UNITS (if any)
Scripps Inst. Oceanography, LaJolla, Ca.
Div. Food Sci., Penn State Univ., University Park, Pa.
Mote Marine Lab., Sarasota, Florida

LAB/BRANCH
Developmental and Metabolic Neurology Branch, DMN

SECTION
Physiology and Metabolism

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (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, we 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, bioelectrogenesis in the electric eel and CNS degeneration in the spawning Pacific salmon. During the past year we have developed an improved technique for the preparation of milk fat globule membranes and molecular constituents thereof. In collaboration with Prof. Patton the effect of membrane antibodies on membranogenesis and excretion was investigated. We continued our work on membrane development in unicellular eukaryotes. Specifically we studied the distribution and development of membrane ecto-ATPases and ecto-5'-nucleotidases in developing sea urchin embryo, acanthameba and the symbiotic dinoflagellate, zoozanthella. While all eukaryotes exhibit ecto-ATPase activity, apparently only those who participate in syncytic organization possess ecto-5'-nucleotidase.

Project Description:

Objectives: To exploit for the study of neurobiology the great variety and abundance and the biochemical specialization of oceanic life forms. 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, substantial interest has been shown 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.

Methods: The primary mission of the Section on Physiology and Metabolism, is to prove into the molecular basis of bioelectrogenesis and neurochemical transmission. In the exploration of marine organisms we have therefore made primary use of model systems which allowed a facile interrogation of plasma membranes, or provided us with fairly simple CNS structures (as in elasmobranchi), or seemed to offer an analogy to certain diseases of the central nervous system in man. The laboratory methodology is essentially classical but modified for the species under investigation. Occasionally more unusual methods were required e.g. in order to maintain a salmon heart beating for several hours on a shipboard laboratory or to obtain EEG tracings from free swimming elsmobranchi.

Major Findings: The emphasis of this project during the reporting period was on the comparative biochemistry of plasma membrane ecto-enzymes. Recent studies in our laboratory had indicated that ecto-ATPases functioned in the modulation of cellular permeability while ecto-5'-nucleotidases appeared to mediate cell-cell recognition phenomena. Our observations on unicellular eukaryotes showed that such organisms (developing sea urchin embryo, amoebas, dinoflagellates) lacked ecto-5'-nucleotidase as long as there was no need for cell-cell interaction, while ecto-ATPase was present. The characteristic activation pattern of several of the ecto-enzymes were studied and found to be similar to those observed in complex organisms. Kinetics were also similar to those found in vertebrates. Current investigations probe the possible role of ATP as a transmitter or membrane modifyer in acanthamoeba.

The presence of ecto-enzymes was originally observed by us during experiments with intact milk fat globules and milk fat globule membranes. It is now widely accepted that milk fat globule membranes are identical in composition to mammary gland epithelial membrane. In collaboration with Prof. Patton we have continued our investigations of the role of plasma membrane enzymes in milk secretion. It was shown that antibodies could be produced to purified milk fat globule membrane 5'-nucleotidase by affinity chromatography and the production of antibodies to the purified membrane enzyme is under way.

We have joined with Prof. Benson and his staff in a pilot study on mucus production on the cell surface in pelagic organisms. We are also participating in a study conducted by this team on the uptake and utilization of arsenic by marine algae. In sea water which is very poor in nutrients, the concentration of P and As are equivalent (about 10^{-6}M) and As is utilized by algae for phospholipid synthesis. These compounds eventually enter the food chain, but virtually nothing is known about the long term toxicity of such materials.

Proposed course: We shall extend our investigations on ecto-enzymes in unicellular eukaryotes to the nervous system of invertebrates. In addition we shall make a pilot study of the role of insecticides in nerve function. This study will be conducted jointly with the Environmental Protection Agency who found that nerve Ca^{2+}-ATPase was inhibited by DDT. If chlorinated hydrocarbons can function as ATPase inhibitors we might use this to discriminate between Ca^{2+}-transport ATPases and ecto-ATPases.

Publications

1. Trams, E. G. and Lauter, C. J.: A comparative study of brain Ca^{2+}-ATPases. Comp. Biochem. Physiol. 59B: 191-194, 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 01481-11 DMN

PERIOD COVERED
October 1, 1977 through September 30, 1978

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,	DMN, NINCDS
	C. J. Lauter, Chemist	DMN, NINCDS
	N. Salem, Staff Fellow	DMN, NINCDS
	M. Rajacic-Stojanov, Visiting Associate	DMN, NINCDS

COOPERATING UNITS (if any)
NONE.

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Physiology and Metabolism

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	3.3	0.3

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 objective of this project is to elucidate the inter-relationship between molecular composition and topographic arrangements of membrane building blocks with reference to plasma membrane functions. Bioelectrogenesis, transport and other metabolic phenomena are based on the proper function of characteristic membrane subassemblies. Plasma membranes are studied after isolation from tissues or in intact cells of CNS derived tissue cultures. In particular the function of membrane ecto-enzymes has been the objective of this study. Experiments on seizure-prone animals indicate that malfunctioning ecto-enzymes in the brain are related to the biochemistry of convulsive disorders. The biochemical lesion in animal models of idiopathic epilepsy seems to be associated with a derangement of nucleotide metabolism in glia cells. Molecular probes are used to characterize individual membrane molecules. Synaptosome-like vesicles enveloped in plasma membrane are exfoliated from cells and appear to serve transport function.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To elucidate what role the molecular composition and topographic arrangement of membrane building blocks play in defining the physiological functions of the plasma membrane. Furthermore, to inquire if cell pathology in certain diseases of the CNS is associated with a derangement of function of known membrane components.

Methods: One of the primary tools used in this investigation is a stock of cultured cell lines which originated from a variety of CNS cells. Established clones of murine and human neuroblastoma, gliomas as well as several primary cell cultures developed in our laboratory are employed. Primary use of these cultures is in the study of cell surface biochemistry.

A variety of animal models for idiopathic epilepsy have been employed in experiments which were designed to delineate molecular differences between seizure-prone and control animals. Mice of several strains, chicken, hamsters and grasshopper mice were used in this project. Covalently binding reagents are used to label plasma membrane constituents in situ and the effect of the reagents on membrane enzymes and lipids is investigated. These molecular probes are used to track membrane molecules through isolation procedures or determine the microenvironment of membrane proteins in terms of lipid composition.

Major Findings: In the preceding reporting period we described the existence of a deficient Ca^{2+}-stimulated ATPase in brains of seizure prone mice. Similar derangements in adenine nucleotide metabolism were found in other animal models of epilepsy. We have now observed a significant deletion of ecto-ATPase in cultured glia cells raised from neonatal, seizure-prone mice (DBA). Ten separate glia cell cultures were obtained from explants of newborn mouse brains, five from DBA mice and five from C57 (control) mice. Assays for ecto-ATPase and several other membrane enzymes (as controls) were performed on intact monolayer cultures which were superfused with the appropriate substrates. Comparison of the ecto-ATPase activities as a function of cell density revealed a large difference between the two cell lines during the proliferative phases of the cultures in the early passages. When growth rates were markedly diminished, however, (i.e. in the stationary phase) the ecto-ATPase activity of the epileptic glia line had reached about 85% of that of the controls. This seemed to be consistent with earlier findings that the susceptibility of DBA mice to audiogenic seizures dissappeared during the later stages of brain maturation. We propose that the temporary insufficiency of brain Ca-ATPase in the DBA mouse was due to the delayed expression of a more mature ATPase isoenzyme. Some form of differentiation also takes place when cell layers become confluent and growth subsides, therefore it appears that also in culture, the activity pattern of ecto-ATPase

resembles the differentiating pattern in the animal. Thus, we have shown that the deficiency of Ca^{2+}-ATPase, found earlier in seizure-prone animals, was present in an amplified form in cultured astroblasts from the same strain. The ATPase incompetency observed in membrane enriched homogenates, therefore was associated with a lesion in ecto-enzyme activity of the plasma membrane; moreover, it appears to be a property of the glial elements of the afflicted brain. These results further suggest that a key to the metabolic error in convulsive disorders may be found in the modulating action of ATP on membrane excitability thresholds and the interrelated function of plasma membrane ecto-ATPase.

We have also begun an investigation of the in situ association of membrane proteins, such as the ecto-enzymes, with the phospholipids of the membrane. To this purpose we have observed the effects of covalently reacting compounds on membrane proteins and lipids in living cells. Monolayer cultures were treated with either difluorodinitrobenzene (DFDNB, a cross-linking reagent) or with fluorodinitrobenzene (FDNB) or trinitrobenzenesulfonic acid (TNBS); both of the latter are monofunctional reagents. These reagents readily form additional compounds with primary amines. Varying concentrations of the reagents were superfused onto monolayer cultures under iso-osmotic conditions at pH 8.1. After removal of the reaction mixtures the activity of ecto-ATPase, ecto-5'-nucleotidase and cholinesterase were determined. We have found that ecto-ATPase was selectively inhibited by 0.05 mM DFDNB while ecto-5'-nucleotidase was inhibited only at substantially higher concentrations of both DFDNB and TNBS.

Acetylcholinesterase on the other hand required in excess of 1 mM or 5 mM DFDNB or FDNB respectively for inhibition. Comparative experiments with other cell lines gave the same results, which implied that the observed molecular interactions were specific. Both the ATPase and 5'-nucleotidase could be protected from inhibition by DFDNB if substrates or substrate congeners were superfused onto the cells before addition of reagent. We concluded that the protective effect obtained by the addition of substrate also revealed a specific interaction with these reagents. We plan to exploit this for the biochemical characterization of ecto-ATPase and ecto-5'-nucleotidase.

We have made further studies on the functions of these two ecto-enzymes. Working with a variety of cultured cell lines we have found that ecto-enzymes were released into superfused medium. The released enzymes are exfoliated in the form of synaptosome-like particles with an average diameter of about 1,000 nm. They contain smaller vesicles with a diameter of about 500 Å. We have referred to the larger particles as exosomes. The exosomes are constituted of plasma membrane vesicles and the membrane encloses the exosome right-side-out. The production of the particles has been observed in a variety of neoplastic and non-transformed cell lines. We suspect that the primary function of the exosomes is transport of cell growth factors, or similar substances

from one type of cell to another, e.g. from a glia cell to a neuron. Preliminary experiments have indicated that exosomes from one cell may be recognized by a heterologous cell and that ecto-5-nucleotidase may play an important role in the transfer of substances carried by the exosomes.

Proposed Course: We will pursue the different aspects of this project in accordance with the most promising leads. Priority will be accorded to experimentation with biocatalysts on the cell surface. We will, in particular, extend our investigations on the so-called exosomal particles which we have recently discovered. Initial experiments will be directed to establish the nature of the exosomes and to exclude the possibility that we are dealing with artifacts peculiar to cultured cells. We will also exploit the leads offered by the results with the cross-linking studies. Here our aim shall be to obtain a more precise description of the molecular composition of the cell surface. Further research on animal models of epilepsy is planned with the aim to uncover a common denominator with respect to lesions of nucleotide metabolism and convulsive disorders.

Publications:

1. Rosenblatt, D. E., Lauter, C. J., Baird, H. R. and Trams, E. G.: ATPases in animal models of epilepsy. J. Mol. Med. 2: 137-144, 1977.

2. Trams, E. G.: On the asymmetric composition of plasma membranes. In: Bazan, N., Brenner, R. and Giusto, N. M. (Eds.) Function and Biosynthesis of Lipids. Academic Press, New York, 1977, pp. 153-173.

3. Duffard, R. O., Fishman, P. H., Bradley, R. M., Lauter, C. J., Brady, R. O. and Trams, E. G.: Ganglioside composition and biosynthesis in cultured cells derived from CNS. J. Neurochem. 28: 1161-1166, 1977.

4. Marangos, P. J., Goodwin, F. K., Parma, A., Lauter, C. and Trams, E. G.: Neuron specific protein (NSP) in neuroblastoma cells: Relation to differentiation. Brain Res. 143: 49-58, 1978.

5. Trams, E. G. and Lauter, C. J.: Ecto-ATPase deficiency in glia of seizure-prone mice. Nature 271: 270-271, 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 01808-09 DMN

PERIOD COVERED
October 1, 1977 through September 30, 1978

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	DMN	NINCDS
OTHER:	R. O. Brady, Chief	DMN	NINCDS
	L. J. McIntyre, Visiting Fellow	DMN	NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Developmental & Metabolic Neurology Branch, NINCDS

SECTION
Myelin and Brain Development

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	2.1	0.6

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 major protein in myelin of the peripheral nervous system is a glycoprotein. Myelin purified from the central nervous system also contains a glycoprotein, but it is quantitatively a minor component of the isolated myelin. This CNS glycoprotein appears to be selectively concentrated in membranes which are transitional between compact myelin and the oligodendroglial surface membrane. We are purifying this glycoprotein which is in the myelin-oligodendroglial complex so that its chemistry and immunogenicity can be studied. This glycoprotein undergoes a chemical change in developing rat brain which involves the polypeptide chain or carbohydrate moieties. Since glycoproteins are known to be involved in recognition and contact phenomena, we are investigating its role in developing brain as the oligodendroglial surface membrane makes contact with axons and is spiraled and compacted to form mature myelin. Glycoproteins are also known to be cell surface antigens and receptors for viruses. Therefore, we are investigating the possible role of the myelin-associated glycoprotein in the autoimmune or infectious aspects of multiple sclerosis and other demyelinating diseases.

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 subfractioned 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. Animals are immunized with the glycoprotein, and antibodies are detected by a radioimmunoassay utilizing a [^{3}H]fucose-labeled test antigen and anti-IgG serum.

Major Findings: The emphasis has continued to be on the isolation and characterization of the major myelin-associated glycoprotein (MAG). The utility of the simple extraction procedure involving lithium diiodosalicylate (LIS), which was first reported last year, has been explored further. When this technique is applied to adult rat myelin it results in a highly selective extraction of MAG. We have now found that the technique is also effective for extracting the slightly larger form of MAG from immature myelin (see below) and for isolating MAG from human myelin. Although the preparations obtained by this very simple procedure are pure enough for some purposes, they do contain small and variable amounts of other proteins. These trace contaminants can be removed by preparative polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate to give a homogeneous preparation of MAG. However, for achieving this final purification we are now exploring column techniques which do not involve the highly denaturing detergent, sodium dodecyl

sulfate, and which would be more convenient for purifying large amounts of MAG.

Antisera to MAG was prepared by injecting the highly purified protein, obtained by LIS extraction and preparative gel electrophoresis, into a rabbit. The radioimmunoassay which we developed, utilizing (^{3}H) fucose-labeled MAG, indicated that the rabbit developed a high titer of antibodies to this glycoprotein. This antisera is currently being used by the Section on Cellular Neuropathology (LNNS) to localize MAG in tissue sections by immunohistochemistry. Preliminary results indicate a highly specific staining of oligodendrocytes and myelin. We are currently evaluating the specificity of this antisera from the biochemical point of view. We have also studied additional rabbits immunized with whole myelin, and confirmed the preliminary finding reported last year that rabbits given experimental allergic encephalomyelitis by this procedure develop antibodies to MAG.

We have continued to carry out experiments to elucidate the chemical reason for the developmental decrease in the mol. wt. of rat MAG which correlates well with the progress of myelinogenesis in this species. Trypsin treatment of purified myelin fragments cleaves both the mature and immature forms of MAG to a molecule with mol. wt. of 80,000 and in the process eliminates the developmental difference. By contrast, trypsin treatment of isolated MAG under the same conditions degrades the molecule to very small mol. wt. peptides. These findings suggest that when MAG is in its normal position in the myelin membrane, the 80,000 mol. wt. portion is protected from proteolytic degradation. They also indicate that the difference between the mature MAG (mol. wt.100,000) and the immature MAG (mol. wt. 90,000) resides in the part of the molecule which is susceptible to proteolysis. Taken together with our previous findings, that there appears to be little difference between the oligosaccharide moieties of the mature and immature glycoproteins (see last years' report), these results suggest that the larger immature glycoprotein may be converted to the mature form by limited proteolysis during myelinogenesis. We are currently comparing peptide maps of the mature and immature MAG in an attempt to obtain direct evidence for this hypothesis.

