ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE
Fiscal Year 1975
Volume I

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

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ANNUAL REPORT

July 1, 1974 through June 30, 1975

PROJECTS LISTED NUMERICALLY

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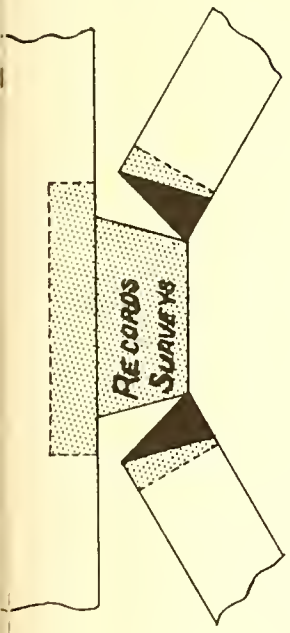

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LAB. OF BIOPHYSICS

SURGICAL NEUROL. BRANCH

CNS STUDIES LAB.

DIRECTOR'S REPORT

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ANNUAL REPORT
July 1, 1974 through June 30, 1975
National Institute of Neurological and Communicative Disorders and Stroke
National Institutes of Health

Director's Report

This year, 1975, is a rather special year for American neurology. The oldest neurological society in the world, the American Neurological Association, celebrated the centennial of its first meeting held in June of 1875. Seventy-five years later the efforts of key ANA members, several voluntary organizations, and interested Congressmen came to fruition on August 15, 1950, when President Truman signed the legislation creating the National Institute of Neurological Diseases and Blindness, and Dr. Pearce Bailey was appointed its first Director. To mark the Institute's 25th anniversary, we have invited several hundred scientists to contribute chapters to a three-volume record of the new knowledge and clinical advances in the neurological and communicative disorders generated by the research grant and training support provided by the Institute over the past quarter century. These silver anniversary volumes are now in press and will be published before the end of 1975.

Perhaps appropriately two major changes were also implemented during fiscal year 1975. The name of the Institute was modified from the now familiar NINDS to NINCDS, the National Institute of Neurological and Communicative Disorders and Stroke. This corrects, at last, a long-standing oversight, by recognizing the Institute's significant commitment to research and research training in the communicative disorders--disorders of hearing, speech, language and special sensory systems that affect more than ten percent of our population. Last year the Institute opened its first intramural research laboratory in this program area, the Laboratory of Neuro-otolaryngology. The name modification this year signals extension of the recognition of the NINCDS commitment to the total program.

Coincidentally the extramural and collaborative and field research divisions of the NINCDS have been amalgamated in a reorganization, from an adminsitrative structure oriented to mechanisms of support (grants, contracts) to one oriented to program areas. Four extramural research program areas have been established--in fundamental neurosciences (directed by Dr. Karl Frank); communicative disorders (directed by Dr. Wesley Bradley); neurological disorders (directed by Dr. Kiffin Penry); and stroke and central nervous system traumma (directed on an acting basis by Dr. Murray Goldstein)--each headed by an associate director of the NINCDS, with administrative support provided by an extramural activities program unit (directed by Drs. Murray Goldstein, O. Malcolm Ray and W. Watson Alberts). In addition to providing better focal points for the various groups of disorders, each of the program areas will utilize whichever support mechanism (research grant, contract, training award) is most appropriate for the achievement of program objectives. As pointed out by others elsewhere in this Annual Report,

reorganization into a programmatic mode is no guarantee of a more successful extramural program and carries special problems of its own. The NINCDS is the last NIH institute to reorganize its extramural program, and we reached the decision to do so with reluctance. Nevertheless, there is a consensus that the advantages of the programmatic mode clearly outweigh the disadvantages of abandoning an established, successful system and of coping with the problems of transition. There are real concerns over the general trends to increased emphasis on directed or targetted research and on "applied" research at the expense, potential or real, of fundamental research, but the NINCDS reorganization includes the appointment of external advisory committees for each of the program areas and the establishment of an Office of Program Planning and Evaluation--both of which provide a means for NINCDS surveillance of these problems.

During fiscal year 1975 a number of noteworthy workshops or conferences were sponsored wholly or in part by the NINCDS. One was on gamma-aminobutyric acid (GABA), the first since the original GABA conference in 1960, wih special reference to the role of GABA as an inhibitory transmitter in the brain and spinal cord and its relevance to Huntington's disease, Parkinson's disease, epilepsy and the like. The conference and the monograph of the proceedings were supported jointly by the Kroc Foundation and the NINCDS.

At the behest of the Secretary, DHEW, the Institute convened two workshops on the current status and future potential of cochlear (or auditory) implants. The recommendations from these workshops are being translated into research grant and contract projects to provide needed additional experimental and bioengineering data and to evaluate in depth the small group of profoundly deaf patients already fitted with implants.

At midyear, the NINCDS brought together in Bethesda representatives from most of the professional associations and voluntary organizations concerned with neurological and communicative disorders. Since these 60-plus groups seldom, if ever, all meet each other, the conference catalyzed valuable interchanges but its principal thrust was a discussion of how best to avoid fragmentary efforts and to concentrate on unified approaches to the problems posed by the neurological and communicative disorders. This conference represents one example of how the interface between an NIH institute and the relevant health-care community can be facilitated.

After much planning, the workshop on the fundamental aspects of spinal manipulative therapy was held at Bethesda in February 1975. At this workshop some 70 chiropractic, osteopathic, and medical practitioners and research scientists gathered for a three-day, in-depth examination of what is known and what is not known about the bases for spinal manipulative therapy as practised by those three groups of practitioners. The exchanges were frank but gratifyingly constructive, so that the Institute can anticipate the generation of a number of research proposals addressed to the important gaps in knowledge identified at the workshop.

Throughout fiscal 1975 a series of workshops were held on various aspects of central nervous system regeneration, sponsored by the special subcommittee of the NINCDS Advisory Council. Each of the five workshops provided exciting new experimental data in a milieu which brought together investigators from many disparate disciplines and catalyzed their communal interests in various facets of the regeneration problem. The proceedings of the workshops have been published in summary form and the recommendations of the NINCDS Advisory Council subcommittee are about to be submitted for publication. Clearly the potentiality for central regeneration exists; the task ahead is to learn how to utilize this potential effectively for the paraplegic, the stroke victim, and others with interrupted central nervous pathways. There is much basic research still to be done, but these workshops have identified the research areas in which to initial significant forward steps in this endeavor.