Our studies on the interactions of myelin and myelin-glycoproteins with various lectins have been completed and are being prepared for publication. There are substantial species differences with regard to the capacity of purified myelin to be agglutinated by the lectins. Concanavalin A and two galactose-specific lectins strongly agglutinate mature and immature rat myelin, but only weakly agglutinate bovine and human myelin. A glucosamine-specific lectin weakly agglutinated rat and bovine myelin, but not human myelin,whereas fucose-specific lectins did not agglutinate any myelin fractions. Binding of (^{3}H) Con-A to myelin glycoproteins after they have been separated on SDS gels indicated that MAG is one of the major Con A-binding proteins in rat, bovine and human myelin. This technique provides a very

sensitive method for detecting myelin glycoproteins in species in which in vivo labelling procedures with radioactive sugar precursors cannot be used. MAG is also a major Con-A binding protein in immature rat myelin, although the relative importance of other glycoproteins in binding Con A was increased relative to mature myelin. Solubilized rat MAG bound to mannose-, galactose, N-acetylglucosamine-, and fucose-specific lectins which had been immobilized on agarose, providing a useful step in its isolation. All of these studies indicate that MAG is probably a principal lectin receptor on the surface of myelin and other oligodendroglial membranes.

The subcellular fractionation technique which was developed for isolating oligodendroglial-derived membranes, which are enriched in MAG, the major Wolfgram protein, and 2'3'-cyclic nucleotide phosphohydrolase from rat CNS, was shown to give similar results with guinea pig spinal cord. This fractionation technique was then applied to Strain 13 guinea pigs with acute or chronic EAE obtained from the Immunology Section (OSD, NIAID). The purpose of these experiments was to determine if the composition of myelin or other oligodendroglial membranes was being altered in these autoimmune demyelinating diseases which were induced by injection of isologous spinal cord. Although the fractions are still being analyzed, we have found some interesting changes in chronic EAE whereas the fractions from animals with acute EAE appear to be the same as those from controls. In chronic EAE, the yield of myelin and some of the other myelin related fractions was decreased slightly. However, the most interesting finding so far is that the content of myelin basic protein in the whole tissue and in purified myelin is significantly decreased while other myelin proteins are unchanged, implying a specific degradation of myelin basic protein in chronic EAE.

We have begun an investigation of glycoprotein synthesis in myelinating explants of mouse cerebellum with the hope of using this system as an *in vitro* model in which to study the role of glycoproteins in myelinogenisis. These cultures very actively incorporate radioactive fucose into glycoproteins. About two-thirds of the labeled glycoproteins are in the explant itself, whereas one-third are released into the culture media. Polyacrylamide gel electrophoresis revealed that the glycoproteins in the explants are very similar to those synthesized *in vivo* after intracranial injection of the labelled fucose. Interestingly, the glycoproteins released into the media are different from those staying in the explant, being enriched in small mol. wt. components. When we attempted to isolate fucose-labelled myelin from the cultures by adding unlabeled cerebellum as carrier, the labelled glycoproteins in the myelin fraction were heterogeneous and not enriched in MAG. At this time, we do not know if this is because the myelin formed by the cultures does not have MAG, orif the culture myelin is not isolated with the standard myelin fraction.

The biochemical analyses of Xenopus myelin has been extended to tadpole brain which developmentally corresponds more closely to the tadpole optic nerve used in the Section on Cellular Neuropathology (LNNS, NINCDS) as a test system for demyelinating agents. In general, the results are similar to those reported last year for mature Xenopus myelin and support the appropriateness of the tadpole optic nerve as test system for demyelinating agents acting on human myelin. Purified tadpole brain myelin had a large amount of high mol. wt. proteins in comparison to mature Xenopus myelin but did contain basic protein and proteolipid. An interesting difference, found both for mature Xenopus and tadpoles, in comparison to mammals is a two to three-fold higher specific activity for 2'3'-cyclic nucleotide phosphohydrolase in the purified myelin fraction. Quantitative lipid analyses of mature and immature Xenopus myelin are in progress.

Significance: The probable localization of the major myelin-associated glycoprotein of the CNS in oligodendroglial derived surface membranes, as suggested by the subfractionation and surface probe studies, has important implications for processes of myelination and demyelination. In this localization the glycoprotein would be accessible for interactions with other cells and with pathological agents such as antibodies and viruses. With regard to myelin formation, there is considerable evidence in the literature indicating that cell surface glycoproteins are involved in recognition phenomena and in specific interactions between cells. The myelin-associated glycoproteins of the CNS could be involved in interactions between oligodendroglial and axonal membranes or between different layers of myelin as they are spiraled and compacted. Many demyelinating diseases such as multiple sclerosis are believed to involve autoimmune and/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 demyelinating diseases. Our demonstration that this glycoprotein is highly antigenic, and particularly the finding that rabbits with experimental allergic encephalomyelitis induced with whole myelin have antibodies directed against the glycoprotein, enhance this possibility. For example, in multiple sclerosis a viral induced change in the sugars on the glycoprotein could cause it to be recognized as a foreign antigen and subject to autoimmune attack. For these reasons, information about the chemistry immunogenicity, and localization of myelin-associated glycoproteins will increase our understanding of the molecular mechanisms of myelinogenesis and demyelination. Development of the convenient LIS-phenol procedure for its isolation is a major step toward obtaining more information of this type.

The finding that myelin basic protein is specifically reduced in the whole tissue and in the myelin fraction of Strain 13 guinea pigs with chronic EAE is an important advance in understanding the mechanism of chronic demyelination in this condition which has been suggested to be a better model for multiple sclerosis than the usual acute EAE. The potential role of proteases in autoimmune demyelinating diseases is a subject of considerable interest in many laboratories at this time.

Proposed Course: Alternative procedures to preparative polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate will be developed to remove the minor contaminants from the relatively pure MAG preparation which we can now obtain readily by the LIS-phenol procedure. We will also isolate and characterize fragments of the glycoprotein prepared by proteolytic or chemical cleavage. These smaller molecules may be easier to work with than the intact glycoprotein. In these studies, emphasis will be placed on elucidating the chemical cause for the developmental change in molecular weight. Carbohydrate and amino acid analyses will be done on the intact glycoprotein and fragments with the ultimate objective of determining its overall molecular structure.

Full advantage will be taken of the antisera which we have now prepared to MAG. Absorption and inhibition studies will be carried out to determine its specificity and the nature of the antigenic sites. The immunohistochemical studies in collaboration with LNNS will be continued at both the light and electron microscope level to more precisely localize MAG in myelin and oligodendroglial membranes. The immunohistochemical staining obtained with antisera to MAG will be compared with that obtained for other myelin constituents. The antisera to MAG will also be tested for demyelinating activity on cultured brain explants. These experiments are designed to determine if the demyelinating factor in the serum of multiple sclerosis patients or animals with experimental demyelinating diseases such as EAE is an antibody to the glycoprotein. Finally, after immunological procedures are adequately worked in experimental animals, multiple sclerosis patients will be tested for humoral or cellular immunity to the glycoprotein.

Publications:

1. McIntyre, R.J., Quarles, R. H., Webster, H. deF., and Brady, R.O.: Isolation and characterization of myelin-related membranes. J. Neurochem. (in press)

2. McIntyre, L. J., Quarles, R. H. and Brady, R. O.: Regional studies of myelin-associated glycoprotein in rat central nervous system. Brain Res. (in press)

3. 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., (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, (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 02024-06 DMN

PERIOD COVERED
October 1, 1977-September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cerebral Strokes in Patients with Primary Hyperlipoproteinemia.

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

PI:	Anatole S. Dekaban, M.D., Ph.D.	Associate Chief	DMN	NINCDS
Other:	Jan K. Steusing	Research Assistant	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 20014

TOTAL MANYEARS:	PROFESSIONAL:	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)

This project has been terminated and no work has been done.

PHS-6040
(Rev. 10-76)

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 02162-04 DMN

PERIOD COVERED
October 1, 1977 through September 30, 1978

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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Compounds were synthesized which are glycolipid analogs and which yield chromophoric moiety upon enzymatic hydrolysis. These compounds are used in enzymatic research and as diagnostic reagents in Niemann-Pick, Gaucher's and Krabbe's disease.

PHS-6040
(Rev. 10-76)

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: Further work was done related to improvements of the synthesis of 2-hexadecanoylamino-4-nitrophenyl phosphorylcholine (HNP). This substance resembles sphingomyelin but has a benzene ring instead of the aliphatic chain and due to its nitrophenyl moiety, yields an intense coloration upon enzymatic cleavage. It is a reliable chromogenic substrate used 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 is sold now by that company. We also developed a simplified synthesis of HNP based on free phosphorylcholine as the starting material. This improvement could be realized due to the availability of free phosphorycholine for which we developed a practical synthesis. Based on the chemistry of HNP, the research on non-radioactive substrates were extended to other lipidoses. Compounds were synthesized which could be used as substrates for measuring gluco and galactocerebrosidase levels in tissue extracts. 2-Hexadecanoylamino-4-nitrophenyl glucoside was shown to be a useful compound for the dianosis of Gaucher's disease. 2-Hexadecanoylamino-4-nitrophenyl galactoside can be used for the diagnosis of Krabbe's disease. Work is also underway on the synthesis of a substrate for the chromogenic diagnosis of Farber's disease, a disorder characterized by a deficiency of ceramidase.

Significance: The new compounds were thoroughly tested and they have been found useful for the diagnosis of lipid storage diseases. These findings are a major breakthrough because the 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 basic idea established by this project, compounds will be synthesized with chromophoric moieties for the detection of other enzyme deficiency 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 02163-04 DMN

PERIOD COVERED
October 1, 1977 through September 30, 1978

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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

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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)

New analytical techniques were developed and used in enzymatic research and in clinical investigations of lipidoses. Enzyme purification was studied by affinity chromatography. The lipid content in human tissues, the diagnosis of lipid storage diseases by gas, thin-layer chromatography and other techniques were studied at the microgram level. Also preparative work was done and used in connection with further synthetic work and for the preparation of lipid standards.

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: 1. Work on the separation of enzymes is related to affinity chromatography, a technique whereby an enzyme is temporarily fixed to a column containing a molecule (ligand) which reacts with an enzyme by immobilizing it. An insoluble inert support (such as agarose) is bound to a spacer arm which contains the ligand. The latter is usually a synthetic compound similar in its complexity to the substrate with which the enzyme interacts. A great number of ligands were synthesized by us and the corresponding affinity columns were assembled. 2. The development of methods for the determination of lipids in small samples of biological materials of human origin such as erythrocytes, leukocytes, fibroblast 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.

Quantitive 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: Improved purification of the enzymes was achieved by using affinity chromatography systems. Considerably more work has to be done before the advantage of these procedures could be evaluated in gains in man-hour work. 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 use of affinity chromatography should provide a significantly simplified method. 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: Efforts to improve the purification of enzymes by affinity chromatography or by other chemical operations will continue as well as by utilizing other advanced techniques. 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:

None.

ANNUAL REPORT
October 1, 1977 to September 30, 1978
Laboratory of Neuropathology and Neuroanatomical Sciences, IRP
National Institute of Neurological and Communicative Disorders and Stroke

Igor Klatzo, Chief

As in the past, the six sections of this Laboratory have been engaged in several research areas related to normal and abnormal structural physiology of the nervous system.

The Section on Cerebrovascular Pathology has pursued research on the elucidation of pathomechanisms involved in cerebral ischemia from two directions: 1) studies on disturbances of the blood-brain barrier (BBB), especially with regard to abnormal passage of catecholamines, such as norepinephrine (NE), and 2) attempts to modify the clinical course of cerebral ischemia.

The studies on the disturbances of the BBB in cerebral ischemia revealed further a differential and selective character of these changes. Whereas in ischemic injury produced by air embolism the leakage of protein tracers was almost instantaneous, this occurred only after considerable delay and for a short time following release of the clamp in ischemia due to occlusion of the common carotid artery in gerbils. Generally, the micromolecular tracers such as C^{14} sucrose or sodium fluorescein showed much more prolonged or more extensive character of abnormal passage than that of protein tracers. The abnormal passage of NE was evaluated quantitatively, by radioautography and by specific histofluorescence observations. The peak of abnormal penetration of systemically injected NE into the cerebral hemisphere on the side of occlusion appeared 72 hours after release of one hour carotid clamping. This was evident in counting H^3 labeled tracer, as well as in radioautographs. The histofluorescent observations revealed formation of bizarre noradrenergic structures around the foci of severe ischemic tissue damage.

The attempts to modify the clinical course of experimental cerebral ischemia were eminently successful with regard to application of hypothermia. Whereas in the control groups subjected to 15 minute bilateral occlusion of carotids the mortality rate after one month of observation was 67%, gerbils in which, shortly before and during carotid occlusions, the body temperature was lowered to approximately 30° C, were almost all alive after one month of observation. A substantial improvement in survival rate was also achieved by subjecting gerbils in connection with ischemic insult to the tapping of cerebrospinal fluid (CSF) from cisterna magna and intraperitoneal injection of distilled water (85% v. 33% controls). The mechanisms responsible for such striking improvements in survival rate remain obscure and are subjects of current investigation. Interestingly, both hypothermia and CSF tapping are still effective when applied 2 hours after an ischemic insult (but not longer) and this fact may be important in possible clinical applications in human patients.

The contribution of the Section on Neurocytobiology to the studies on pathophysiology of cerebral ischemia consisted primarily in elucidation of various changes occurring in cerebral endothelium due to an ischemic insult. These investigations were carried out on isolated capillaries derived from the normal and subjectied to ischemic brain tissue, as well as on cultures of endothelium grown in vitro from pia arachnoid.

A simplified procedure for isolation of metabolically active cerebral capillaries was used in observations on various transport processes occurring in the brain under ischemic and post-ischemic conditions. In capillaries isolated from the brain tissue subjected to ischemia and from post-ischemic periods the change in 2-deoxy-D (^{3}H) glucose (^{3}H-2DG) uptake was evident by the reduction and the transient increase of ^{3}H-2DG uptake, respectively. Observations on capillaries derived from the normal brain which were exposed to incubation in nitrogen atmosphere suggested an oxygen dependent nature of ^{3}H-2DG uptake. This is in contrast to the previously generally accepted idea that under physiological conditions the glucose and glucose analogues are transported by facilitated carrier mediated mechanism, which does not require any energy. The observations on uptake of various amino acids in isolated capillaries differed from that of the ^{3}H 2-DG in the fact that the former was either increased or unchanged, but never decreased. This suggests an oxygen independent mechanism of amino acids transport in contrast to that of glucose and its analogues.

The isolated capillaries took up the ^{3}H norepinephrine and the labeled substance increased with the duration of incubation (2-60 minutes). The uptake of ^{3}H 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 ^{3}H 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 1.2mM. Pyrogallol, the known inhibitor of catechol-O-methyl transferase competitively inhibited the uptake of ^{3}H norepinephrine in the isolated capillaries. Moreover the preincubation of the capillaries with pargyline, the inhibitor of monoamine oxidase (MAO) led to a decreased level of ^{3}H labeled substance in the capillaries.

Preliminary investigations of the accumulated substances in the capillaries were so far found to be the methylated metabolites of norepinephrine namely normetanephrine and metanephrine.

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 to enter or leave the brain.

In the Section on Cellular Neuropathology, a major new research effort concerned the use of immunocytochemical methods to localize myelin components in oligodendroglia and myelin sheaths during development and in normal and diseased white matter. Technical problems have made it difficult to use antibody conjugates or the unlabelled antibody (peroxidase-antiperoxidase) method to study myelin constituents. By using a new fixative and appropriate pretreatment, basic protein (BP) has been demonstrated in oligodendroglia of neonatal rat CNS before myelin sheaths are formed and before it can be detected biochemically. Oligodendroglial staining intensity with BP antiserum increases to a peak and then starts to decrease before myelin formation reaches maximum levels. Myelin sheaths were also heavily stained by cerebroside antiserum which does not stain oligodendroglia. It also has been possible to modify our method and obtain excellent localization of BP and cerebroside in paraffin sections of human nervous tissue. Studies of lesions found in multiple sclerosis and metachromatic leukodystrophy are now in progress.

Studies of the CNS myelin lesions produced by hexachlorophene (HCP) in tadpole optic nerves have continued. In freeze-fracture replicas, particle distributions on membrane faces lining the intramyelinic vacuoles generally resembled those found in control myelin. Focal alterations were seen that were associated with small blister-like projections into the lumens of some vacuoles. Tight junctions between myelin layers have been identified in transmission electron micrographs of thin sections. They correspond to those seen in freeze-fracture replicas and limit the interlamellar spread of extracellular tracers. They also are preserved at the margins of HCP induced vacuoles and probably also prevent extensive breakdown of myelin sheaths in both HCP and triethyl tin intoxication.