The momentum generated last year in the area of multiple sclerosis (MS) has continued this year. A conference-workshop was held in Bethesda at which current progress in the virological, immunological and genetic aspects of MS was discussed at some length. Abstracts of the presentations and a summary of the proceedings have been published. The contract for the epidemiological survey of MS in the Shetland and Orkney Islands is nearing completion. Most of the results have not yet been fully analyzed, but it is already clear that in these islands where the prevalence of MS was known to be the highest in the world, the prevalence is even higher than had been previously suspected. In May 1975 a small delegation of U.S. scientists went to Moscow under the U.S.-USSR exchange program to meet with their Soviet counterparts of an exchange of information on MS. The four-day conference covered epidemiology, virology, immunology and clinical therapy, with comprehensive review presentations by scientists from each country. The reviews of Soviet epidemiological and clinical studies were frankly presented and particularly valuable, since much of the information has not been readily available outside the Soviet Union. The unique situation of significant "gradients" of MS prevalence within the confines of the Soviet Union seemed to warrant further evaluation in conjunction with studies of histocompatibility antigen and viral antibody profiles, and such a study is under consideration.

Because of the reorganization of the NINCDS extramural programs already noted, this is the last annual report in which the traditional extramural subdivisions of the Institute are represented. In the extramural grants and training programs (EP) the fiscal year has been characterized by a very long delay (until almost the fourth quarter) before the FY 1975 appropriation was allocated to fund new and renewal research grants. The delay was occasioned first by the slowness with which the appropriation was passed and signed--in mid-December, almost midway in the fiscal year. Immediately thereafter the recission exercise occurred, imposing another three-month delay. The program management problems imposed by these delays were compounded by a prohibition on the use of multiyear funding of a portion of the new research grants to lessen the impact on the future commitment base.

The appropriation for NINCDS for fiscal year 1975 at $142.5 million was approximately equal to the FY 1974 appropriation plus the FY 1973 "impounded" funds released during FY 1974. Of the $142.5 million, approximately $100 million was available for the EP research grants and training programs. Thus during FY 1975 a total of 1198 research grants (individual, program project and outer grants) were funded at a cost of $86.6 million. By comparison in FY 1971, a total of 1256 grants were funded but the total cost was only $53.6 million--an indication of the inflation factors operating over the past five years. Not only has the average cost per research grant nearly doubled but the proportion devoted to indirect (overhead) costs has risen on the average from about 30 percent to about 45 percent. Thus the purchasing power of our 1975 dollar for actual research is disturbingly much less than it was only a few years ago. This is a factor not regularly taken into account when the slow pace of research advances and clinical applications are decried.

Fiscal year 1975 was an extraordinarily complicated year for training programs. The phase out of the traditional training grant programs continued, so that by the end of FY 1976 essentially none of these programs will be in existence. The substitute fellowship program instituted by the Secretary, DHEW--the socalled Weinberger fellowships--are also being phased out as a consequence of Congressional legislation creating the National Research Service Award program with both individual fellowships and institutional grants (the latter fixed at 25 percent of the total available funds). The legislation required studies by the National Academy of Sciences to certify manpower needs before fellowships in the various disciplinary or clinical areas could be awarded. But time did not permit adequate studies before the end of the fiscal year, so that existing guidelines were recommended for another year. The confusion in the scientific community over these many mutations on the training theme has been understandably total. Fortuitously the NINCDS had undertaken manpower surveys in its major basic and clinical areas, which together with a recently published DHEW-HRA survey of health manpower provide the Institute with sound bases for its training program priorities. One casualty(hopefully only temporary) has been the NINCDS minorities training program in the neurosciences; its suspension has been lifted and we hope to reinstate it during FY 1976.

The spectrum of EP research projects being supported by NINCDS this year continues to be wide ranging and exciting. Some examples follow. Support for targetted research comprised 101 program project and clinical research center grants, including: 15 stroke clinical research centers and 11 stroke acute care research units; 4 head injury and 5 acute spinal cord injury clinical research centers; 9 centers for epilepsy, 4 for neuromuscular disorders and 7 for sclerosing disorders (MS, ALS, etc) clinical research; 4 parkinsonism clinical research centers; and 5 clinical or outpatient research centers in the communicative disorders. The total direct costs for these 101 targetted research projects approxi---mated $28.2 million in FY 1975.

Among the 1000-plus individual research grants, projects are being supported in the following areas: synaptogenesis studied in organ culture;

of motor disorders and of x-chromosome-linked characteristics, utilizing the fruit fly, Drosophila; application of scanning electron microscopy to localized alterations of vascular endothelium during lipemia or ischemia; evaluation of geographic as well as ethnic differences in stroke mortality; trials of immunosuppression of gliotic scars with "astrocyte maturing factor"; monitoring of evoked potentials as an indicator of spinal cord integrity after injury; correlation of edema and local circulatory changes with post-traumatic changes in levels of norepinephrine and of tyrosine hydroxylase activity; continuing and expanding application of computerized axial tomography (EMI scanning) to stroke, trauma, and a variety of other neurological problems; evidence that susceptibility to experimental allergic encephalomyelitis (EAE) may be controlled by a gene linked to the major histocompatibility antigen locus, suggesting that the gene controls T-cell reactivity directed against the encephalitogenic portion of myelin basic protein; suppression of EAE by treatment with myelin basic protein before or at the onset of clinical symptomatology; demonstration of a non-myelin antigen for the lymphocyte migration test and for EAE; association of the HL -A 3 histocompatibility antigen with higher antibody titers (e.g. measles antibodies in MS); development of a new generation of anti-parkinsonism drugs (such as bromocryptine) that are dopamine agonists and a primate experimental model for testing such drugs; isolation and purification of each of the enzymes involved in the metabolism of gamma-aminobutyric acid (GABA), preparation of antibodies to these enzymes, and ultrastructural localization of the enzymes in central inhibitory neurons; confirmation of the profound reduction of GABA levels in the mid-brain of patients with Huntington's disease; evidence for widespread effects of epileptogenic foci on neuronal circuits such that epileptogenic neurons appear to functionally deafferent themselves during ictal events; correlations of Purkinje cell firing with cerebrocortical epileptic focus activity and anticonvulsant drug action; profound inhibition of protein synthesis in brain during seizures such that in neonatal rats with seizures brain development is impaired and behavioral milestones are delayed; development of a variety of animal models to study slow and latent virus infections of the nervous system and their immunological concomitants, e.g. a hamster model for SSPE, the mouse hepatitis animal model for PML, and visna in sheep as a model for EAE; production of brain tumors in hamsters after intracerebral inoculation with papova virus isolated from human cases of PML; occurrence of severe neurological and neuropathological changes in hamsters after intracerebral inoculation with the 6/94 parainfluenza virus isolated from human cases of multiple sclerosis; reproduction of myasthenia gravis in animals in association with the development of antibodies to the acetylcholine receptor protein; observation of "antibody" blockade at the neuromuscular junction in human cases of myasthenia; evidence for a membrane locus as the site of the genetic defect in muscular dystrophy, as indicated by scanning electron microscopic studies of erythrocytes from dystrophy patients; indications that ototoxic drugs or pathological noise produce atrophy of the stria vascularis of the cochlea before loss of hair cells occurs; studies of the psychophysics of binaural hearing to ascertain how the human subject detects signals with differences in interaural timing or levels or both; examination of the vestibular apparatus by electron microscopy

(including scanning EM) illustrating hitherto unknown fine structural details, demonstration of the relevance of speech processing to short-term memory; distinction by conditioning studies between taste as an identification function and olfaction as a limbic (sexually-oriented) function; and design of visual and vibrotactile coders as cues for speech perception (lip reading) in the profoundly deaf.