The Section on Neurocytology was engaged in four major projects: 1) to transplant the fragments of superior cervical ganglion (SCG) into the IV ventricle and to study any interactions between the intact brain and the SCG transplant; 2) to ascertain the nature and function of the intramembranous assemblies of particles within astrocytes; 3) to see whether anterograde axonal transport of exogenous protein can be stimulated physiologically and to characterize within the neuron changes in enzymatic activity that accompany pinocytosis of exogenous origin and 4) to follow, immunocytochemically, changes in enolase content of neurons and glia in developing brains and in vitro and to see whether neural-crest derived cells can also be identified by their enolase.

1) The most unexpected effect of transplanting SCG fragments to the intact pial surface of the cerebellum was the arrest of cells belonging to the external granule layer (EGL) in the superficial molecular layer of the cerebellum and the atypical migration of some of these cells: out of the brain parenchyma and into the transplant. In normal rat brains, almost all of the EGL cells completed their migration to form the internal granule layer 28 days after birth. In the transplant hosts, however, whole laminae of EGL cells remained in the subpial zone of the molecular layer four months after the

transplantation, some 3 months after the normal migration internally should have been completed. Some of the EGL cells actually migrated in the opposite direction to invade the transplant along with accompanying neuropil: synaptic terminals of mossy fibers and the internal granule layer as well. Sequential plastic sections, 1μ thick, have been examined one hour after transplantation to be certain that no appreciable damage has been done to marginal glial or ependymal surfaces.

The graft itself flourished in the CSF compartments. When placed over choroid plexus, the graft sometimes became covered by choroidal epithelium and was incorporated into the choroidal stroma. The graft retained its normal, fenestrated vessels even when placed over the glial surface. Although most of the SCG ganglion cells died, many survived together with a profusion of unmyelinated axons encompassed by Schwann cells. Although few if any of these axons appeared to have penetrated the brain surface, many became myelinated by the sixth month after transplantation. The formation of nodes of Ranvier indicated cooperative myelination on the part of Schwann cells. The Schwann cells often covered all available surfaces: neuronal, glial and ependymal.

This system, in which the transplantation site is easily accessible with a minimum of trauma lends itself to the study of some underlying mechanisms of the regulation and outgrowth of both central and autonomic neurons. The cultivation of neurons and their targets within CSF compartments, which can be readily irrigated, should provide further information on competitive interactions between foreign neurons and brain cells.

2) When the cell membranes of astrocytes are fixed, frozen, cleaved and replicated, orthogonal assemblies of particles (about 5-7nm in diameter) are revealed on the inner, cytoplasmic or P face and complementary lattices of tiny grooves are found on the outer or E face of the split membrane. Such assemblies which may be square, rectilinear or of irregular contour are most numerous in astrocytes fronting cerebrospinal fluid (CSF) or blood vessels and are less numerous in ependymal cells and absent from choroid plexus epithelium.

To elucidate the role of the assemblies it would be important to change their number, collective surface area, shape and number of subunits. However, before such manipulated changes can be assessed, it is first necessary to establish a base line of these parameters from region to region in the adult and in the developing brain of the rat. The most accessible gliocyte for these studies is the marginal or subpial astrocyte. The studies carried out in the Section indicated that there is an ontogenetic progression in the number of assemblies and the area of membrane face that they occupy. In the dorsal cortex of the cerebrum in 19-day-old fetuses, the number of assemblies is relatively small: $10/\mu^2$ of membrane face area. In the newborn rat up to 12 hours old, the number increases almost fivefold ($46/\mu^2$). In the adult dorsal cortex the number is $310/\mu^2$. Although doublets of the 5-7nm subunit particles emerge in fetal astrocytes, assemblies do not appear to arise by lateral migration of single subunits to form the orthogonal aggregate. In adult brains,

the number of assemblies varies, with some consistency, between different regions. With respect to the area of membrane face occupied by the assemblies, astrocytes of the dorsal cortex have the highest values, those of the medulla less, and least in the lateral and basal cortex.

Having established the base line for different regions it is intended to assess the effects of experimental manipulation. In order to examine the "nature" of assemblies, i.e. whether or not they are proteinaceous, there will be attempts to prevent their progressive increase by administering cycloheximide, an inhibitor of protein synthesis, to newborn rats. A failure to increase in number and area would reinforce the notion that the assemblies consist of protein. It is also planned to examine reactive, proliferating astrocytes that constitute a subtle glial scar resulting from transplantation of ganglia to the pial surface. In order to test the conjecture that the assemblies might influence the composition of CSF, it is intended to infuse intraventricularly a number of substances such as acetazolamide, potassium, and sodium maleate. These treatments might have an effect on the number, area and configuration of assemblies.

3) The evidence for anterograde transfer of protein within axons is not well substantiated. It was decided to see whether a group of neurons, whose function can be experimentally modified without damaging them, could be induced to incorporate horseradish peroxidase (HRP), whether the protein was then transported orthogradely, and whether the enzymatic activity of the storage depots, the lysosomes, is affected. Since the modifying stimulus for one chosen nucleus, the neurosecretory one, increases its synthesis and "packaging" of hormones, it was also asked whether there was an enzymatic change in the Golgi complex of these stimulated neurons. A prerequisite for demonstrating orthograde transport is that the neuron be intact. Intraventricular infusion of HRP spares neuron structure while permitting a high concentration of protein to bathe the soma and dendrites. Although the cell bodies of craniomotor neurons such as the XII n cells incorporated HRP from the extracellular clefts, the number of labeled lysosomes was about half of that labeled retrogradely. Within the neurosecretory system, small amounts of HRP appeared within the axons and their terminals in the posterior lobe of the pituitary gland. Appreciably more HRP entered axons and their neurohemal endings in mice dehydrated by being given 2% NaCl solution to drink for 5 days prior to the intraventricular injection of HRP. The cytochemical accompaniments of HRP uptake in such animals was an increase in the number of lysosomes showing acid phosphatase activity in their supraoptic neurons. There was, at first unexpectedly, a concomitant fall in such activity within the Golgi related smooth endoplasmic reticulum associated with lysosomes (GERL). There was also a remarkable increase in the thiamine pyrophosphatase activity in the Golgi complex of the neurons in the dehydrated mice, an increase that was presumably related to the enhanced synthesis and packaging of antidiuretic hormone and neurophysin. Investigations are currently in progress to determine whether these osmotically stimulated changes are reversible and to compare them with those regenerating nerves.

4) A cytosol protein, specific to neurons and identical to Moore's 14-3-2 protein, is the glycolytic enzyme, enolase. This nerve-specific enolase (NSE) differs physically and chemically from an isoenzyme, designated as non-neuronal enolase (NNE). After purification, both isoenzymes have been used as antigens to raise corresponding antibodies so that the isoenzyme can be located immunohistochemically. By this means, neurons may not only be distinguishable from glial neighbors but any changes in isoenzyme type that might accompany or presage neuronal differentiation during development can be followed. A further expectation that has been borne out is that certain endocrine cells derived from neural crest may be identified. These cells, dubbed by Pearse as APUD cells (amine precursor uptake and decarboxylating cells), have been only equivocally identified in some organs. In the current studies, it was shown that NSE was indeed specific to neurons whereas NNE stained astrocytes, oligodendrocytes, ependyma and choroid plexus epithelium of rats. Both labeled antibodies stained the entire cytoplasm, as expected for a soluble enzyme. After appropriate fixation, fine detains of glial structure, including perivascular and pial endfeet, were stained. In the cerebellum, glial staining was unaccompanied by neuronal staining whereas incubation of alternate sections in dilute NSE antiserum stained Prukinje and granule cells only. The same specificity was present for other regions of the adult brain. Monolayers of neurons maintained in tissue culture were also selectively stained by NSE. The epithelioid glial cells have not, so far, reacted convincingly with NNE antiserum. APUD cells, derived from neural crest stained vividly with anti-serum NSE in monkey tissues. Thus the parafolicular cells that produce calcitonin were dark in contrast to the barely discernible follicular cells. Islet cells of the pancreas stood out against the neighboring exocrine parenchyma and adrenal medullary cells against cells of the adrenal cortex.

Preliminary observations indicated that in developing rat brains, the neuroblasts contained NNE and only after differentiation did they acquire their complement of NSE. This observation must be corroborated. It would also be of interest to see if the de-differentiated glial cells in vitro truly lack NNE which might be induced to reappear with immersion in dibutyryl cyclic adenosine monophosphate, a compound that converts them to their differentiated asteroid shape.

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 plamalemma. 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 sections 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, amebocytes 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).

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 releases calcium.

The development of sensitivity to acetylcholine in cultured muscle fibers has been followed with the freeze-fracture technique. Patches of increased sensitivity, "hot spots," turn out to correspond to patches of intramembrane particles similar to those found in the postsynaptic membrane of mature synapses. However, the hot spot is not a homogenous collection of membrane particles but instead is divided into discrete patches of 20-40 particles which are sometimes found, still in patches, in the mouths of vesicular invaginations of the sarcolemma. These findings suggest that preformed groups of receptors have been leaving or entering the plasmalemma. Further work should show whether these low receptors are exchanged between the plasmalemma and the inside of the muscle cell.

The influences of receptors on synapse formation were examined in sympathetic ganglion cultures grown in the presence of antibody to purified nicotinic receptors. Synapse development proceeded normally but the mature synapses subsequently detached from the sympathetic ganglion cells. Therefore, the antibody to receptor effects maintenance of synapses but not their formation. A technique was also developed for exposing postsynaptic surfaces from intact tissues for examination with a scanning electron microscope.

The Section on Experimental Neuropathology was involved in the following projects: (I) the mechanism in the formation of "solitary" dark neurons and argentophilia of neuronal perikarya and perikaryal neurofibrils, (II) the cause of inconsistent demonstration of neuronal glycogen by current histochemical methods, and (III) the basis for the diffuse retrograde neuronal reaction after severance of a small facial nerve branch.

Dark neurons induced by postmortem trauma to unfixed nervous tissue are formed regularly and in large numbers within circumscribed regions when brains are fixed by immersion, but they do occur as isolated elements when brains are fixed by perfusion. In the latter material, however, these neurons are so rare that they may be easily overlooked, and contemporary investigators have regarded their presence in experimental material fixed by perfusion as the result of an intravitam process, disease or trauma to the brain. In an effort to clarify this ambiguity in determining the specificity of dark neurons in material fixed by immersion and by perfusion, a large material fixed by perfusion under varied conditions was examined. Solitary dark neurons tended to be formed in the transitional zone between gray and white matter where a stress force may be activated during removal of the brain. This may happen when the white matter is less well fixed than the gray matter because of inadequate flow of the fixative through the white matter or because of perfusion of small amounts of the fixative. The demonstration of such neurons in the transitional zone if the brain is removed shortly after perfusion may indicate that speed of fixation of the white matter was relatively slow because the amount of fixative reaching this region was smaller than that flowing through the gray matter (differences in minute volume). Under these circumstances, the neurons located in the transitional zones remain unfixed and vulnerable to the pressure effect of a stress force activated by removal of the brain.

Silver impregnation of neuronal cell bodies as well as of fibrils in this part of the neuron has been axiomatically accepted by most investigators to be characteristic of the normal neuron but by a few others to be the result of a pathologic process. A tendency of the metal to be deposited on scattered neurons has not been unequivocally explained. The morphologic similarities between dark neurons and silver impregnated neurons and the simultaneous occurrence of the two cell types could suggest an association between them, and this has recently been proven by two methods. Contiguous sections stained alternately with a basic

aniline dye for dark neurons and with a silver method for impregnation of neurons were searched for large neurons displaying these characteristics. When the same field in two contiguous sections containing the two types of neurons was projected one on top of the other on the photographic ground glass, the position of the silver impregnated neuron was found to match that of the dark neuron. That the silver is actually deposited on the dark neurons was subsequently confirmed by photomicrography of dark neurons in a section stained with an aniline dye (cresyl violet) and then of the same cells after restaining of the sections with silver. A review of material exposed to the effect of postmortem trauma and fixed by different methods demonstrated a variable degree of thickened and displaced neurofibrils in the cell bodies of the argentophil neurons while these neurofibrils in the neuronal cell bodies are normally not impregnated with silver when the material is fixed by perfusion and free of dark neurons. A paradox not yet understood is the observation that in newborn rats, rabbits and cats, the brain stem removed prior to fixation contained large neurons displaying intense impregnation of fibrils in the cell bodies even though in these instances no dark neurons are identifiable; if fixed by perfusion, the fibrils are no longer impregnated with silver.

After repeated efforts to improve current histochemical methods for the demonstration of glycogen, it has finally been possible to formulate a method by incorporating various recommendations, each of which alone was found to be ineffective. By such a modified method, the stainability of neuronal glycogen has been intensified, and reproducible results have been obtained from animal to animal. In certain parts of the brain, glycogen was detected in neurons which previously were thought to be devoid of this metabolically important substance.

The material used for the study of glycogen was originally obtained for an investigation of a previously unknown widespread acute neuronal reaction following transection of a small facial nerve branch. Besides the rabbit, other animals have now been operated so as to determine whether this phenomenon is typical of rabbits only. Although the specificity of this reaction is uncertain, the demonstration that other neurons than those affected by the operation are involved is puzzling and suggests that some caution in interpretation of experimental results must be exercised; so far, it appears that the operation of a nerve induces a chain reaction spreading from the directly affected neurons.

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 02281-02 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Pathophysiologic correlations in the disturbed cerebrovascular permeability due to cerebral ischemia produced by occlusion of carotid artery or air embolism in Mongolian gerbils

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

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Other:	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)
None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.9	.8	.1

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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)

Occlusion of carotid artery or air embolism in Mongolian gerbils produced changes in cerebrovascular permeability which were differential and selective in character with regard to various tracers tested. This indicates a variety of mechanisms involved in abnormal transendothelial passage. The disturbances of the BBB could not be topographically correlated either with the changes of regional CBF or with the changes in regional glucose utilization. The latter appeared in various anatomical structures indicating a wide extension of metabolic changes in cerebral ischemia. This project has been completed.

Project Description:

Objectives: The main objective of this study has been to interrelate various changes which follow abnormal cerebrovascular permeability produced by interruption of blood supply by occlusion of carotid artery or by air embolism in gerbils. It was also intended to elucidate a possible relationship of BBB disturbances to changes in the cerebral blood flow (CBF) and to their metabolic effect on the brain parenchyma, as expressed by glucose utilization radioautographic assay.

Methods Employed: Cerebral ischemia was produced in Mongolian gerbils by clamping the left common carotid artery for different periods of time, the animals being sacrificed at certain time intervals following release of occlusion. Also Mongolian gerbils were subjected to injection of 0.03-0.05 ml of air into the external carotid artery through a catheter introduced near the bifurcation of the common carotid artery, the injected air travelling with the blood via the internal carotid artery into the brain.

For study of the abnormal permeability of the BBB, 2% Evans blue solution, 10% sodium fluorescein and C^{14} sucrose solution were used. The changes in water content were measured by specific gravity determinations of the tissue samples. The CBF was radioautographically assayed after administration of C^{14} antipyrine one minute before the sacrifice. The glucose utilization was studied radioautographically sacrificing the animals 45 minutes following injection of C^{14} 2-deoxy-D-glucose. Frozen sections were prepared and photographed for the localization of fluorescent (Evans blue and sodium fluorescein) BBB tracers. The same sections were subjected to radioautography to demonstrate the distribution of C^{14} antipyrine and glucose analogue.

Major Findings: Evaluation of brain water content revealed within 5 minutes following carotid occlusion or air embolism a significant increment in water in the affected left hemisphere.

The observations on BBB disturbances showed definite differences in duration and intensity with regard to extravasation of individual tracers. Whereas in ischemic injury produced by air embolism the leakage of Evans blue-albumin was almost instantaneous, this occurred only after considerable delay and for a short time following release of the clamp in ischemia due to occlusion of the carotid artery. Generally, the micromolecular substances such as C^{14} sucrose or sodium fluorescein showed much more prolonged or more extensive character of abnormal passage than that of a protein tracer. The local disturbances of BBB to various tracers could not be related topographically to radioautographic patterns of CBF. The radioautographic assays on local glucose utilization revealed in both models of ischemic injury abnormal patterns characterized by areas of conspicuously increased or reduced grain density which showed no spatial relationship to regions marked by abnormal passage of BBB tracers.

Significance to Biomedical Research and Program of the Institute: The homeostatic regulation of the optimal biochemical environment by the BBB is severely disturbed in a number of pathological conditions. This study has been focused on elucidation of interrelationships between various changes related to abnormal vascular permeability in order to recognize operative pathomechanisms in the development of resulting brain tissue injury. It can be assumed that an understanding of sequences in pathogenic events can provide an opportunity to modify these changes and influence the extent and intensity of resulting brain lesions.

Proposed Course of the Project: This project has been completed. The major findings were presented at two international symposia: 1) International Erwin Riesch Symposium on the Pathology of Cerebrospinal Microcirculation, September 7-10, 1977, Berlin, 2) International Symposium on the Pathophysiology of Cerebral Energy Metabolism, September 18-22, 1977, Belgrade.

Publications:

Nishimoto, K., Wolman, M., Spatz, M., and Klatzo, I.: Pathophysiologic correlations in the blood-brain barrier: Damage due to air embolism. Adv. Neurol. 20: 237-244, 1978.

Nishimoto, K., Kakari, S., Pappius, H., Spatz, M., Walker, J. T., Jr., and Klatzo, I.: Behavior of the blood-brain barrier (BBB) in cerebral ischemia. In Mrsulja, B. B., Rakic, Lj. M., and Klatzo, I. (Eds.): Pathophysiology of Cerebral Energy Metabolism. New York, Plenum Press (in press).

MITH... IAN SCIENCE INFORMATION EXCHANGE (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH S.../I NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02282 02 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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

PI:	M. Smialek	Visiting Scientist	LNNS NINCDS
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	J. T. Walker, Jr.	Biol. Lab. Tech.	LNNS NINCDS
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.85	.85	0

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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)

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_2O was not given. The group of gerbils subjected to CSF tapping and H_2O 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%.

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 eluciation 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 H_2O at different periods and in groups of animals in which H_2O 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 H_2O was modified it was evident that CSF tapping was effective even when performed 48 hours before carotid occlusion, whereas beneficial effect of H_2O lasted between 2 hours before occlusion and the time of occlusion.

Significance to Biomedical Research and 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.

Proposed Course of the Project: The preliminary findings from this project were presented at the 3rd International Joint Meeting on Stroke and Cerebral Circulation, Feb. 16-18, 1978, New Orleans, La. Further investigations on this project will be concentrated on the elucidation of mechanisms which account for the remarkable difference in survival. Biochemical assays in various groups of animals will be extended to include biogenic amines, transport phenomena and dynamics of the CSF.

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 02322-01 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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

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	J. T. Walker, Jr.	Biol. Lab. Tech.	LNNS NINCDS
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	K. Nishimoto	Visiting Fellow	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)
None

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1.85	1.85	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Abnormal penetration of exogenous norepinephrine (NE) through the BBB was evaluated in cerebral ischemia 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 ^{3}H 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.

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 ^{3}H 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 noradrenergeric 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 ^{3}H 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: These investigations will be extended to include biochemical assays on the behavior of various enzymes related to NE metabolism and also attempts will be made to visualize these enzymes by immunochemical methods on the ultrastructural level.

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 02323-01 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.6	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Mongolian gerbils in which bilateral occlusion of common carotid arteries for 15 minutes was carried out in conditions of hypothermia (bocy temperature approx. 30°C) showed a remarkable improvement of survival rate when compared with control group subjected to carotid occlusions alone (98% v. 33%).

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 folloiwng 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: Further investigations on this project will be concentrated on biochemical evaluation of various parameters of ischemic injury, such as: changes in energy metabolism, transport phenomena, biogenic amines, water increment, etc.

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 01999-06 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)
T. Fujimoto, Department of Neurosurgery, Tokyo Medical and Dental University, Tokyo, Japan

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	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)

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 02000-06 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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 Neurocytobiol.	LNNS NINCDS
Other:	K. Abe	Visiting Fellow	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.2	0

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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)

Experimentally, cerebral ischemic edema 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 wet and dry weight and the specific gravity 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. Path. 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 period of ischemia and various recovery periods as compared to the long period of ischemia.

Method 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: 268-271, 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 shows little variations 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 can be divided into two groups of which one shows 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 increase 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.

Proposed Course of the Project: The study of brain edema in ischemia will be concerned with the continuous effort to differentiate the early cytogenic from the secondary vasogenic component of the cerebral edema. The investigation will include electron microscopic and radioisotopic evaluation of the ischemia brain in gerbils.

Publications: See Project No. Z01 NS 02279-02 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 02001-06 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (30 characters or less)

Amino acids transport in hypoxia, hypercapnia and hypocapnia

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. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

T. Fujimoto, Department of Neurosurgery, Tokyo Medical and Dental University, Tokyo, Japan

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

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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)

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 02083-05 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Uptake of glucose analogues in cerebellar culture

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:	M. R. Murray	Research Biologist	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

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NINCDS, NIH, Bethesda, Maryland 20014

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0	0	0

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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)

This project has been completed and the resulting paper has been published.

Renkawek, K., Spatz, M., Murray, M. R., and Klatzo, I.: Uptake of radiolabeled glucose analogues by organotypic cerebellar cultures. J. Neurobiol. 9: 111-119, 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 02084-05 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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

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

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0	0	0

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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)

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 02103-04 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Correlation of ^{3}H isoleucine uptake in pia arachnoid with culture of fibroblasts

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. R. Murray	Research Biologist	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)
None

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	.4	.7

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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 correlative studies of ^{3}H isoleucine uptake in pia arachnoid explants and in the fibroblastic culture of skin have shown that the uptake is saturable and could be cross inhibited by L leucine and cycloleucine in both types of cultures. However, the pia arachnoid was found to be different from the fibroblasts since it (1) couldn't accumulate the amino acid as did the fibroblasts and (2) couldn't be cross inhibited by D leucine, thus displaying stereospecificity for the L form of the amino acid. Therefore, the pia arachnoid may represent a site for regulation of amino acid transport in the central nervous system.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Facilitated carrier mediated glucose transport was described in organotypic pia arachnoid cultures recently (Spatz, M., Renkawek, K., Murray, M. R., and Klatzo, I.: Brain Res. 100: 710-715, 1975). This is a continuation study of pia arachnoid cultures in order to characterize its functions under normal and pathological conditions.

Methods Employed: Pia arachnoid explants from newborn rats were cultivated in the Maximow double cover slip depression slide assembly according to the technique of Allerand and Murray, 1968. Fetal fibroblasts were grown in MEM Eagle's media containing 1 mM glutamine and 10% fetal calf serum. Radiolabeled uptake of isoleucine and the non-metabolizable cycloleucine were studied in 14 day old cultures. The experiments were performed in triplicate in saline (BSS) washed cultures. They were incubated in .05 ml of BSS containing .5 µCi of the ^{3}H labeled isoleucine or cycloleucine and .025 µCi of ^{14}C inulin at pH 7.4 for 2-15 minutes. Various concentrations of either unlabeled cycloleucine or isoleucine or L-leucine or D-leucine were added to the incubation media for the inhibition studies. Thereafter, the cultures were washed, extracted with 15% trichloroacetic acid and the amount of isotope was determined by liquid scintillation counter. The protein was assayed according to Lowry et al. technique (J. Biol. Chem. 193: 265-275 1951).

Major Findings: The pia arachnoid uptake of ^{3}H isoleucine was lower than in the fibroblastic cultures. AFter 1 minute of incubation in the ^{3}H isoleucine, the concentration of the isotope in the pia arachnoid explant didn't exceed the concentration of ^{3}H isoleucine in the medium, while the fibroblastic culture's uptake was about 12 times higher than the level of ^{3}H isoleucine in the medium. A linear increase of ^{3}H isoleucine took place for 1-5 minutes in both cultures but the concentration of the isotope was always lower in the pia arachnoid than in the fibroblasts. Moreover, in both cultures the uptake of ^{3}H isoleucine was saturable with addition of unlabeled isoleucine in increasing concentration to the incubating medium containing the labeled substance. The uptake could be also cross inhibited by addition of unlabeled L-leucine and cycloleucine. However, D-leucine affected only the isoleucine uptake of the fibroblasts but not the pia arachnoid cultures. A manuscript is in preparation.

Significance to Biomedical Research and the Program of the Institute: The pia arachnoid explant due to its relatively simple composition of pial membrane and vessels is an excellent model for the investigation of its function. The determination and evaluations of the uptake of various substances by pia arachnoid will permit us to assess the permeability of these structures. Thus, these studies will be helpful in defining its properties and possible role in the relation to blood and spinal fluid in the normal and diseased states.

Proposed Course of the Project: The continued investigations will be concerned with defining and eliciting the factors responsible for the amino acid uptake under normal conditions. Therefore, the kinetics and the influence of electrolytes, metabolites, and drugs will be determined in both types of cultures. Thereafter, this model will be used to study the amino acids uptake under pathological conditions.

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 02166-04 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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	Chief, Section on Neurocytobiology	LNNS NINCDS
Other:	D. Micic	Visiting Fellow	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)
None

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Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.2	.3

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 effect of cerebral ischemia and postischemia on the synaptosomal uptake of some neutral amino acids was determined in Mongolian gerbils at various periods of time. A transiently increased uptake of ^{3}H isoleucine, ^{14}C cycloleucine and ^{3}H phenylalanine in the synaptosomes was found after 3 minutes of bilateral common carotid occlusion which returned to normal at 30 minutes of reestablished cerebral blood circulation.

Project Description:

Objectives: In our continuous effort of evaluating the effect of cerebral ischemia on the function of various cerebral compartments we investigated the amino acid uptake in synaptosomes obtained from brains of gerbils subjected to ischemia.

Methods Employed: Radiolabeled isoleucine, cycloleucine and phenylalanine were used for the uptake studies in synaptosomes separated from brains of gerbils subjected to bilateral ischemia and postischemia of different duration. The assay procedures were the same as those used for the synaptosomal uptake of ^{3}H 2-deoxy-D-glucose (^{3}H 2-DG) except for a shorter incubation period of 2 instead of 15 minutes (Spatz et al., Brain Res. 120: 141, 1977).

Major Findings: The specific synaptosomal uptake of the tested amino acids was found to be increased in cerebral ischemia of 3 minutes duration. This increased synaptosomal amino acid uptake was transient since no significant changes of either of these amino acids' uptake were seen in the synaptosomes obtained from brains of gerbils subjected to 1, 15 and 30 minutes of cerebral blood flow deprivation as compared to controls. However, a further increase in the uptake of these amino acids was seen in synaptosomes separated from brains of animals with 3 minutes of arterial clipping and 1 minute clip release. Thereafter, the uptake progressively decreased and returned to normal at 30 minutes of reestablished circulation after 3 minutes of arterial occlusion.

Significance to Biomedical Research and the Program of the Institute: The results of these investigations shed some more light on the effect of ischemia and postischemia on the synaptosomal function. It appears that the deprivation and reestablishment of the cerebral blood supply diversely affects the uptake of neutral amino acids as compared to the glucose analogues, since a transient increase instead of decrease of the respective substrate's uptake was seen in the synaptosome. The absence of reduced neutral amino acid uptake in ischemia strongly suggests that the transport of these amino acids is most likely oxygen and energy independent. The basic understanding of the synaptosomal function in ischemia may be helpful in elucidating the mechanism responsible for the pathophysiological changes seen in the cerebrovascular disease.

Proposed Course of the Project: The effect of ischemia on the uptake of other amino acids will be studied in order to obtain a full picture of the altered synaptosomal function in this disease.

Publications:

Micic, D., Swink, M. E., Klatzo, I., and Spatz, M.: Transient ischemic alteration of synaptosomal neutral amino acid uptake. Experientia (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 02197-03 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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

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

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	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)

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 02193-03 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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 Neurocytobiol.	LNNS NINCDS
Other:	D. Micic	Visiting Fellow	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)

None

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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.2	.3

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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)

Radiolabeled neutral amino acids uptake was studied in isolated cerebral capillaries obtained from gerbils subjected to bilateral common carotid occlusion with and without release. Both ischemia and postischemia and the postischemic recirculation of the cerebral blood lead to a transient increase of capillary neutral amino acid uptake.

Project Description:

Objectives: The isolated cerebral microvessels have been useful in the investigation of transport processes occurring on the blood-brain level. Recently, we have shown that ischemia and anoxia reduce the capillary ^{3}H 2-DG uptake which could be recovered either by reestablishment of cerebral blood circulation or by substituting nitrogen with oxygen atmosphere. The purpose of this investigation has been to evaluate the effect of cerebral ischemia and postischemia on the capillary uptake of substances which are transported across the blood-brain barrier (BBB) by a specific carrier mediated process other than the one for hexose.

Methods Employed: Cerebral ischemia and postischemia were produced in gerbils by bilateral common carotid occlusion (1-30 minutes) and clip release for 1-120 minutes after 3 or 6 minute arterial clipping. The procedures for the determination of ^{3}H and/or ^{14}C labeled neutral amino acids in the isolated cerebral capillaries were the same as those used for the uptake of 2-deoxy-D-(^{3}H) glucose except for incubation period of 2 instead of 5 minutes (Spatz et al., Brain Res. 120, 1977).

Major Findings: The cerebral capillaries isolated from brains of gerbils subjected to bilateral common carotid artery occlusion for 1-30 minutes duration showed a transiently increased uptake of isoleucine, cycloleucine and phenylalanine at 3 minutes. The capillary glutamine uptake was not affected by cerebral ischemia. The increased capillary amino acid uptake dropped to a lower level at 1 minute and remained significantly higher than the one of controls up to 10 minutes but returned to normal values after reestablishment of cerebral blood circulation. Moreover, an augmented uptake of these amino acids was observed in microvessels separated from brains of animals subjected to 6 minutes of bilateral carotid artery occlusion and 1-30 minutes release. Two hours later the capillary uptake of tested amino acids returned to normal levels. In both circumstances the increased isoleucine and phenylalanine uptake could be inhibited by various concentrations of cold cycloleucine or phenylalanine to the same degree as in controls, respectively. Although the postischemic effect on the amino acid uptake is similar to the one described in the capillary 2-DG but the 6 minutes occlusion resulted in normal entry of the amino acids while a reduction of 2-DG uptake was seen in the cerebral capillaries at the same time.

Significance to Biomedical Research and the Program of the Institute: Based on our investigation, the cerebral capillaries are useful for the study of some parameter 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: Similar model will be used for the stud/ of other amino acid capillary uptake as well as release in ischemic and post-ischemic gerbils.

Publications:

Spatz, M., Micic, D., Fujimoto, T., Mrsulja, B. B., and Klatzo, I.: Tranport phenomena in cerebral ischemia. In Mrsulja, B. B., Rakic, L. M., and Klatzo, I. (Eds): Pathophysiology of Cerebral Energy Metabolism. New York, Plenum 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 02275-02 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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

BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.3	.7

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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)

Cerebral capillary endothelial cell cultures have been established from isolated cerebral capillaries of 2-day-old rats. The cells have been growing slowly as sheets of elongated cells (with plump nuclei) forming bands and occasionally loops. So far, we have a 2nd and 3rd generation of these cultures.

Project No. ZO1 NS 02275-02 LNNS

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 both the organotpyic explant and dissociated cells. These cells grow very slowly as sheets of elongated cells with plump nuclei forming bands and occasonally an attempt of loop formation was seen in these cultures. However, the organotypic explants of cerebral capillaries have not been suitable for multiple passages in obtaining large yields for the planned histochemical and biochemical studies. Therefore, we continue to concentrate our efforts to obtain more cultures from the dissociated cells of cerebral capillaries. At the present time, we have a second and third generation of the cultures.

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 02276-02 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Ischemic and postischemic effect on the activity of cytochrome oxidase and acetylcholinesterase in Mongolian gerbils

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

PI:	D. Micic	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
NIH, NINCDS, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.4	.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 cytochrome oxidase and acetylcholinesterase activities were assayed during short and long term ischemia as well as in various recovery periods after 1 hour of cerebral blood flow deprivation. 1) The cytochrome oxidase activity was affected only in the recovery period after one hour ischemia and after 5 hours of continuous ischemia. At that time the activity of cytochrome oxidase was significantly reduced when compared to the controls. In contrast, no significant changes in the activity of acetylcholinesterase were observed in the experimental as compared to control gerbils. 2) Both enzymes, the cytochrome oxidase and acetylcholinesterase were transiently reduced in the animals anesthetized with pentobarbital. This project is completed.