In the collaborative and field research (C&FR) division of the NINCDS, over 100 research and service contracts were funded during fiscal year 1975, and on-campus laboratory research continued to be active and productive. The Perinatal Research Branch reports completion of the assessment of the 8-year-olds in the collaborative perinatal project, with analyses of the overall project data in the final stages. Target dates for completion of these analyses and preparation of the respective monographs are still the end of fiscal year 1976. As this project is finally phased out, plans are being laid for transition to a developmental neurology sub-program within the newly established neurological disorders extramural research program. As part of the reorganization, the C&FR Epidemiology Branch has been abolished. Most of the extant activities were transferred to the Intramural Research (IR) Program, with laboratory research in virology and immunology absorbed into the IR Laboratory of Central Nervous System studies and the Guam facility and its programs placed under the Director of Intramural Research. The section on genetics and epidemiology has been transferred to the IR Infectious Diseases Branch to implement studies on histocompatibility antigen profiles in various infectious diseases. This section has reported on the two forms of torsion dystonia, the autosomal dominant type in which serum levels of norepinephrine and of its synthesizing enzyme, dopamine-beta-hydroxylase, are both elevated; and the autosomal recessive type in which both these serum components are normal. This is still another example of complexity of genetic disorders of the nervous system, in which the clinical picture may provide few clues to the hetrogeneity underlying it. Also as part of the reorganization the C&FR Office of Biometry has been returned to the OD-NINCDS as the Office of Biometry and Epidemiology. An epidemiologist has been recruited, with major responsibilities for overseeing the survey studies on head and spinal cord injuries, intracranial neoplasms, and multiple sclerosis that are already under way, and those on stroke and on epilepsy that are planned for FY 1976. The Office of Biometry continues to provide backup for the perinatal collaborative project data analyses and will play a vital role in the development of data systems for the projected Office of Program Planning and Evaluation.

The other C&FR elements are also being assimilated into the reorganized extramural programs structure. The C&FR Communicative Disorders Section provides the staffing and programmatic nucleus for the Communicative Disorders Extramural Research Program. During FY 1975 the staff was strengthened by additions of a speech pathologist, and the contract-supported projects have continued on detection of hearing disorders in children, on adult aphasia, on field testing the fitting of master hearing aids, on tactile and visual aids for the deaf, and on noise especially as it affects the hearing impaired child during speech acquisition. The

noise research activities in DHEW, supported by NINCDS, NIEHS, and NIOSH, continue to be coordinated through an informal committee of representatives of the three institutes, as part of the overall noise pollution effort being coordinated by the Environmental Protection Agency.

As a result of the NINCDS reorganization the C&FR Applied Neurological Research Branch has been split, with the Epilepsy Section joining with the C&FR Perinatal Research Branch to provide the staffing and programmatic nucleus for the Neurological Disorders Extramural Research Program, and the Stroke and Head Injury Section providing the nucleus for the Stroke and CNS Trauma Extramural Research Program. In Epilepsy, during FY 1975, two new anticonvulsants reached the market: carbamazepine (Tegretol) for complex partial seizures and clonazepam (Clonopin) for absence seizures. The latter is especially interesting because it is effective in doses at the microgram (10^{-6} g.) level and at blood levels in the nanogram (10^{-9} g.) range. Another very promising anticonvulsant drug, dipropylacetate (Depakine) is ready for definitive clinical trials. It is unique in being the first clinically effective drug whose mechanism of action appears to be a modulation of GABA metabolism. A major adjunct to anticonvulsant drug development is the initiation under contract of a drug screening program involving a cooperative study with a number of pharmaceutical companies and a university pharmacology department. The year's major development in the epilepsy program has been the completion of the 11 feasibility studies and the review of some 20 proposals for full-scale comprehensive epilepsy programs. Three contract-supported programs have been awarded: in Oregon at the University of Oregon, Portland; in Minnesota jointly at the Mayo Clinic in Rochester and the University of Minnesota in Minneapolis; and in Virginia at the University of Virginia, Charlottesville. If funds permit additional awards may be made in FY 1976, the quality of these three proposals, the geographic distribution, and the populations involved would seem to augur well for the initiation of this program.

The C&FR Stroke and Head Injury Section has collaborated with the Office of Biometry in the ongoing or planned surveys on head and spinal cord injury and on stroke. Some 1300 patients have been accessioned into a study on transient ischemic attacks to evaluate risk factor screening in stroke-prone patients. A collaborative study is under way to provide indicators for prognosis in comatose patients. Several studies have been contracted for to ascertain the ability of computerized axial tomography (EMI scans) to resolve problems in stroke and trauma patients where the nature of the pathology is clinically uncertain. Contract support is also being provided for the applicability of recently developed epidural pressure monitoring devices to the continuous surveillance of intracranial pressure in stroke and trauma patients.

Extensive reviews of the NINCDS information network components were completed by the middle of fiscal year 1975. Each of the three components was site visited *in extenso* and the recommendations of the site visitors were carefully evaluated by the Institute's scientific information advisory committee and by the NINCDS Advisory Council. Term-

ination of the communicative disorders component at Johns Hopkins was recommended and the phasing out of this operation is in progress. The clinical neurology component at Omaha received high marks and will be continued as a project continued as a project under the Neurological Disorders Extramural Research Program. It was recommended that activities of the brain information service at UCLA be markedly modified and stream--lined, with improvement in advisory input, management and consumer orientation. These changes are being implemented and the project will be continued under the Fundamental Neurosciences Extramural Research Program.

In the NINCDS intramural research (IR) programs significant organizational changes took place during FY 1975. The intramural program has been subdivided into a laboratory research division under Dr. Richard Irwin as assistant scientific director for laboratory research and a clinical research division under the Clinical Director, with recruitment of Dr. Donald Calne, for that post. The new laboratories of neuroimmunol--ogy under Dr. Dale McFarlin and neuropharmacology under Dr. Thomas Chase have become operational during the year, despite the personnel ceiling restrictions which continue to curtail flexibility within the IR programs. The EEG Branch has been reorganized into a Clinical Neurosciences Branch with a new section on functional neurosurgery to be headed by Dr. Van Buren, who has relinquished direction of the Surgical Neurology Branch. Recruitment of his successor for the latter post is now in progress. The Laboratory of Biophysics has moved two of its three sections to quarters in the Marine Biological Laboratory at Woods Hole, Mass., where year-round accessibility to the requisite marine forms upon which their research depends can be more efficiently insured. The Infectious Diseases Branch was transferred from C&FR to IR, and the neural pros-thesis program was transferred from IR to the new Fundamental Neurosciences Extramural Research Program--both these latter moves taking cognizance of the essential intramural and extramural natures, respectively, of these activities. In addition a number of C&FR administrative-type activities were moved out of Bldg. 36 to space in the Federal Bldg. in Bethesda, so that space for the new IR laboratories and for existing overcrowded laboratory activities could be recovered. The contract to renovate Bldg. 376 at the Frederick Cancer Research Center has finally been awarded, so that access to the animal and laboratory containment facilities for the slow virus program in the Laboratory of Central Nervous System Studies can be anticipated within about a year.

Almost two thirds of the NINCDS intramural research projects have involved collaboration with units in other NIH institutes or extramurally, and some 50 visiting scientists and fellows and guest workers supplemented the regular staffs in every IR laboratory or branch. With additional clinically oriented projects (in neuroimmunology, neuropharmacology and infectious diseases) the IR clinical research program has become more diversified. Obviously the competition for beds has intensified, so that outpatient, ambulatory, motel and nursing home adjuncts are being explored to spare inpatient beds for the most critical research studies. The major problem which continues to confront the intramural program is the personnel problem. Additional ceiling reductions were imposed

during the year to an all time low of 513. An analysis of the long-term impact of repeated reduction (totalling 138 since 1968) indicates an erosion primarily of support personnel (technical and secretarial) from a ratio of two support to one professional in 1968 to a one-to-one ratio today. The latter is clearly too low for efficient laboratory operation. Constraints on the numbers of nontenured professionals (research and clinical associates and staff fellows) are severe and further weaken the thrust of ongoing projects. There is clearly a need for greater flexibility than the present NIH personnel system permits. There is an urgent need to be able not only to phase out the present NIH laboratories or branches that are no longer productive or relevant to the Institute's missions but also to be able to move unneeded personnel out so that the slots can be recovered for use in higher priority new initiatives. The problem is particularly acute in the NINCDS where the intramural personnel complement is 69 percent of that in 1968, in comparison to percentages of 80 percent or better for every other institute at NIH. But the problem is NIH-wide and demands prompt, constructive attention. A corollary to the problem is the detrimental effect on many related EEO programs, such as Stride, Upward Mobility and our commitment to achieve a better balance for minority and women employees in our personnel structure.

In spite of such formidable constraints the NINCDS intramural program continues to flourish. Two Institute scientists were especially honored during the year: Dr. Roscoe Brady was elected to the National Academy of Sciences, and Dr. Carlton Gajdusek was awarded the DHEW Distinguished Service Award and was selected as the Dyer Lecturer at NIH. Some of the scientific highlights from IR projects follow: use of freeze-fracture techniques in conjunction with electron microscopy to demonstrate discharge of synaptic vesicles only at specific points at the presynaptic membrane by a mechanism of exocytosis (vesicle fusion with the membrane); demonstration in Gaucher's disease of sustained decrease in the elevated levels of glucocerebroside after a single trial of enzyme replacement therapy, such that glucocerebroside levels were 50 percent of controls at one year after enzyme injection--representing a calculated clearance of 4-year's accumulation of lipid; apparent activation of ineffective enzymes in patients with Fabry's or Gaucher's disease following enzyme replacement therapy; observation of neuropathological changes as early as 4 weeks after intracerebral inoculation of agents of the kuru-Creutzfeldt-Jakob group, with multinucleated neurons, intracytoplasmic vacuoles and concentric laminar bodies--all months before EEG changes and a year or more before clinical symptomatology; isolation of a possible C-type virus particle from brains of Guamanian patients dying of amyotrophic lateral sclerosis (ALS); discovery of a new focus of ALS in western New Guinea with a prevalence several-fold higher than that on Guam or the Kii peninsula in Japan; development of an air cushioned microelectrode that permits continuous artifact-free single-cell recordings from human cerebral neurons during neurosurgical procedures; the finding that no specific RNA sequence is required to produce defective interfering viral particles, so that the interference phenomenon might be utilized to abort virus infections; demonstration of a circulating "factor" in the serum of myasthenia gravis patients that blocks the binding of alpha-

bungarotoxin to the muscle acetylcholine receptor; use of freeze-fracture techniques together with immunoperoxidose techniques in electron microscopy to visualize viral antigens and the budding of viruses from host nerve cells; confirmation of a defect in pyruvate oxidase activity in tissue samples and cell cultures from patients with Friedreich's ataxia; the observation that clearance of potassium ions released by firing epileptogenic neurons is slowed from areas of reactive astrocytic gliosis; experimental production of hydrocephalus in primate fetuses after inoculations with influenza or Venezuelan equine encephalitis viruses; utilization of antibodies to isolated acetylcholine receptors to demonstrate the appearance in muscle of extrajunctional receptors after denervation, and their suppression after reinnervation; clinical efficacy of new dopamine receptor agonists like bromocryptine for the treatment of Parkinson's disease--drugs which obviate a number of the troublesome side effects associated with L-Dopa therapy; and the exploitation of simple invertebrate nervous systems to study central processing when two sensory pathways (vestibular and visual) are stimulated simultaneously while recording intracellularly from the neural network. These brief examples indicate why the NINCDS continues to take pride in its intramural research program.

The general ferment noted in last year's report has continued unabated during fiscal year 1975. The Assistant Secretary for Health, DHEW, and the Director of NIH have both changed again, with the newly installed incumbents each NIH alumni. Hopefully the transitions will be smoother and the policies more responsive than in previous changes of administration. We must also now anticipate a new regime at the level of the Secretary, DHEW, with the inevitable get-acquainted period that must ensue. Three new institute directors, for NHLI, NIAID and NIDR, will be appointed shortly; the search continues for an NIA director; and the impending departure of the Clinical Center director will create another top level vacancy. Against this sort of background the NIH and its bureaus, institutes and divisions are confronted with a number of major developments and challenges. An indepth review of the NIH and of ADAMHA is underway by the President's Panel on Biomedical Research. The problems of the ethics and safeguards for human experimentation are being scrutinized by another Congressional commission, at a time when there are also increasing Congressional pressures to bring more of the fruits of biomedical research to the physician and the patient. In more than a few situations these two Congressional initiatives may prove to be mutually exclusive. A comparable paradox is presented by two recent pieces of legislation that provide on the one hand for freedom of information in terms so broad that the peer review system may be in jeopardy and on the other hand for protection of individual privacy such that legitimate epidemiological and clinical research may be severely compromised. In addition there is a burgeoning of commissions for specific diseases or disorders, such as diabetes and arthritis and probably epilepsy and Huntington's disease, that tend to distort research emphases at a time when managerial resources in terms of dollars and personnel are being acutely strained and even eroded. Moreover, the delays in voting and allocating appropriations tend to lengthen year by year, so that program planning and funding must be deferred until the

final few months of the fiscal year, to the detriment of sound long-term planning on the Institute's part and stability of research projects on the investigators part. And there are numerous other roadblocks to orderly and efficient conduct of our business.