Project Description:

Objectives: Cerebral ischemia appears firstly to affect the mitochondria, then the endoplasmic reticulum and ribosomes (Hagen et al., 1960; Hills, 1964; Clendenon et al., 1971). However our recent studies have shown that one of the mitochondrial enzymes, the monoamine oxidase, is not affected during short term ischemia but in the recovery period or after prolonged continuous ischemia of 5 hours. Therefore we investigated another mitochondrial enzyme, the cytochrome oxidase, which is involved in electron transport system and correlated with the behavior of synaptosomal enzyme, acetylcholinesterase activity in cerebral ischemia and postischemia of gerbils.

Methods Employed: Several groups of anesthetized animals (pentobarbital 20 mg/kg i.p.) were subjected to unilateral carotid artery occlusion for 1 hour and various periods of release or continuous occlusion of 1-5 hours. Sham operated gerbils and gerbils without pentobarbital treatment served as controls. The mitochondrial cytochrome oxidase activity was assayed (Hess, H. and Pope, A., J. Biol. Chem. 204: 295, 1953).

The synaptosomal activity of acetylcholinesterase and butyrylcholinesterase was determined by the Ellman spectrophotometric method (Biochem. Pharmac. 7: 88, 1961), using respective substrates.

Major Findings: The cytochrome oxidase activity was significantly reduced at 20 hrs and remained low at 72 hrs and 1 week after the reestablishment of cerebral blood circulation in 1 hr occluded gerbils. A significantly decreased activity of this enzyme was also observed after 5 hrs of continuous cerebral flow deprivation. In contrast the acetylcholinesterase activity was not affected by cerebral ischemia or postischemic cerebral recirculation of blood. Pentobarbital anesthesia reduced the cerebral activity of cytochrome oxidase and acetylcholinesterase transiently. Butyrylcholinesterase and the nonspecific cholinesterase were not affected by the barbiturate treatment.

Significance to Biomedical Research and the Program of the Institute: These studies indicate that the cytochrome activity is affected the same way as previously studied (mitochondrial enzyme) MAO by cerebral ischemia and postischemia. The reduction of this enzyme occurs only after long term continuous ischemia or in the recovery period from short term ischemia. The absence of changes in the acetylcholinesterase activity indicates that the deprivation and reestablishment of cerebral blood circulation has no effect on the enzyme responsible for the metabolism of cholinergic neurotransmitter. However, barbiturate anesthesia affects both the mitochondrial and the synaptosomal enzymes in the brain. Our findings suggest that the increased brain acetylcholine level reported to occur after barbiturate anesthesia most likely is due to the inhibition of acetylcholinesterase activity.

Proposed Course of the Project: This project is completed and another manuscript is in preparation.

Publications:

Micic, D., Micic, J., Klatzo, I., and Spatz, M.: Effect of pentobarbital on the synaptosomal activity of acetylcholinesterase in Mongolian gerbils. Experientia 34: 169-170, 1978.

NOTICE OF INTRAMURAL RESEARCH PROJECT | Z01 NS 02277-02 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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 L. Rogac, Institute of Biochemistry, and B.. J. Mrsulja, Institute of Biology, Faculty of Medicine, Belgrade, Yugoslavia

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Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.4	.1	.3

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The activity of several enzymes has been investigated in isolated cerebral microvessels versus total brain homogenate obtained from normal and ischemic gerbils. These studies suggested that the capillary enzymes are affected by ischemia prior to the brain parenchyma. DHE pretreatment had a beneficial effect on the ischemic changes of various enzymes in the brain parenchyma but not in the capillaries.

(10-76)

Project Description:

Objectives: The simple technique for isolation of cerebral capillaries (Mrsulja et al., Brain Res. 110: 361, 1976) provides an opportunity to study metabolic events occurring in the microvessels under normal and pathologic conditions.

Methods Employed: Several enzyme activites were assayed in the cerebral capillaries versus total brain homogenate isolated from normal and ischemic gerbils. The cerebral ischemia was produced by bilateral common carotid artery occlusion for 1-9 minutes. The effect of dihydroergotoxine methane pretreatment (DHE, 1 mg/kg body weight, i.p.) on the capillary versus parenchyma enzymes was evaluated in ischemia.

Major Findings: In normal animals glutamyl transpeptidase, butyrylcholinesterase, alkaline phosphatase and leucine aminopeptidase are found in the capillary fraction, while enzymes of energy and amino acid metabolism as well as acetylcholinesterase are present mainly in the brain parenchyma. In bilateral ischemia the following enzymes were found to be reduced: HK, PK, LDH, AChE, BuChE and ATPase, while PFK, G-6-Pase and gamma-GT activities are increased. There was no change of G-6-PDH, AChE and BuChE activities in the brain parenchyma, although NAD-dependent, isocitive dehydrogenase and HK activites were decreased. A beneficial effect of DHE pretreatment on the enzymatic changes occurring in ischemia was seen in parenchyma but not in the capillaries.

Significance to Biomedical Research and the Program of the Institute: The metabolic studies of microvessels isolated from brain may lead to the understanding of factors regulating BBB permeability in normal and pathologic conditions. These events are of great importance in order to elucidate many disease processes and to select the best therapeutic approaches.

Proposed Course of the Project: The metabolic study of the microvessels will be extended to the postischemic period.

Publications:

Djuricic, B. M., Rogac, Lj., Spatz, M., Rakic, Lj. M., and Mrsulja, B. B.: Brain microvessels. I. Enzymic activities. Adv. Neurol. 20: 197-205, 1978.

Mrsulja, B. B., Djuricic, B. M., Mrsulja, B. J., Rogac, Lj., Spatz, M., and Klatzo, I.: Brain microvessels. II. Effect of ischemia and dihydroergotoxine on enzymic activities. Adv. Neurol. 20: 207-213, 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 02278-02 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Effect of anoxia on 2-deoxy-D (^{3}H) glucose 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:	D. Micic	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

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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.55	.45	.1

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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)

2-Deoxy-D (^{3}H) glucose (^{3}H 2-DG) uptake was studied in isolated normal cerebral capillaries subjected to various gas mixtures during incubation. Under 100% N_2 atmosphere, the specific ^{3}H 2-DG capillary uptake was markedly decreased but could be recovered by substituting the N_2 gas for oxygen or normal air. Free fatty acid serum albumin prevented the reduction of ^{3}H 2-DG capillary uptake under anaerobic conditions, which could be abated by addition of individually tested saturated and unsaturated free fatty acids. This project is completed.

Project Description:

Objectives: A decreased 2-deoxy-D-(^{3}H) glucose (^{3}H 2-DG) uptake was observed in the capillary fraction isolated from brains of gerbils subjected to bilateral cerebral ischemia (Spatz et al., Brain Res. 120: 141, 1977). In order to clarify some aspects of the pathophysiological mechanism responsibil for that phenomenon, we investigated the effect of O_2 deprivation on the ^{3}H 2-DG uptake in the cerebral capillary fraction separated from the nonvascular normal brain tissue.

Methods Employed: The procedures for the capillary isolation and ^{3}H 2-DG studies were the same as the ones described previously (Mrsulja et al., Brain Res. 110: 361, 1976). However 26 mM K phosphate buffer containing 146 mM of sucrose was used instead of Ringer solution for the incubation in the atmosphere of various gases.

Major Findings: A markedly reduced (75%) specific ^{3}H 2-DG uptake was found in the capillaries incubated in nitrogen atmosphere as compared with the one exposed to normal air or 100% oxygen or 95% N_2 and 5% O_2. The decreased capillary ^{3}H D-DG uptake could be recovered by substituting the atmosphere with normal air or oxygen after 7.5-15 minutes exposure to N_2 gas. The anoxic inhibition of ^{3}H 2-DG uptake couldn't be restored by the addition of either energy phosphate metabolites or mono-, bivalent ions, except for $MgCl_2$, to the incubation buffer. The addition of essentially fatty acid free serum albumin prevented the ^{3}H 2-DG reduction of ^{3}H 2-DG capillary uptake under anaerobic conditions, which was partially or fully abated by the presence of individually tested saturated and unsaturated fatty acids in the incubating medium.

Significance to Biomedical Research and the Program of the Institute: The results of this investigation are of great importance for the transfer of this primary brain nutrient may be oxygen dependent, in contrast to previously accepted concepts. Moreover it appears that the transport of glucose may be closely related to free fatty acid metabolism, which has not been considered to have such a role. The basic understanding of the cerebral capillary function may help in elucidating the mechanism of cerebrovascular disease and possibly the altered function of other neurological diseases.

Proposed Course of the Project: This project is completed, but an attempt will be made to define the relationship of oxygen-dependent glucose uptake and the free fatty acid metabolism.

Publications:

Micic, D., Micic, J., Swink, M. E., and Spatz, M.: The anoxic effect on 2-deoxy-D-^{3}H glucose uptake in the isolated cerebral capillaries. Proc. Soc. Exp. Biol. Med. 158: 318-322, 1978.

Spatz, M., Micic, D., Mrsulja, B. B., and Klatzo, I.: Cerebral microvessels as mediators of cerebral transport phenomena. Adv. Neurol. 20: 189-196, 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 02279-02 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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:	K. Nishimoto	Visiting Fellow	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. Neuroanat. Sci.	LNNS NINCDS

COOPERATING UNITS (if any)
H. Pappius, Montreal Neurological Institute, Montreal, Canada; T. Fujimoto, Department of Neurosurgery, Tokyo Medical and Dental University, Tokyo, Japan.

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.3	.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The analysis of the electrolyte brain content in ischemic cerebral edema has been in progress. The results suggested a biphasic alteration of electrolyte levels in the ischemic brain corresponding to the changes in water content of the brain. The peak of the reduced K^+ content and the elevation of Na^+ level in the affected brain coincided with the occurrence of the increased BBB permeability to proteins.

Project Description:

Objectives: In human cerebral ischemia brain edema is considered to be an important factor in causing mortality (Shaw et al., 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 and Payan, Exp. Neurol. 16: 252-255, 1966; Kohn, C., Arch. Path. 69: 544-553, 1972; Ito et al. Acta Neuropath. 32: 209-223, 1975). In our recent studies a development of biphasic ischemic cerebral edema was observed after a short term ischemia of 1 hr duration (Spatz et al., in Dynamics of Brain Edema, Berlin Heidelberg, Springer-Verlag, 1976, pp. 181-186). The present investigation has been concerned with the evaluation of cerebral electrolyte levels under the same conditions in order to elucidate further the pathophysiological mechanism responsible for the development of ischemic edema.

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 and sham operated animals were used as controls. The cerebral water content was determined by wet and dry weight. The sodium and potassium were analyzed by flame photometry.

Major Findings: This project is in progress but the preliminary results demonstrate a biphasic alteration of electrolyte levels in the ischemic brain corresponding to water content of the brain. After 1 hour of occlusion, the concentration of K^+ is already decreased while Na^+ level is increased in the affected hemisphere as compared to controls (experimental K^+ 369 ± 21.9 meg/kg dry weight, Na^+ 48.7 ± 5.9 meg/kg wet weight; controls K^+ 432 ± 16.7 meg/kg dry weight and Na^+ 40.3 ± 2.5 meg/kg wet weight). Both the K^+ and Na^+ concentrations appear to return to normal at 5 hrs but the peak of secondary alterations occurs at 10 hrs after release of 1 hour occlusion. At this time the greatest reduction of K^+ and elevation of Na^+ level is seen in the ischemic hemisphere as compared to controls (K^+ 339 meg/kg dry weight, Na^+ 74.8 meg/kg wet weight; K^+ 432 meg/kg dry weight, Na^+ 40.3 meg/kg wet weight, respectively). These changes are taking place at the same time as BBB permeability increased to albumin and substances of similar molecular size (Spatz et al., in Dynamics of Cerebral Edema, Berlin Heidelberg, Springer-Verlag, 1976, pp. 181-186). One week after the release of occlusion a reversibility of the electrolyte change is seen in about 50% of cases.

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.

Proposed Course of the Project: To correlate the level of electrolytes in ischemic cerebral edema with the water content of the brain and with the BBB permeability under the same conditions in an attempt to define more closely the dynamics of this process.

Publications:

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., and Klatzo, I. (Eds.): Pathophysiology of Cerebral Energy Metabolism. New York, Plenum 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 02280-02 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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:	D. Micic	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

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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.55	.35	.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)

The effect of cerebral ischemia and postischemia on MAO activity was investigated in Mongolian gerbils. Ischemia of 1 hour duration doesn't alter the MAO activity until 20 hrs later when the reaction of MAO activity becomes apparent and persists for 1 week. In prolonged ischemia, the decrease in MAO activity is not seen prior to 5 hours of continuous unilateral common carotid artery occlusion.

Project Description:

Objectives: In our previous experiments we postulated that the monoamine oxidase activity couldn't be responsible for the observed ischemic and post-ischemic reduction of biogenic amines and accumulation of its metabolites in the brain (Mrsulja et al., Acta Neuropath. 36: 1, 1976, and Brain Res. 98: 388, 1975). In order to elucidate further the metabolic fate of these substances we investigated the cerebral monoamine oxidase (MAO) activity during ischemia and postischemia of Mongolian gerbils.

Methods Employed: Several groups of anesthetized gerbils (pentobarbital 20 mg/kg i.p.) were subjected to unilateral carotid artery occlusion for 1 hour and various periods of release or to continuous occlusion for 1-5 hrs. Sham operated and animals without barbiturate treatment served as controls. The mitochondrial MAO activity was assayed by microfluorimetric methods (Biochem. Pharmacol. 14: 1686, 1965).

Major Findings: In the short termed ischemic animals, the activity of MAO was significantly reduced in the hemisphere ipsilateral to 1 hour occlusion and release for 20 and 72 hrs and 1 week as compared to the contralateral to the one from control and sham operated animals [ischemia = 16.05 ± 1.35 (7), 12.99 ± 1.59 (7), 13.23 ± .76 (6) nmoles 4 HOQ/mg P/hr, respectively; control = 21.91 ± .79 (12) nmole 4 HOQ/mg P/hr]. However, normal levels of the enzyme were found 4 weeks after the release of 1 hour occlusion. In the continuously ischemic gerbils the MAO activity was found to be only reduced at 5 hours [15.79 ± 1.0 (7) nmoles 4 HOQ/mg P/hr].

Transient inhibition of MAO was also observed in pentobarbital treated gerbils. The MAO activity was 18.27 ± 18 and 17.06 ± 58 nmoles 4 HOQ/mg P/hr. In the anesthetized animals at 90 and 150 minutes, respectively (controls = 22.2 ± 1.8 nmoles 4 HOQ/mg P/hr), the activity of the MAO returned to normal levels 270 minutes (3 hours) after the pentobarbital injection.

Significance to Biomedical Research and the Program of the Institute: The ischemic and postischemic reduction in MAO activity observed in these experiments substantiates our previous postulate that such alteration of the MAO couldn't be responsible for the decreased level of cerebral biogenic amines and accumulation of its metabolites in gerbils subjected to ischemia and recirculation of cerebral blood (Brain Res. 98: 388, 1975).

The transient depression of MAO activity is noteworthy since pentobarbital may considerably affect the level of neurotransmitters in the brain.

Proposed Course of the Project: This aspect of the project is completed and another manuscript is in preparation. However, in the future the MAO activity will also be determined in isolated cerebral capillaries from ischemic as compared to controls, since the presence of this enzyme plays

a role in the BBB as enzymatic barrier for catecholamine (see Project No. Z01 NS 02324-01 LNNS)

Publications:

Micic, D., Klatzo, I., and Spatz, M.: The effect of sodium pentobarbital on some mitochondrial enzymes. J. Neurochem. (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 02324-01 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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	Guest worker	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

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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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 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 ^{3}H 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 ^{3}H 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 ^{3}H norepinephrine was determined by adding pyragallol 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 ^{3}H norepinephrine and the labeled substance increased with the duration of incubation (2-60 minutes). The uptake of ^{3}H 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 ^{3}H 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. Pyragallol, the known inhibitor of catechol-O-methyl transferase competitively inhibited the uptake of ^{3}H norepinephrine in the isolated capillaries. Moreover, the preincubation of the capillaries with pargyline, the inhibitor of monoamine oxidase (MAO) led to a decreased level of ^{3}H labeled substance in the capillaries.