At the same time the NIH and each of its institutes are being challenged with respect to their roles in the health care delivery system and in the quality and standards of health care delivery. There are increasing pressures to take a more positive role in the translation of research advances into clinical utilization. The current effort in information mandated by Congress last year may not satisfy, despite the fact that during FY 1975 the NINCDS, for example, handled 3200 public inquiries (in addition to Congressional inquiries), filled over 400,000 requests for publications and has underway an active program of videotaping lectures and courses for distribution to physicians. The NIH is seemingly being urged in the direction of more demonstration and control programs a direction perceived by many as the entering wedge for full scale health care delivery service programs. And the companion pressures for expertise in dealing with the demands of PSRO legislation accentuate the problem. The dilemma is the difficulty of recruiting the service and educational arms of the federal, state and local organizations to joining the effective channelling of research advances to the clinical market place. In the partial vacuum that exists, should the NIH fill it and thus spread its available resources so thinly that the future of biomedical research is jeopardized? Or should the NIH resist and run the risk of alienating its supporters because they view it as an introspective ivory-tower operation with no community conscience?

It is worth taking note of Eric Sevareid's commentary on the last major speech by Secretary Weinberger. The Secretary pointed out that if social programs continue at the same rate for the next 20 years they will cost more than one half of our entire gross national product (i.e., all the goods and services produced in this country). Sevareid comments that we lack a method of deciding what programs to keep and what to throw away; that the nation needs a balance sheet that it can understand. And he reminds us of the warning by John Stuart Mill that, in time, the good done by charity to its recipients would come into collision with the general good. The NIH cannot escape entanglement with these sorts of issues. Clearly as the NINCDS embarks on its second quarter century the tasks of its leadership and of its scientific and lay constituencies are not simply those of hastening the acquisition of new knowledge to conquer the chronic and debilitating disorders of the nervous system--important as those endeavors may be. But in addition the Institute and its community must deal responsibly and constructively with the increasing number of important socio-economic issues confronting us.

Office of Scientific and Health Reports

NINCDS information and publications and public affairs activities are centered within the Office of Scientific and Health Reports, formerly the Office of Public Affairs. The work of the Office is divided into three sections: Scientific Publications, Health Reports, and Public Inquiries.

The Office is responsible for advising the Director and his executive staff on the Institute's public and professional image and on the effective interpretation and reporting of Institute-conducted and supported research findings. These findings must be made available for many audiences, including Congress, the Department and other agencies of Government, scientists, physicians, voluntary health agencies, and the general public. The program is one of initiating and implementing many projects, as well as responding to Congressional, Departmental, internal, and public requests.

Considerable time was spent in Fiscal 1975 obtaining materials for the Secretary to be used as background in forming policy decisions on various bills introduced in Congress relating to a proposed study of Huntington's disease, a proposed national epilepsy commission, and a proposed screening center for Tay-Sachs disease patients. Extensive correspondence relating to these decisions was drafted.

This year and each year, the Office of Scientific and Health Reports has been responsible for producing eight Special Reports on disease categories designated by the House Subcommittee on Appropriations. These describe the disorder, present the Institute's program as it relates to this area, reviews research advances of the past year including details of new therapy, and reviews briefly the outlook for the future. The Special Reports this year included the following: Stroke, Parkinson's Disease, Epilepsy, Cerebral Palsy. Spinal Cord Injury, Multiple Sclerosis, Neuromuscular Disorders, and Communicative Disorders.

Coordination of the Institute program with the work of private agencies and professional societies is a major function of the Office of Scientific and Health Reports (OSHR). For example, in January 1975 the Office organized and managed a 2-day meeting with representatives from some 60 organizations and societies working in the fields of neurological and communicative disorders. The Office serves as a focal point for continuing contacts with these groups, supplying various types of support as requested.

Last year, the Senate Appropriations Committee instructed NIH to increase its research information to physicians and the public. A Committee on Dissemination of Research Results was established last Fall. The Chief of the Institute's Office of Scientific and Health Reports was a member of the Committee and contributed to its report, which has now been transmitted to the Senate. A permanent Committee on Communications

is now being established and Dr. Donald Calne, NINCDS Clinical Director, has been nominated to serve on the Committee.

In the day-to-day operation of the OSHR, the three sections produce publications, audiovisual materials, reports, and answers to public inquiries, and also serve as an information resource on the many disease categories within the Institute's purview.

Public Inquiries Section

Staff members of this section spend a large part of their time responding to letters and phone inquiries. In the past year, more than 3,200 inquiries required individually written letters answering specific questions about neurological, communicative, and sensory disorders. For many of these, research was necessary. For many, it was necessary to coordinate information with intramural scientists and with Institute grantees.

Congressional mail was especially heavy last year as a result of continuing budget changes. Letters asking that the recommendations of the National Advisory Multiple Sclerosis Commission be implemented have come in on a steady basis A letter of reply and a leaflet detailing Institute plans for implementation was sent in response to all Congressional and White House letters.

Similar upswings in mail volume occurred as a result of an NINCDS Workshop on the Research Status of Spinal Manipulative Therapy in February, and the meeting of Voluntary Health Agencies and Professional Societies in January. Additional thousands of inquiries were answered with printed materials. In all, NINCDS responded to requests for 406,357 publications.

The Office of Scientific and Health Reports interacts on a continuing basis with the 60 national voluntary health agencies and professional societies which make up the National Committee for Research in Neurological Disorders. This includes providing material on Institute research, programs, and personnel to the editor of the NCRND Newsletter, a publication of the National Committee.

Through an Exhibits Program in this section, the Office of Scientific and Health Reports brings the Institute's extramural activities to the attention of the professional societies and health agencies at national scientific meetings. Last year, a Program Exhibit was shown at the American Academy of Ophthalmology and Otolaryngology meeting in Dallas, the American Speech and Hearing Association meeting in Las Vegas, the American Academy of Neurology meeting in Bal Harbor, Florida, and the American Neurological Association meeting in New York City. All arrangements for scheduling and manning the exhibit were handled by staff of this section. These showings generated hundreds of requests for information about Institute programs.

Members of this section were involved in planning activities related to Institute participation in the NIH Reunion and NIH Open House. This included planning exhibits, gathering information for the geneology charts, coordinating preparation of the charts with Medical Arts, and distributing NINCDS materials at the exhibits.

Members of this section also keep abreast of Advisory Council meetings, plan the annual Council dinner, and update a Council Directory. Additionally, the head of this section is the liaison person with the extramural area, is responsible for providing grantee information for use in Institute reports and publications, and writes annual special reports for Congress on cerebral palsy and spinal cord injury.