Preliminary investigations of the accumulated substances in the capillaries were so far found to be the methylated metabolites of norepinephrine namely normetanephrine and metanephrine.

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 to enter or leave the brain.

Proposed Course of Project: These investigations are still in progress and several kinetic parameters have to be established before extension of this study to other members of the catecholamine family.

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 02325-01 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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:	K. Nishimoto	Visiting Fellow	LNNS NINCDS
	I. Klatzo	Chief, Lab. Neuropath. & Neuroanat. Sci.	LNNS NINCDS
	M. Spatz	Head, Section on Neurocytobiol.	LNNS NINCDS

COOPERATING UNITS (if any)

None

BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.8	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)

The permeability of the blood-brain barrier has been evaluated in gerbils subjected to bilateral cerebral ischemia and postischemia. Preliminary results suggest that the BBB permeability is altered in postischemic period but not during cerebral ischemia.

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 so far used for the evaluation of the BBB: NaFl, Evans blue, ^{14}C sucrose and ^{3}H inulin.

Major Findings: This project is in progress but 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.

Generally, the behavior of the BBB to sucrose was similar to the one found using NaFl. However, the percentage of animals showing an increased BBB permeability to inulin was lower than those for sucrose and NaFl. The increased extravasation of Evans blue was seen in about 10% of cases 48 hours following the release of arterial clipping.

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: Several other diffusible substances of various molecular weights will be used to assess the behavior of BBB following bilateral cerebral ischemia. Thereafter the evaluation of substances will include a variety of substrates which pass the BBB by active or passive facilitated carrier mediated process.

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 02326-01 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.2	.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)

Changes in the capillary lactate and 2-deoxy-D-glucose uptake were studied in the developing brain. The cerebral capillaries isolated from the brain of young and adult rats were found to have a reciprocal affinity for these substances which is in agreement with the reported differences in BBB permeability for lactate glucose analogues observed during development.

Project Description:

Objectives: Many characteristic features attributed to various specific transport systems of the blood brain barrier (BBB) observed in adults were also found to be present in young animals. However, during development the brain uptake and the cerebral levels of certain substrates were distinctly different from the one found in the mature brains. Recently, it had been shown that the brain uptake of the monocarboxylic organic acids was higher while that of glucose was lower in the weanling than adult rats. Since the isolated cerebral microvessels have been proven to be suitable for uptake studies which conceivably could reflect some of the described transport phenomena occurring in the brain, we investigated the capability of capillary uptake of ^{14}C L-lactic acid and 2-deoxy-D(^{3}H)glucose in the rats postnatally as compared to adults.

Methods Employed: The cerebral microvessels were separated from non-vascular brain tissue of newborn, 2-21 day and 9 week old Osborne-Mendel rats. The method of capillary isolation was the same as the one described previously except for the sucrose gradient in which the molarity of sucrose was changed from 1 and 1.5 to 1 and 1.8 solutions. Duplicate or quadruplicate aliquots (.1 ml) of each sample were used with .1% albumin Ringer solution (.5 ml) containing either ^{14}C L-lactate acid (spec. act. 146.9 mCi/mM) or 2-deoxy-D(^{3}H) glucose (spec. act. 8.26 Ci/mM) obtained from New England Nuclear Company at pH 7.4 for 15 minute incubation at 37°C. The nonspecific uptakes were determined by addition of unlabeled (cold) lactic acid and 3-methyl-D-glucose [3-MG (200 mM)] to their respective labeled mixtures. The Km for ^{14}C L-lactice acid was determined by the addition of L-lactive acid in concentration of .5-4 mM. Cold pyruvic acid in concentrations of 2.5-7.5 mM and unlabeled 3-MG in concentrations of 5-20 mM was used for cross inhibition of capillary uptake of lactic acid and 2-DG, respectively.

Major Findings: The capillary uptake: ^{14}C L-lactic acid was 49599.7 $\pm$ 2070 (10) and 4001 $\pm$ 179 CPM/mgP (10) and ^{3}H 2-DG was 6335.9 $\pm$ 1639 (8) and 271767 $\pm$ 9308 CPM/mgP (16) in newborn and adults respectively. However, the nonspecific capillary lactic or 2-DG uptake was the same (10-15%) in the cerebral microvessels obtained from all the tested brain irrespective of age. The saturability and the Km values were also the same whether estimated in 2 day or 9 week old animals and close to the one described by Oldendorf (Km 2.5 and 3 mM, respectively). The lactic acid could be cross inhibited by pyruvic acid and 2-DG by 3-MG to the same degree in newborn as in the adults.

Significance to Biomedical Research and the Program of the Institute: The various levels of the specific capillary lactic acid and 2-DG uptakes seen in postnatal life and in adults indicate that the cerebral capillaries have a distinct function during brain development which most probably is responsible for the extent of substrate passage across the BBB. Moreover, these observations strongly suggest that the capillaries isolated from brains of immature and weaning animals are as suitable as the one separated from adult brain for transport and metabolic investigations.

Proposed Course of the Project: The properties of isolated cerebral capillaries for the uptake of various substances will be determined in the developing brain in order to establish their BBB role under physiologic and pathologic conditions.

Publications:

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. Brain Res. (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 02327-01 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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

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
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SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.7	.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 uptake of biogenic amines and a precursor, L-dopa, into the cells of pial cultures is studied by histochemical techniques with special attention to capillary endothelial cells.

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^{-2}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^{-5}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: Further studies on the subject are in progress to further explore the characteristics of the uptake by means of known uptake of blocking agents and metabolic inhibitors.

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 02328-01 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.6	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)

The inhibition of cholinesterases, especially acetylcholinesterase, in the developing neuroblast of the spinal and sympathetic ganglion 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 role of acetylcholinesterase in the maturation of the neurons.

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 cholinoceptive 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: Eserine, inhibitor of both acetyl- and non-specific cholinesterase had an inhibitory effect on maturation of the neurons of both ganglia. This was observed as rapid degeneration of the cultured neurons within a few days of culture (10^{-4}-10^{-3}M concentrations), or as an inhibition of the growth of nerve fibres, and of the lack of increase in the size of the nerve cell bodies accompanied by an increased number of degeneration and dying neurons (the 10^{-6}-10^{-5}M concentrations).

Iso-OMPA, inhibitor of non-specific cholinesterase, did not have effect on the parameters studied at low concentrations (10^{-6}-10^{-4}M concentrations) and even at concentration of 10^{-3}M it did not cause as widespread inhibition of growth and development as eserine at the concentration of 10^{-6}M.

BW 284 C 51, an inhibitor of acetylcholinesterase caused an inhibition of growth and degeneration of the neurons at the concentration of 10^{-6}M and at 10^{-7}M abnormal granulation was observed in the cytoplasm of the developing neurons.

Paraoxon, inhibitor of both acetyl- and non-specific cholinesterase caused increased granulation of the cytoplasm of the neurons at 10^{-5}M concentration.

DFP, an inhibitor of both acetylcholinesterase and non-specific cholinesterase caused increased granulation of the cytoplasm and abnormal degenerative cell figures at concentrations 10^{-5}-10^{-4}.

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: The preliminary observations should be confirmed and further studies should be carried out at different stages of the maturation of the neurons and with variable exposure times.

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 01995-06 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Morphological studies of CNS myelin formation, demyelination, and remyelination in model systems

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
	T. Tabira	Visiting Fellow	LNNS NINCDS
	M. Kies	Chief, Section on Myelin Chemistry	LCM NIMH

COOPERATING UNITS (if any)
Department of Neurology, Johns Hopkins Medical School, Baltimore, Maryland; J. M. Matthieu and P. J. Honegger, Departments of Pediatrics and Physiology, University of Lausanne, Lausanne, Switzerland

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Cellular Neuropathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.1	4.1	2.0

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)

The long range goal of this project is to use morphological methods such as light and electron microscopy, immunochemical staining, freeze-fracture techniques, and electron dense tracers to study the lamellar structure of myelin and cellular mechanisms of myelin formation and breakdown. Tissues used include neonatal rats, aggregating cultures of mechanically dissociated fetal rat brains, the optic nerves of Xenopus tadpoles, and the myelinated epiretinal fibers of the rabbit eye. Current projects are: 1) use of immunocytochemical techniques to localize myelin constituents during myelin formation and breakdown, 2) an electron microscopic study of aggregating cell cultures during their differentiation and growth in vitro, 3) a study of myelin sheath remodelling in optic nerves of Xenopus tadpoles during metamorphosis, and 4) an investigation of the electron microscopic appearance of interlamellar tight junctions in CNS myelin sheaths.

Project Description:

Objectives: 1) To modify the unlabelled antibody (peroxidase-antiperoxidase) method in order to achieve better tissue penetration and study the distribution of basic protein (BP) in newborn and developing rat brain. 2) To examine the fine structure of aggregating cultures maintained in vitro for 4-40 days and identify patterns of neuronal and glial differentiation, especially the onset and progression of myelin formation. 3) To study whole mounts and sections of metamorphosing Xenopus tadpoles and young frogs and define the frequency, distribution and evolution of myelin sheath changes. 4) To investigate the distribution of interlamellar tight junctions in CNS myelin and test their permeability in normal and hexachlorophene intoxicated tadpoles.

Methods Employed: 1) Groups of neonatal rats were perfused with a variety of buffered and unbuffered aldehyde fixatives. Vibratome sections of brain, brainstem and spinal cord were pretreated and then immunostained with antisera to BP and cerebroside according to the peroxidase-antiperoxidase method (Sternberger, 1970). The sections were mounted on slides in glycerine and examined with a differential-interference microscope. Semiquantitative estimates of oligodendroglial staining intensity were measured with an Optomax image processor and used to calculate optical densities. 2) Aggregates of mechanically dissociated fetal rat CNS cells that had been maintained for 4, 19, 26, and 40 days in vitro by Dr. Honegger were processed for light and electron microscopic study so that diameters of aggregates could be measured and the distribution of cell types could be determined semiquantitatively during differentiation. 3) After perfusion fixation, optic nerves from stage 52-65 tadpoles were either mounted whole, measured and examined light microscopically or were processed for electron microscopic study. 4) Groups of normal and hexachlorophene intoxicated tadpoles were fixed by perfusion. Lanthanum chloride, an electron dense tracer, was injected around the optic nerves of some tadpoles in each group 30-60 min. before sacrifice. After fixation, the optic nerves were embedded in Durcupan and sectioned for electron microscopic study.

Major Findings: 1) Of the many fixatives tested, an unbuffered formaldehyde-mercuric chloride mixture provided the best penetration and staining of myelin sheaths by BP and cerebroside antisera. This method proved to be reproducible, highly sensitive and specific with no staining of neurons, astrocytes, ependyma or blood vessels. In the anterior commissure of 5-7 day old rats, oligodendroglia contained BP before the onset of myelin formation (10-18 d). Oligodendroglia and myelin sheaths in the brainstem of newborn rats are stained by BP antiserum. Measurements showed that the staining intensity increased to a maximum at 5 days and then decreased while the rate of myelin formation was still rising. Cerebroside antiserum only stained myelin sheaths; oligodendroglia and Schwann cells remained unstained during myelination. 2) The cells rapidly formed spherical aggregates 300-400 μm in diameter. At 4 d in vitro the cells were undifferentiated but by 26 d, central regions of aggregates contained neurons with processes, growth cones, and synapses. Astrocytes, oligodendroglia and myelin sheaths were present

also. 3) During metamorphosis, large redundant myelin loops appeared in many of the larger sheaths in the center of the optic nerve. These loops were broken down into segments and ovoids within oligodendroglia and were no longer present in nerves of young frogs. There was no significant axonal degeneration and measurements showed that during metamorphosis optic nerve length increased 40-50%. 4) The interlamellar tight junctions found in freeze-fracture replicas of tadpole optic nerves were similar to those already described in other species. In thin sections of Durcupan embedded nerves, it was clear that these junctions were formed by fusion of adjacent intraperiod myelin lamellae. In transverse sections, they occurred in register and appeared as a series of lines traversing the sheath radially where the tongue processes joined the compact layers of the myelin spiral. These tight junctions were impermeable to Lanthanum chloride and were almost always found at the margins of intramyelinic vacuoles produced by hexachlorophene.

Significance to Biomedical Research and the Program of the Institute: 1) Our new modification of the unlabelled antibody method has made it possible to investigate the developmental sequence of BP synthesis in oligodendroglia and rapidly growing myelin sheaths. Intense staining of oligodendroglial processes and myelin sheaths early in myelination makes it possible to count the number of sheaths being formed by a single oligodendrocyte. Since the method is both sensitive and specific, it should be extremely useful for studying the distribution of BP and other myelin constituents during development, the evolution of lesions associated with myelin breakdown, and the regeneration of CNS myelin. 2) Aggregating cultures of mechanically dissociated rat CNS cells differentiate morphologically into a population of neurons and glia that resemble those developing in vivo. A major advantage of this culture system is that the tissue yield is sufficient to permit biochemical and morphological study of a relatively small group of cultures without having to use micro methods. Currently we are investigating the effect of vitamin E on myelin formation and maintenance. 3) The findings in Xenopus tadpoles strongly suggest that during metamorphosis, myelin sheaths and axons are remodelled as the optic nerves shorten. This appears to be a striking example of oligodendroglial and axonal plasticity that deserves further investigation by testing the effects of varying levels of tyroid hormone and environmental temperature. 4) Our observations on optic nerve myelin sheaths embedded in Durcupan have shown that interlamellar tight junctions can be identified in thin sections. They correspond to the "radial component" described earlier by others and to the rows of junctional complexes seen in freeze-fracture replicas. The tracer experiments show that these tight junctions probably play a major role in preventing myelin breakdown in spongioform encephalopathies such as hexachlorophene intoxication.

Proposed Course of the Project: To be continued. The above findings were presented at annual meetings of the American Society for Neurochemistry, the American Association of Anatomists, the Winter Conference on Brain Research, and the VIII International Congress of Neuropathology.

Publications:

Webster, H. deF., Tabira T., and Reier, P. J.: Examination of the developing nervous system of Xenopus tadpoles with differential-interference microscopy: A new assay procedure for neurotoxicologists. In Roizin, L. et al. (Eds.): Neurotoxicology. New York, Raven Press, 1977, pp. 403-411.

Sternberger, N. H., Itoyama, Y., Kies, M. W., and Webster, H. deF.: Immunocytochemical method to identify basic protein in myelin-forming oligodendrocytes of newborn rat CNS. J. Neurocytol. 7: 251-263, 1978.

Sternberger, N. J., Itoyama, Y., Kies, M. W., and Webster, H. deF.: Myelin basic protein demonstrated immunocytochemically in oligodendroglia prior to myelin sheath formation. Proc. Nat. Acad. Sci. USA. 75: 2521-2524, 1978.

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. (in press).

Trapp, B. D., Honegger, P., Richelson, E., and Webster, H. deF.: Morphological differentiation of mechanically disassociated fetal rat brain in aggregating cell cultures. Brain Res. (in press).

Cullen, M. J., and Webster, H. deF.: Remodelling of optic nerve sheaths and axons during metamorphosis in Xenopus Laevis. J. Comp. Neurol. 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 01996-06 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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	Research Chemist	DMN NINCDS

COOPERATING UNITS (if any) Developmental and Metabolic Neurology Branch, NINCDS; G. H. DeVries, Medical College of Virginia, Richmond, Va.; J. M. Matthieu, University of Lausanne School of Medicine, Lausanne, Switzerland; M. J. Cullen, University of Southern California School of Medicine, Los Angeles, California

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Cellular Neuropathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	.7	.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 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 frogs and tadpoles, the developmental origin of proteins and glycoproteins in myelin sheaths of the rabbit optic nerve and the electron microscopic appearance in freeze fracture replicas of myelin and axolemma enriched membranes isolated from rabbit optic nerves.

Project Description:

Objectives: 1) To isolate myelin from the CNS of Xenopus frogs and tadpoles, study its electron microscopic appearance, and characterize it biochemically. 2) To study the contribution of axonal transport to the production of proteins and glycoproteins found in myelin sheaths of the rabbit optic nerve. 3) To isolate myelin and axolemma enriched fractions from rabbit optic nerves, prepare freeze fracture replicas and study their electron microscopic appearance.

Methods Employed: 1) Brains and spinal cords of Xenopus frogs and tadpoles were used to isolate purified myelin according to the method of Norton and Poduslo. Part of each fraction was fixed, embedded, sectioned and stained for electron microscopic study. The remainder was used for biochemical analysis. 2) Intraocular and intracerebral injections of differently labelled precursors were done in each of a group of rabbits. Myelin and an axolemma-enriched fraction were isolated from optic nerves, chiasm and tracts; these fractions were studied biochemically and aliquots of each were fixed, embedded and sectioned for electron microscopic study. 3) We prepared freeze fracture replicas of myelin and axolemma enriched fractions isolated from rabbit optic nerves and compared their fine structure with that observed in myelin and axolemma found in replicas of rabbit optic nerves removed after fixation in vivo.

Major Findings: 1) Myelin purified from the central nervous system of Xenopus Laevis frogs contained the same major lipid and protein components as human myelin. Minor differences in proteins included a higher apparent molecular weight for basic protein than the corresponding mammalian protein. Also the absolute specific activity of 2',3'-cyclic nucleotide 3' phosphohydrolase (CNP) was considerably higher in Xenopus myelin than in mammals. In myelin isolated from Xenopus tadpoles, the percentage of high molecular weight proteins and the specific activity of the myelin associated enzyme, CNP, were higher than in Xenopus frogs. 2) Typical myelin proteins and glycoproteins were only significantly labelled by precursors injected intracerebrally. The pattern of proteins and glycoproteins in myelin labelled by intraocular injection was very similar to that obtained in the axolemma-enriched fraction by the same route. In electron micrographs single membranes comprised most of the profiles seen in the axolemma enriched fraction; multilamellar membranes with the appearance and periodicity of compact myelin were seen in the myelin fraction. 3) When rabbit optic nerves were fixed in situ, removed, frozen, and fractured, the distribution of intramembranous particles in myelin and the axolemma was similar to that described in other species. In freeze-fracture replicas of myelin and axolemma-enriched fractions, many areas were free of intramembranous particles. Clusters of globular and rod shaped particles were present in other areas of the myelin fraction and some membranes in the axolemma enriched fraction had clusters of particles typical of those seen on the axolemma.

Significance to Biomedical Research and the Program of the Institute: 1) Since the biochemical compositions of Xenopus and human CNS myelin are

similar, the use of Xenopus CNS tissue as a test system for myelinotoxic agents important in the pathogenesis of human demyelinating diseases seems justified. 2) The results noted above indicate that neuronal metabolism and axonal transport do not contribute significantly to the synthesis of specific myelin proteins and glycoproteins. They also suggest that the components of myelin fractions which are labelled by intraocular injection are contaminants of axolemmal origin. One of these glycoproteins may prove to be a useful marker of axolemmal membranes. 3) Our electron microscopic observations support the concept derived from biochemical data that the 0.8/1.0 and 1.0/1.2 fractions are enriched in axolemmal membrane. The clustering of intramembranous particles also suggests that isolation of membrane fractions is associated with lipid and/or protein associations not seen in vivo.

Proposed Course of the Project: To be continued. The above findings have been presented at annual meetings of the American Society for Neurochemistry and the American Association of Anatomists.

Publications:

Matthieu, J.-M., Webster, H. deF., Beny, M., and Dolivo, M.: Characterization of two subcellular fractions isolated from myelinated axons. Brain Res. Bull. 2: 289-298, 1977.

Reier, P. J., Tabira, T., and Webster, H. deF.: Hexachlorophene-induced myelin lesions in the amphibian central nervous system: A freeze fracture study. J. Neurol. Sci. 35: 257-274, 1978.

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.

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. 1978 (in press).

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. 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 01805-10 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Membrane Structure

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

PI:	J. J. Anders	IPA Biologist	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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	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)

Within the freeze-fractured membranes of marginal astrocytes, the orthogonal arrays or "assemblies" of small particles are counted and the area they occupy measured in fetal, newborn, young, and mature rats. Regional differences, i.e. those between dorsal and lateral cerebral cortex and medulla in adult rats are beirg assessed to provide a base line for any changes brought about by different treatments. The greatest number of assemblies are in the adult dorsal cortex. The least number of assemblies by far are in the 20 day old fetus in all regions of the brain. The number rises appreciably during the first 72 hours. The assemblies within glial scars are also being examined.

In order to differentiate glia from neurons in situ and in vitro an immunocytochemical localization of nerve specific enolase (NSE) and non-neuronal enolase (NNE) is proving highly useful. Cells derived from neural crest, such as islet cells of pancreas, adrenal medulla, and parafollicular (calcitonin) cells of the thyroid are also selectively stained by NSE antibody, as might be such cells after neoplastic transformation.

Project Description:

Objectives: To determine ontogenic changes in number and area of particle assemblies within cell membranes of developing astrocytes and astrocytic scars and the type of enolase antigen in neuroblasts and in cells derived from neural crest.

Methods Employed: Twenty-day old fetuses, newborn and mature rats are fixed briefly with aldehydes and the membranes of marginal astrocytes are freeze-cleaved. Newborn rats are injected subcutaneously with 3 to 6 mg/kg body weight of cycloheximide which inhibits protein synthesis. "Subtle" glial scars are produced by placing autonomic neurons on the surface of the medulla and fixing the brains 1 month later.

A fixative consisting of dilute aldehydes and picrate enable the reliable binding of peroxidase labeled antibody to NSE and NNE in tissue culture and in 20 μm thick slices of brain, pancreas, thyroid and adrenal medulla.

Major Findings: In 20 day old fetuses and newborn rats there are only few assemblies (10-46/μ^2) which increase with age of the rats (310/μ^2 in adults). Regionally, the assemblies are most numerous in dorsal cortex and medulla and less in lateral cortex.

NSE antibody labels spinal cord neurons *in vitro* and most neurons throughout the adult brain and cells derived from neural crest: islet cells, adrenal medullary, and parafollicular (calcitonin) cells of the thyroid.

Significance to Biomedical Research and the Program of the Institute: By manipulating the intramembranous particles of astrocytes we may learn something of how these cell membranes are involved in the activity of normal glial cells and, eventually, of developing, gliotic and neoplastic astrocytes.

The localization of NSE and NNE during development should provide information on metabolic maturation in brain cells and, perhaps, early detection of neural-crest-derived neoplasms.

Proposed Course of the Project: Now that the base-line of assembly number and area has been established, we may be able to detect changes following different treatments.

To determine whether NSE is also a marker for neuroblasts before or after the presumptive neurons become differentiated.

Publications: Prescott, L. and Brightman, M. W.: A technique for the freeze-fracture of tissue culture. J. Cell Sci. 30: 37-43, 1978.

MITHSONIAN SCIENCE INFORMATION EXCHANGE ROJECT 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 02086-05 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Regeneration in Vertebrate and Invertebrate Nerves

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

PI:	J. M. Rosenstein	Guest Worker	LNNS NINCDS
Other:	M. W. Brightman	Head, Sect. on Neurocytology	LNNS NINCDS

COOPERATING UNITS (if any)

None

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	0.3

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

(a1) MINORS ☐ (a2) INTERVIEWS

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

he interaction between transposed autonomic ganglion and the intact, still-eveloping brain of 6 to 14 day old rats have been examined by light and lectron microscopy. When 1 mm^3 fragments of the superior cervical ganglion SCG) are transplanted to the undamaged pial surface of the cerebellum in young ats, cells - both neuronal and glial - of the external granule layer (EGL) are ested in the molecular layer. Three months after they should have migrated o form the internal granule layer, whole laminae of these cells not only fail o migrate in the normal direction but some leave the cerebellum and, together ith cerebellar neuropil, migrate into the transplant. Within the transplant, ome of the ganglion cells die but there is a marked proliferation of axons. nmyelinated axons are enclosed by Schwann cells and these, in turn, by ibroblasts. After six months, many axons have become myelinated. The rmation of nodes of Ranvier indicates cooperation between Schwann cells ch also bedeck ependymal and glial surfaces as well as neuronal processes.

S-6040
ev. 10-76)

Project Description:

Objectives: To determine whether isolated pieces of peripheral and central nervous system (CNS) can grow within cerebrospinal fluid (CSF) compartments and to induce the regenerating axons to grow into selected regions of the CNS across intact ependyma and glia.

Methods Employed: Fragments of superior cervical ganglion (SCG) from 21 day old rats are marked with carbon black and inserted gently upon the floor of the IV ventricle beneath the cerebellar nodulus of recipient rats 6 to 14 days old (homografts). Other implants are placed over the pia of the medulla so as to overlie glia instead of ependyma and choroid plexus. Care is taken not to injure either glia or epithelium. After various periods, the recipient is fixed with aldehydes and osmium for light and electron microscopy.

Major Findings: Surprisingly, where SCG pieces are placed on the undamaged cerebellar surface, whole laminae of external granule layer (EGL) cells of the cerebellum are arrested months after they should have migrated to the internal granule cell layer. Some of the EGL cells together with cerebellar neuropil actually invade the SCG graft which, by the 6th month, contains many myelinated axons.

Significance to Biomedical Research and the Program of the Institute: This system, which is readily accessible with little or no trauma and which can be irrigated *in situ*, permits examination of interactions between regenerating neurites and brain surface.

Proposed Course of the Project: To determine whether non-neuronal tissue elicits the same migration of neurons out of the brain and what the factors are that "coax" neurites from brain into implant and, ultimately, in the opposite direction.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPAPTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02144-04 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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:	S. I. Rapoport	Neurophysiologist	LNP NIMH
	K. Dorovini-Zis	Visiting Fellow	LNNS NINCDS

COOPERATING UNITS (if any)
J. Robinson, Evanston Hospital, Evanston, Illinois
Laboratory of Neurophysiology, NIMH

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SECTION
Section on Neurocytology

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

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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 mechanisms whereby the blood-brain barrier (BBB) to proteins, e.g. horseradish peroxidase (HRP) is opened in response to hyperosmotic agents are being scrutinized with a tracer smaller than HRP or colloidal lanthanum. The inference that the openings may be parajunctional channels through the endothelium rests on our demonstration that vesicular transfer cannot account for the escape of protein; the exudate pattern is the same before fixation or after fixation, which halts vesicular movement. In order to see whether junctions or channels are implicated, a tracer not much larger than the hydrated sodium ion, i.e. ionic lanthanum is to be used. The threshold concentration of 1.6 M arabinose that has been used for rats and of 2.0 M urea used for rabbits will be used on rat specimens only. The disadvantage of ionic lanthanum is that it cannot be detected by light microscopy. The great advantage is that the tracer, being ionic, is more interesting physiologically and the results may be applicable to other di- and tri-valent cations. About 2 hours after arabinose administration, 90% of the barrier has been re-established. We shall examine these reversibly "closed" vessels along with the few that are still permeable.

Project Description:

Objectives: To assess morphological correlates of reversible closure of the blood-brain barrier (BBB) to protein after osmotic opening and to see whether tracer size (ion vs. protein) determines barrier passage during osmotic opening.

Methods Employed: To infuse into one carotid artery, 1.6 M arabinose which is above threshold for opening the barrier to HRP (horseradish peroxidase). Six to 12 hours later, when the opening to sucrose has been reversed by about 90%, to inject HRP systemically and to fix in order to examine, ultrastructurally, the remaining 10% of still permeable vessels.

To repeat this experiment with 1.0-1.2 M arabinose, below the threshold of opening of the BBB to peroxidase, to see whether the much smaller ionic lanthanum can pass the barrier at lower osmotic threshold than does the much larger peroxidase molecule.

Major Findings: Although very few, if any, endothelial junctions have HRP passing through them at sites of exudation, the junctions are widely separated in some specimens that had been fixed prior to the infusion of HRP via the aorta.

Significance to Biomedical Research and the Program of the Institute: An understanding of how the BBB may be opened reversibly without damage to brain tissue and the conditions that effect the escape of ions from capillaries may be useful in considering therapeutic substances of different sizes normally excluded from the brain by its barriers.

Proposed Course of the Project: To detect how, where and when ionic lanthanum escapes from brain capillaries during hyperosmotic changes of cerebral vessels and the distribution of tracers after re-establishment of the barrier.

Publications: Brightman, M. W.: Morphology of blood-brain interfaces. Exp. Eye Res. 25: 1-25, 1977.

ITHSONIAN SCIENCE INFORMATION EXCHANGE JECT 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 04 LNNS

ERIOD COVERED
October 1, 1977 to September 30, 1978

TLE OF PROJECT (80 characters or less)

Identification of Neurons having Terminals in the Median Eminence and Area Postrema

S, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHE ESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. D. Broadwell	Staff Fellow	LNNS NINCDS
Other:	M. W. Brightman	Head, Section on Neurocytology	LNNS NINCDS

OOPERATING UNITS (if any)

None

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NINCDS, NIH, Bethesda, Maryland 20014

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1.0	1.0	0

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

(a1) MINORS ☐ (a2) INTERVIEWS

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

ie number of HRP positive organelles in undamaged motoneurons and neuro-ecretory neurons after retrograde transport of the protein is about twice hat following orthograde loading. The orthograde axoplasmic flow of HRP is ery slight in these two classes of neurons in hydrated mice. After 5 days f drinking 2% NaCl solutions, the anterograde flow is appreciably increased neurosecretory neurons but not in motoneurons. That the HRP laden vacuoles neurons are lysosomes is shown by simultaneous demonstration of HRP and id phosphatose (ACP) activities in the same vacuole. In the GERL (Golgi sociated smooth endoplasmic reticulum and lysosomes) cisterns of the somata neurosecretory cells, the ACP activity diminishes while there is a ncomitant rise in thiamine pyrophosphatose activity in the adjacent Golgi mplex. Thus, prolonged osmotic stimulation results in an increase in tabolic and degradative enzymatic activities at the same time that the hograde transfer of protein increases. The fall in ACP activity in the RL might reflect a diversion of degradative enzymatic activity to the sosomes which are faced with an increased burden of protein.

S-6040
ev. 10-76)

Project Description:

Objectives: To follow the orthograde and anterograde transport of protein from blood and CSF in neurons of dehydrated mice.

Methods Employed: Peroxidase (HRP) is perfused ventriculo-cisternally for 10 minutes in normal mice and in those given 2 per cent sodium chloride solution to drink for 5 days. The mice were fixed from 6 to 24 hours later. Acid phosphatase, thiamine pyrophosphatase and HRP activity were localized at light and electron microscope levels.

Major Findings: About twice the amount of HRP enters undamaged somata of cranial motoneurons and neurosecretory cells by retrograde axonal transport than by anterograde transfer from dendrites and the somal membrane. The anterograde flow of HRP within neurosecretory axons of dehydrated mice is considerably greater than in hydrated mice. We have demonstrated, for the first time, a marked increase in thiamine pyrophosphatase activity in the Golgi complex of such osmotically stimulated neurons. These changes are reversed when the mice are rehydrated.

Significance to Biomedical Research and the Program of the Institute: The increased anterograde transfer of exogenous protein along the axons of neurosecretory cells and the heightened enzymatic activity of their Golgi complex demonstrates a reversible adaption of these neuroendocrine cells to osmotic stress.

Proposed Course of the Project: The findings are 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 02200-03 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Freeze-Fracture of Cell Membranes Intercalated with Lipids

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

PI: M. W. Brightman Head, Section on Neurocytology LNNS NINCDS

COOPERATING UNITS (if any)

M. Sato, Kyoto University, Kyoto, Japan

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The polyunsaturated fatty acid (FA), linolenic acid, when injected into the blood, crosses cerebral vessels by passing directly across the endothelial cell membrane. Some of the FA fills endothelial pits and vesicles and may represent that portion of the FA circulating in combination with serum albumin to which it is normally bound. Within endothelial, smooth muscle and adventitial cells, FA droplets penetrate the perinuclear cistern but are not visible within the nucleus. Droplets also enter choroid plexus epithelium from blood or CSF by passing directly across the cell membrane rather than in vesicles.

Project Description:

Objectives: To determine the distribution of fatty acid (FA) visible by both light and electron optics, within blood vessels, choroid plexus and neurons.

Methods Employed: High concentrations of linolenic acid, which is strongly osmiophilic, is infused into one carotid artery or through the cerebral ventricles of rats and the tissue prepared for electron microscopy and freeze-fracture.