The Office of Scientific and Health Reports also serves as the focal point for the Institute in discharging its responsibilities under the Freedom of Information Act. Under this Act, the Public Inquiries Section responded to 115 requests for summary minutes of the meetings of these groups and for lists of their members. The section also provided the Department with quarterly reports of this activity.

Scientific Publications Section

The Scientific Publications Section produces and distributes publications, both for the general public and for the various scientific audiences. Publications production services are requested by various administrative units of the Institute, ad hoc committees preparing reports, and outside organizations in the neurological field when sufficient need is demonstrated. The services include planning, writing, editing, design layout, clearance, distribution, storage, and later revising and reprinting, according to demand. The section works with the NIH Printing Unit, the Medical Arts and Photography Branch, and the Government Printing Office. It also serves as the Institute's supply center for all publications.

The Monograph Series contains neurological science contributions of Institute staff members, committees, and consultants. Included are reviews, reports, nomenclature, classification and methodology aids, and proceedings of meetings sponsored by the Institute. Since 1965, 13 items have been published in this series. Seven of these are still in print and are being distributed by the section. A new monograph, entitled The Scientific Basis of Spinal Manipulative Therapy was in preparation at the close of Fiscal 1975, and will be published in 1976.

Bibliographies are also produced and distributed by the section. The Cerebrovascular Bibliography, a quarterly selection of stroke references from Index Medicus, was changed from a free distribution status (500 recipients) to a paid subscription basis with the January-March 1974 issue. Various other bibliographies, produced in the past, are still being distributed, and an annual bibliography of papers on the NINCDS Collaborative Study on Cerebral Palsy, Mental Retardation, and Other Neurological and Sensory Disorders of Infancy and Childhood was processed.

The latest in the Profile Series, retitled NINDS Research Program Reports--1974, was published in August 1974. This is a 153-page booklet containing special Congressional reports covering research advances and an opening statement by the director on general program directions.

The Institute's highly popular Hope through Research series of leaflets continued to be revised and reprinted as necessary. A new leaflet on myasthenia gravis was written by office staff and is in press. Three others, on shingles, cerebral palsy, and Huntington's chorea were revised, and one on dizziness was reprinted without revision. Accompanying the Hope through Research leaflets is a series of fact sheets, usually shorter, and on relatively rare disorders. A fact sheet on torsion dystonia was written and published during the year. Another, on hydrocephalus, was revised and is in press. Two other fact sheets, on amyotrophic lateral sclerosis and subacute sclerosing panencephalitis, are being revised and will be republished in FY 1976. There are now 16 leaflets in the Hope through Research series, and 6 fact sheets. These publications, three of which are also in Spanish, are the most widely circulated of the Institute's publications. Many go to private voluntary groups which distribute them to patients and health workers. Many others go to students for use in health-related classes and course work.

Other publications produced by the section included:

Epilepsy Research, FY 1973: A Report for the Epilepsy Advisory Committee to the NIH;

Individual leaflets containing the special Congressional reports mentioned above;

Voluntary Health Agencies Working to Combat Neurological and Sensory Disorders (a listing).

Other printing and duplicating services secured by the Section included several programs for meetings, a directory of present and former NANDS Council members, and a directory of professional societies.

The section provided extensive editorial assistance for an NINCDS 25th Anniversary Volume on the Neurosciences. This will be an 1,800-page review, written by some 150 contributors, of the entire field of neurological and communicative sciences. The work included arranging and attending meetings to determine chapter topics and select authors, and scientific editing and proofing of final manuscripts.

Assistance was also provided for a book containing proceedings of a conference held in February 1975 on GABA (gamma-aminobutyric acid). This included: attending the conference, obtaining final manuscripts, and providing editorial assistance to scientific editors.

Health Reports Section

The Health Reports Section prepares Institute research and health reports for Congress, scientists, voluntary agencies and the general public. Reports are written, filmed or audio or video taped. The section also arranges for distribution of reports to as many interested audiences as is possible.

The section cooperated closely with the National Committee for Research in Neurological Disorders (NCRND) which comprises some 60 voluntary health agencies and professional groups in the neurological and communicative areas, in focusing attention of junior and senior high school science department heads on brain research. The Institute sent a packet--an introductory letter, an Institute folder about research programs, a National Committee folder about voluntary service to brain research, and a copy of the booklet "Discovering Yourself in the Brain Age"--to 16,500 members of The National Science Teachers Association (NSTA) who are heads of science departments. The mailing prompted some 1,600 requests each for numerous Institute publications, indicating that the effort increased interest on the part of many teachers to bring more information on the brain to their students. It is hoped it also stimulated many young people to pursue careers in neurology.

Thirty-three lectures about neuromuscular diseases, videotaped on 28 cassettes in 1972, '73 and '74 with cooperation of the Houston Veterans Administration Hospital and the National Naval Medical Center, were set up for free loan distribution and for sale. Under contract with a commercial distributor, NINCDS pays for each use of a cassette. Through GSA's National Audiovisual Center, tapes are for sale at prices ranging from $41 to $65 each, depending upon length of the taped lecture. The tapes were announced in mid-December as "An Intensive Course in Neuromuscular Diseases." By April, some 140 requesting sources had asked for 1,500 tape loans. Because no use factors had been established, only 5 copies of each tape were made for distribution. The heavy response resulted in bookings extending into June 1976. Additional copies of the most requested tapes have been put into the system to alleviate the long wait for some requestors. Loan requests came from medical schools, VA hospitals, military medical units, private hospitals, practicing groups and individual scientists and physicians.

A complete videotape system, purchased at the end of FY 1974, was finally accepted from the contractor in January. Fourteen lectures on "Perspectives in Multiple Sclerosis," presented at an NIH conference February 11-12, were recorded on videotape, and these will enter the distribution system soon--probably through the National Medical Audiovisual Center (NMAC) in Atlanta--and will be announced to the groups who have already requested tapes in the neuromuscular series

Recently, as an experiment, two of the MS lectures were announced in the Calendar of Events for showing at noon on a Tuesday in the Non-print Media Section of the NIH library. Fourteen persons attended the

hour session. The consensus was that videotape presentations of important lectures were much better than transcripts and should be increased. The library staff members, who have copies of all the neuromuscular tapes and will soon be custodians of the Institute's set of copies of the MS tapes, plan to present two TV lectures a week at the library to see what response can be obtained from this method of presenting additions to professional education.

The section is ready to assist all scientific offices within the Institute to film or videotape presentations which will improve communication of Institute research accomplishments. It is engaged in one project to provide videotapes of a clinical session at a New York meeting on Dystonia Musculorum Deformans, another to videotape classes in neuropathology at the American Academy of Neurology meetings in Toronto in 1976, and another to provide audio tape summaries of a special meeting on gamma-aminobutyric acid (GABA) held in February at the Kroc Ranch in California.

The section obtained still photography as requested, maintained a photo file, distributed Institute motion pictures locally on a limited basis, supplied new prints of Institute motion pictures distributed by NMAC to that agency, and coordinated efforts of administrators, scientists and artist personnel of the Medical Arts and Photography Branch, DRS, to produce an exhibit for the NIH Alumni Reunion and the NIH Open House. Finally, several motion picture sequences which the section either filmed or arranged for the filming for the Epilepsy Foundation of America last year are included in that agency's six new films on epilepsy now being released.

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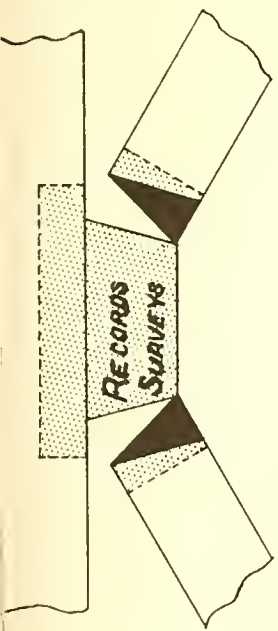

TABBED SEPARATOR SHEET
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(3-56)

LAB. OF BIOPHYSICS

SURGICAL NEUROL. BRANCH

CNS STUDIES LAB.

CL. EEG & NEUROPHSY. BR.

DEVELOP. &

TABLE OF ORGANIZATION, IR

SCIENTIFIC DIRECTOR'S RPT.

LAB. OF NEURAL CONTROL

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LAB. OF NEUROPHYSIOLOGY

MEDICAL NEUROL. BRANCH

Table of Organization

Intramural Research Program

National Institute of Neurological and Communicative Disorders and Stroke
(Personnel on hand May 1975)

Office of the Director

Director of Intramural Research - Thomas N. Chase, M.D.
Assistant Director of Intramural Research - Richard L. Irwin, Ph.D.
Clinical Director - Donald B. Calne, M.D.
Medical Officer - Jacob A. Brody, M.D.
Medical Officer - Choh-Luh Li, M.D.
Administrative Officer - Glenn E. Hammond
Administrative Assistant - J. Loring Jenkins
Administrative Assistant - John H. Jones
Administrative Technician - Doris R. Perry
Secretary (Stenographer) - Margaret Henry
Secretary (Stenographer) - Vernita Bergmeyer
Travel Assistant - Ida M. Chernikoff
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Biological Laboratory Technician (Animal) - Adrian P. Loftis
Procurement Assistant - Patricia D. Williams
Biologist - Minnie Toure
Biological Laboratory Technician (Micro) - Charles E. Sartor
Secretary (Typing) - Lillian L. Wease
Secretary (Stenographer) - Doris Selkowitz
Secretary (Stenographer) - Corinne C. Gillis
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Clerk-Stenographer - Olive Childers
Clerk (Typing) - Andrea C. Gielen
Clerk-Stenographer - Joan E. Kraft
Laboratory Worker - John R. Bowers
Laboratory Worker - Kenneth Oglesbee

Section on Technical Development

Computer Programmer - William H. Sheriff, Jr.
Staff Fellow - Bruce Smith, Ph.D.
Staff Fellow - Susan Hauser, Ph.D.

NINCDS Guam Research Center

Medical Officer - Frank B. Anderson, M.D.
Nurse - Marjorie Gillespie
Health Technician - Jose M. Torres
Health Technician - Manuel T. Cruz

Health Technician - Francisco Leon-Guerrero
Biological Laboratory Technician (Micro) - Luiz T. Munoz
Secretary (Typing) - Mary E. Hernandez

Medical Neurology Branch

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Biologist - Mary A. Oberc
Microbiologist - Priscilla Chauvin
Surgeon - Barry W. Festoff, M.D.
Surgeon - John W. Griffin, M.D.
Surgeon - Adam N. Bender, M.D.
Surgeon - Sidney C. Bean, M.D.
Surgeon - Benjamin Brooks, M.D.
Surgeon - Jay D. Cook, M.D.
Surgeon - Roger W. Kula, M.D.
Surgeon - John L. Trotter, M.D.
Surgeon - David S. Zee, M.D.
Surgeon - Richard Rosenbaum, M.D.
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Biological Aid - Janet M. Harner
Biological Aid - Doris S. Park
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Section on Clinical Applied Pharmacology

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Biologist - Katharine L. Oliver
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Surgical Neurology Branch

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Surgeon - Norman D. Peters, M.D.
Surgeon - Leslie D. Cahan, M.D.
Surgeon - James H. Wood, M.D.
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Visiting Associate - Rodwan K. Rajjoub, M.D.
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Biological Laboratory Technician (Animal) - Clifford A. Seay
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Sr. Staff Fellow - Rodney A. Brooks, M.D.
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Surgeon - Boris A. Vern, M.D.
Surgeon - Barry I. Ludwig, M.D.
Surgeon - David M. Bear, M.D.
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EEG Technician - Martha H. Fair

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Chemist - Roy M. Bradley
Chemist - Jane M. Quirk
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Sr. Staff Fellow - Peter H. Fishman, Ph.D.
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Staff Fellow - John W. Kusiak, Ph.D.

Section on Neurochemical Methodology

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Biological Laboratory Technician (Biochem.) - Frank J. Fash

Section on Clinical Investigation and Therapeutics

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Research Chemist - George Constantopoulos, Ph.D.
Medical Technician (Chem.) - Jan K. Steusing
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Laboratory of Neuropathology and Neuroanatomical Sciences

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Visiting Scientist - Krystyn Renkawek, M.D., D.M.Sc.
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Staff Fellow - Michael J. Cullen, Ph.D.
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Biologist - Sandra K. Crites
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Laboratory of Neurophysiology

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Section on Cell Biology

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Visiting Scientist - Pier Marchiafava, M.D.

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Sr. Staff Fellow - Peter B. Detwiler, Ph.D.
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Visiting Fellow - Tsutom Nishihara, Ph.D.
Visiting Fellow - Patricia Whitman, Ph.D.

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Chemist - Cheryl Marks
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Sr. Staff Fellow - Richard C. Henneberry, Ph.D.
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Visiting Fellow - Yasutaro Fujita, Ph.D.

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Visiting Fellow - Gudrun Stamminger, Ph.D.

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Biological Laboratory Technician (Animal) - William Rodriguez
Surgeon - Howard Kirshner, M.D.
Surgeon - William F. Blank, M.D.
Visiting Associate - Michio Yamaguchi, M.D.
Chemist (Biochem) - Carmen L. Freixas
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Laboratory of Neural Control

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Supervisory Research Psychologist - Herbert Lansdell, Ph.D.
Research Physiologist - William B. Marks, Ph.D.
Engineering Technician - George M. Dold
Physiologist - Joan S. McIntosh
Electronics Engineer - Martin J. Bak
Senior Surgeon - Frederick T. Hambrecht, M.D., Ph.D.
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Visiting Fellow - Kendro Kanda, M.D.