Major Findings: FA droplets, probably in the form of sodium and potassium soap, occur not only randomly across cell membranes and in the cytoplams of endothelial cells but, in segments of the basilar and carotid arteries, the great majority of droplets fill pinocytotic vesicles. Within the cerebral parenchyma, some synaptic vesicles are also labeled by the FA and the postsynaptic web of dendrites are heavily stained. In choroid plexus, FA droplets not only pass between epithelial cells but cross their cell membranes directly to penetrate the double membrane of mitochondria.

Significance to Biomedical Research and the Program of the Institute: Although high concentrations of this FA are required to be visible in both the light and electron microscope, the distribution of FA droplets offers an approximation of two routes by which fatty acid may enter different cell types in the brain: direct passage across cell and cyto-membranes and via vesicles, presumably when the FA is bound to serum protein.

Proposed Course of the Project: To publish findings.

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 01[illegible]-12 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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)
R. L. Gulley, Case Western Reserve University, Cleveland, Ohio
D. M. D. Landis, Massachusetts General Hospital, Boston, Massachusetts

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NINCDS, NIH, Bethesda, Maryland 20014

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.05	.05	0

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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 location and nature of extracellular barriers to colloidal substances at a variety of locations in the nervous system has been determined. In the organ of Corti, extensive systems of tight junctions form continuous bands surrounding cells and occluding extracellular spaces. It is these bands of junctions which control the movement of colloidal and probably ionic substances in intervening extracellular spaces. Furthermore, these membrane structures disappear rapidly in anoxia, suggesting that they depend on metabolic factors for their maintenance. These data on the cellular basis of brain barriers, as well as the new structural approaches used in these studies, afford a basis to design experimental studies of a variety of clinically important conditions which depend on or produce damage to brain barriers. They also give rise to new ideas about the roles of various cells associated with neurons and about the function of barrier systems in special regions, such as the organ of Corti.

Project Description:

Objectives: Determination of the structural basis of biologically and clinically important barriers in the nervous system.

Methods Employed: a) The organ of Corti in chinchillas was examined with the freeze-fracture technique to determine the types and patterns of junctions between the various types of epithelial cells that separate the endolymph from perilymph. The permeability of these junctions to proteins was tested by injecting horseradish peroxidase into the subarachnoid space, from where it diffused into the perilymph, and then using the electron microscope to determine the distribution of peroxidase in the organ of Corti. b) The astrocytic sheaths at the arachnoid surface of the brain and at the interfaces of the brain with blood vessels in mice were examined with the freeze-fracture technique in brain slices incubated in appropriate medium bubbled with O_2 or N_2.

Major Findings: a) Barriers in the organ of Corti of the mammalian inner ear were the object of a collaborative study with Dr. Robert Gulley. The perilymph is separated from the endolymph by tight junctions between the apices of hair and supporting cells. These tight junctions prevent peroxidase injected into the perilymph from reaching the endolymph.

The freeze-fracture technique was used to examine the architecture of these junctions in more detail. They are more extensive, and presumably, "tighter" than in any other tissue studied so far. The unique form of these tight junctions also suggests that they might have functions other than to prevent mixing of endolymph and perilymph and, perhaps, are involved in the first steps in the sensing of mechanical displacements in the organ of Corti.

In the course of studying the tight junctions other nonjunctional specializations of the lateral borders of the hair cells were noted. In fact, these specializations, which are not found on any other types of cell, clearly distinguish the inner from the outer hair cells.

b) The freeze-fracture technique was used to study the structure of astrocytic membranes, particularly where they surround cerebral blood vessels, or form the outermost surface of the brain next to the cerebrospinal fluid. These membranes are characterized by many assemblies of small particles that are never found on neurons and are less frequent elsewhere on astrocytic membranes. These assemblies were examined in slices of cerebellum incubated in artificial cerebral spinal fluid for up to 30 minutes. They were maintained if the medium was bubbled with O_2 but disappeared in N_2. They also disappeared from the intact brain if the circulation was interrupted for 15 minutes or more.

Significance to Biomedical Research and the Program of the Institute: We determined the location of barriers to proteins at significant fluid interfaces in the brain and sense organs. Our determinations depend on cytological

techniques that show specifically which components of which layers are permeable. Thus, we are able to determine the cells as well as the probable mechanisms that control the chemical environment of the brain and related structures. This year we defined, for the first time, the cellular and subcellular basis of the barrier between the endolymph and perilymph. These techniques are now available for clinically oriented research to see the role of pathological changes in these barriers in various diseases of the middle ear. Also of interest is that our recent data indicate a possible role for astrocytes in the brain barrier system. This possibility will focus attention on the role of these glial cells in pathological conditions affecting the brain barrier system.

Proposed Course of the Project: The final papers on the organ of Corti have been published. No further work on this subject is planned. The work on the effects of oxygen lack on the assemblies of small particles in astrocytic membranes was repeated in the last year and we plan to prepare a paper on this subject. This project will receive little attention in the next year.

Publications: Gulley, R.L. and Reese, T.S.: Freeze-fracture studies of the non-junctional membrane specializations in the organ of Corti. Anat. Rec. 189: 109-123, 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 01881-08 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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:	S. Cohen	Staff Fellow	BBB NICHD
	K. J. Lynch	Guest Worker	LNNS NINCDS
	R. L. Ornberg	IPA Biologist	LNNS NINCDS
	D. W. Pumplin	Guest Worker	LNNS NINCDS
	R. P. Rees	Guest Worker	LNNS NINCDS

COOPERATING UNITS (if any)
Behavioral Biology Branch, NICHD
M. Dennis, University of California School of Medicine, San Francisco, CA
C. Franzini-Armstrong, Pennsylvania Muscle Institute, Philadelphia, PA (cont'd. next page)

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NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.5	4.0	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 are being 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):

M. E. Eldefrawi, University of Maryland School of Medicine, Baltimore, MD
A. T. Eldefrawi, University of Maryland School of Medicine, Baltimore, MD
R. L. Gulley, Case Western Reserve University, Cleveland, OH
M. Henkart, Armed Forces Radiobiology Radiation Institute, Bethesda, MD
J. E. Heuser, University of California Medical Center, San Francisco, CA
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 μm 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 msecs later in preparation for either freeze-fracturing or freeze-substitution. The initial stages in secretion have also been studied in Limulus amebocytes (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 amebocytes 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 microelectrodes. 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 exoctyosis 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 neruomuscular 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 the purchase in the next year of a high resolution analytical scanning transmission electronmicroscope.

Publications:

Heuser, J. E., and Reese, T. S.: Structure of the synapse. In Kandel, E. (Ed.): The Handbook of Physiology, The Nervous System I. American Physiological Society, 1977, pp. 261-294.

Pumplin, D. W. and Reese, T. S.: Action of brown widow spider venom and botulinum toxin on the frog neuromuscular junction examined with the freeze-fracture technique. J. Physiol. 273: 443-457, 1977.

Rees, R. P.: The morphology of interneuronal synaptogenesis: a review. Fed. Proc. 37: 2000-2009, 1978.

Gulley, R. L., Landis, D. M. D. and Reese, T. S.: Internal organization of membranes at end bulbs of Held in the antero-ventral cochlear nucleus. J. Comp. Neurol. 180: 707-742, 1978.

Rees, R. P.: Structure of cell coats during initial stages of synapse formation on isolated cultured sympathetic neurons. J. Neurocytol. In press.

Pumplin, D. W. and Reese, T. S.: Membrane ultrastructure of the giant synapse of the squid Loligo peali. Neurosci. In press.

Heuser, J. E. and Reese, T. S.: Synaptic Vesicle Exocytosis Captured by Quick-Freezing. In Schmitt, F. O. (Ed.): Fourth Intensive Study Program in the Neurosciences. MIT Press. In press.

Rheuben, M. B. and Reese, T. S.: Membrane structure at neuromuscular junctions in the moth. J. Ultrastruct. Res. 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 01449-12 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The effect of hormones on the retrograde neuronal reaction

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 Experimental Neuropathology LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Experimental Neuropathology

INSTITUTE AND LOCATION
NIH, NINCDS, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.9	.3	.6

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)

Rabbits subjected to facial nerve transection during cortisone acetate treatment display acute depression of regenerative changes in neuronal perikarya and of reactive mitosis in microglial cells. To determine whether these reactions can result in a more severe impairment of neuronal regeneration, chronic experiments have been started for quantitative studies. A comparison with thyronine treated animals is carried out in order to establish the nature of the action of the two hormones on the central nervous system. This may have some relevance to the prognostication of clinical observations and treatment.

Project Description:

Objectives: To determine what effect hormonal treatment may have on the recovery phases of motor neurons subjected to transection of their axons.

Methods Employed: Rabbits were treated with cortisone or thyronine for a week prior to severance of the facial nerve.

Horseradish peroxidase powder was applied to the cut end in order to assess the intensity of axonal flow in cortisone treated and non-cortisone treated animals. The peroxidase technique was used also for determining the area which is occupied by neurons undergoing retrograde reaction.

After short and prolonged survival, the deeply anesthetized animals were sacrificed and fixed by perfusion.

Serial sections of the brain stem were stained for glycogen and with PAS-gallocyanin. Formulation of a new fixation procedure has intensified the histochemical reaction for glycogen.

Major Findings: Preliminary studies revealed that, after cortisone treatment, there is in the operated animals a depression of reactive mitosis in microglial cells and an acute depletion of glycogen in neurons. The acute neuronal reaction is the same after thyronine treatment as in untreated animals. Preliminary review of a pilot study with horseradish peroxidase indicates that axonal flow is not intensified after cortisone treatment of operated young animals.

The horseradish peroxidase technique revealed (1) the site of perikarya sending axons to various facial muscles differs from that indicated in other studies, and (2) the area with peroxidase tagged neurons is smaller than that occupied by neurons undergoing acute retrograde reaction as demonstrated by conventional staining methods.

After experimental operations designed to study the chronic effect of these two hormones, no differences in clinical manifestations have been detected.

Significance to Biomedical Research and the Program of the Institute: After a study on the acute depressing effect of cortisone on neuronal perikarya and neuroglial elements, it is of interest to determine whether an opposite reaction can be obtained by introduction of another hormone, such as thyronine, which is said to accelerate peripheral nerve regeneration. By counting the number of cells remaining in the facial nucleus after single operation or reoperation, one should be in a position to conclude decisively whether administration of either of the two hormones can be of any benefit or can affect the vulnerability of nervous tissue. Definite guidelines for clinical application must await results of such experiments.

Proposed Course of the Project: (1) To prepare and examine histologic material from hormone-treated animals with acute and prolonged survival after single transection or reoperation. (2) To test the effect of cortisone on the axonal flow with horseradish peroxidase in animals in which the facial nerve was transected at varying ages. (3) To improve the fixation procedure in order to optimize the histochemical staining technique. (4) To compare the staining intensity of glycogen by use of different fixatives.

Publications: None

PROJECT NUMBER (DO NOT use this space)

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 NS 02002-06 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Mast cells in the brain

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

PI: Jan Cammermeyer Head, Section on Experimental Neuropathology LNNS NINCDS

COOPERATING UNITS (if any)
W. Flor, Department of Neurobiology, Armed Forces Radiobiology Institute, Bethesda, Maryland

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Experimental Neuropathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	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)

This project has been temporarily interrupted during installation of an electron microscope. It will be taken up again shortly.

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 02003-06 LNNS

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The thalamo-choroidal body

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 Experimental Neuropathology LNNS NINCDS

COOPERATING UNITS (if any)

E. Westergaard, Anatomy Department C, University of Copenhagen, Copenhagen Ø, Denmark

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Experimental Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	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)

This project has been temporarily discontinued until experience in the use of a newly installed electron microscope has been acquired.

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 02143-04 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Volumetric changes of brains during histologic preparation

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 Experimental Neuropathology 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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	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)

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 02195-03 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Perivascular cerebral siderosis as a marker of blood-brain barrier damage

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 Experimental Neuropathology 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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	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)

This project has been temporarily discontinued until experience in the use of a newly installed electron microscope has been acquired.

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 02284-02 LNNS

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Improvement of current methods of fixation by perfusion

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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.3	.7

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 presence of solitary dark neurons in material fixed by perfusion was noted when white matter as compared with gray matter was poorly fixed, as may happen when (a) amounts of perfusates are small, approximately 1-2% of body weight, (b) the fixative is introduced 5 minutes or longer after interruption of the systemic circulation, or (c) the brain is removed after postmortem intervals shorter than 4 hours when Bouin's solution is used.

The histochemical demonstration of glycogen was intensified after utilization of a perfusion procedure in which various recommendations were incorporated.

This project is completed, and a manuscript is in preparation.

Project Description:

Objectives: (1) To obtain consistent glycogen reaction in neurons and astrocytes, and (2) to prevent formation of "solitary" dark neurons.

Methods Employed: (1) 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.

(2) The distribution of solitary dark neurons is ascertained in paraffin sections of brains fixed by perfusion after modification of the various steps involved in this procedure. Speed of flow of the perfusates and specific gravity of the brains were determined for groups of three animals after change of a single step in the perfusion procedure.

Major Findings: (1) 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.

(2) The speed of flow and the specific gravity of the brain are influenced by changing certain steps in the perfusion procedure such as pressure and temperature without any effect, however, on the quality of neuronal fixation. Contrary to a previous statement, removal of the brain after a brief post-perfusion interval was found to be deleterious to the appearance of the neurons. The original recommendation of a 4-hour interval for material fixed by perfusion with a strongly acid solution was confirmed.

Significance to Biomedical Research and the Program of the Institute: (1) 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.

(2) An assessment of factors contributing to formation of the unusual solitary dark neurons is required to overcome still existing controversies concerning the role of dark neurons in experimental material.

Proposed Course of the Project: This project is completed, and a manuscript is in preparation.

Publications:

Cammermeyer, J.: Is the solitary dark neuron a manifestation of postmortem trauma to the brain inadequately fixed by perfusion? Histochemistry 56: 97-115, 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 02285-02 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.1	.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)

Brains from patients observed in Guam 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 00969-14 CNSS; Z01 NS 00201-23 ODIR).

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.

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 02286-02 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

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 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.1	.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)

The demonstration of cerebral hemorrhages in material fixed by perfusion offers an opportunity to examine factors involved in development of hemorrhages.

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 after vaying postinjection intervals. 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.

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.

Proposed Course of the Project: 1) In order to establish whether an increased venous pressure after arrest of the systemic circulation may cause effusion of erythrocytes, the jugular veins will be cut and the carotid arteries compressed for 10 min. prior to cannulation of the heart. 2) As soon as experience in use of the electron microscope installed in this section has been acquired, experimental oil embolism material will be studied in electron micrographs.

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 02333-01 LNNS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 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 Experimental Neuropathology LNNS NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Experimental Neuropathology

INSTITUTE AND LOCATION
NIH, NINCDS, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.2	.6

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Argentophilia of neuronal perikarya and perikaryal neurofibrils occurred whenever the brain was exposed to postmortem trauma prior to perfusion of the fixative. By two different methods, namely (1) reconstruction of a microscopic field with argentophil and chromophil neurons and (2) restaining with silver of a previously cresyl violet stained section, argentophilia and chromophilia were found to be different tinctorial manifestations of the same neuron. Thus, it has been possible to clarify the artifactual nature of argentophilia. This characteristic is associated with changes in neurofibrils which in the neuronal perikarya are argentophilic.

Project Description:

Objectives: To ascertain the specificity of argentophilia in neuronal perikarya and perikaryal neurofibrils.

Methods Employed: Bodian silver impregnation of paraffin embedded microscopic sections from animals fixed by different methods.

Major Findings: In scattered neurons, the perikaryal cytoplasm is intensely impregnated with silver. The neurofibrils in the perikarya of these neurons are often swollen, displaced and heavily impregnated with silver.

By reconstruction of the same field in contiguous sections stained alternately with silver and a basophil dye, and by restaining of cresyl violet-stained sections with silver, silver-stained neurons and dark neurons were found to be manifestations of the same neuron.

When the brains of newborn infants were fixed by immersion, the motor neurons exhibited marked neurofibrillary changes even though at this age no dark neurons were formed.

Significance to Biomedical Research and the Program of the Institute: The unequivocal demonstration that the argentophil neuron is another manifestation of the dark neuron and thus can be induced by postmortem trauma to the unfixed brain solves a 100 year long uncertainty about the significance of the silver impregnated neurons. In view of the realization that the argentophil neurons are of artifactual origin, opinions about the effect of experimental operations and drugs need to be revised.

Proposed Course of the Project: The results have been included in a manuscript forwarded for publication.

Publications: None


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