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Office of the Chief

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Chemist - Raymond R. O'Neill
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Visiting Fellow - Yoriaki Yamashita, M.D.

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Biological Laboratory Technician - Alfred E. Bacote
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Surgeon - Richard Yanagihara, M.D.
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Staff Fellow - George J. Nemo, Ph.D.
Staff Fellow - Ralph M. Garruto, Ph.D.
Biological Laboratory Technician (Micro) - Helena L. Gilbert
Secretary (Steno) - Marion F. Poms
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Anthropologist - Laura A. Kreiss
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Biological Laboratory Technician - Katherine Kinney
Biological Laboratory Technician - Anthony Montanaro
Biological Aid (General) - Ann C. Rudnick
Health Technician - Ivan M'Baginatao
Biological Aid - Judith P. Chavis
Animal Caretaker - Hubert O. Saville
Animal Caretaker - Eugene D. Webster
Animal Caretaker - Ezra Shafer
Animal Caretaker - Harry Winpigler
Animal Caretaker - Leon J. Vance

Laboratory of Neuro-Otolaryngology

Office of the Chief

Chief, Laboratory of Neuro-Otolaryngology - Jorgen Fex, M.D., Ph.D.
Biologist - Gabrielle Neises
Electronics Engineer - William Livingston
Sr. Staff Fellow - Robert L. Gulley, Ph.D.
Sr. Staff Fellow - Dennis Drescher, Ph.D.
Staff Fellow - Joe C. Adams, Ph.D.
Staff Fellow - Robert Wenthold, Ph.D.
Secretary (Steno) - Elsie Walter
Clerk-Typist - Christine Widgren

Laboratory of Neuropharmacology

Office of the Chief

Chief, Laboratory of Neuropharmacology - Thomas N. Chase, M.D.
Chemist - Nancy Eng
Secretary (Typing) - Sandra M. Meadows
Surgeon - Ronald Kartzinel, M.D.
Surgeon - Ira Shoulson, M.D.
Sr. Staff Fellow - Mark J. Perlow, M.D.
Staff Fellow - Judith R. Walters, Ph.D.
Staff Fellow - Leonard P. Miller, Ph.D.
Biological Aid (Biochem) - Richard Leupold
Clerk - Jeanette Barrow
Visiting Fellow - Marina M. Mata, Ph.D.

Neuroimmunology Branch

Office of the Chief

Chief, Neuroimmunology Branch - Dale E. McFarlin, M.D.
Chemist - Elizabeth Mingioli
Secretary (Steno) - Shirley Burdette
Clerk-Typist - Karen L. Beard

Infectious Diseases Branch

Office of the Chief

Chief, Infectious Diseases Branch - John L. Sever, M.D., Ph.D.
Statistician - Mary R. Gilkeson
Secretary (DMT) - Dianne Edwards
Secretary (Steno) - Ethel M. Ingram
Procurement Clerk - Florence Reid
Clerk-Typist - Sharon K. Painter
Clerk-Typist - Nelva L. Reckert

Section on Experimental Pathology

Veterinarian - William T. London, D.V.M.
Biologist - Blanche Curfman
Biological Laboratory Technician (Animal) - Robert L. Brown
Research Veterinarian - Amos E. Palmer, D.V.M.
Biological Laboratory Technician (General) - Geneva M. Brown
Biological Laboratory Technician (Animal) - Joseph Ricketts
Biological Aid (Animal) - Wayne E. Nusbaum
Biological Aid - Sherry L. Thomas
Biological Aid - Dorene Conley
Laboratory Worker - Mark Simms
Animal Caretaker - Adley J. Atkinson
Biological Aid - Debra L. Young

Section on Immunochemistry and Clinical Investigations

Microbiologist - Anita C. Ley
Nurse - Dorothy Edmonds
Surgeon - Jerome E. Kurent, M.D.
Surgeon - Richard Johannes, M.D.
Sr. Staff Fellow - Michael F. Murphy, Ph.D.
Biological Laboraotry Technician - Gene M. Brashears
Biological Laboratory Technician - Paul P. Becher
Medical Technician - Jennifer Dorosz
Medical Technician - Ampar Strickland
Clerk - Frederick Brownholtz

Section on Virology and Bacteriology

Senior Scientist - David A. Fuccillo, Ph.D.
Research Veterinarian - Luiz Barbosa, D.V.M.
Microbiologist - Flora Moder
Microbiologist - Renee G. Traub
Biologist - Rebecca Hamilton
Biological Laboratory Technician - Frank J. West
Microbiologist - Mary A. Krasny
Nurse Specialist - Helen M. Krebs
Biological Laboratory Technician (Micro) - Aurellla S. Krezlewicz
Biologist - Sandra Fitzgerald
Veterinarian - David L. Madden, D.V.M.
Visiting Scientist - Monique Dubois, M.D.
Microbiologist - Otto Gutenson
Histopathology Technician - Edna Worthington
Biological Aid - Leonard Moore
Biological Aid - Stephen I. Adler
Biological Aid - Kenneth A. Blank
Biological Aid - Mitchell Binder
Biological Aid - Gary Offenbacher

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Use one page for each separation.

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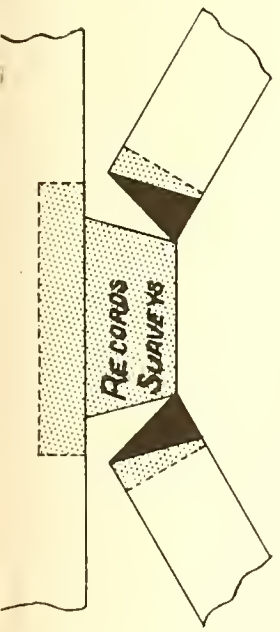
Cut off and discard all tabs except the one covered by tape.



TABBED SEPARATOR SHEET
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(3-56)

LAB. OF BIOPHYSICS
SURGICAL NEUROL. BRANCH
CNS STUDIES LAB.
CL. EEG & NEUROPHSY. BR.
DEVELOP. & METAB. NEUROL. BR.
LAB. OF NEUROPATHOLOGY & SCIENTIFIC DIRECTOR'S RPT.
LAB. OF NEURAL CONTROL
OD, IR
LAB. OF NEUROPHYSIOLOGY
MEDICAL NEUROL. BRANCH

Annual Report of the Scientific Director
of the
National Institute of Neurological and
Communicative Disorders and Stroke
July 1, 1974 through June 30, 1975

The Intramural Research Program of the National Institute of Neurological and Communicative Disorders and Stroke conducts research related to the nervous system and its disorders in laboratories and clinics located in Bethesda, Maryland, as well as in laboratory facilities at Fort Detrick, Maryland, and at the Guam Clinical Research Center. Within this program, a broad array of basic and clinical techniques are applied to the study of nearly every aspect of the form and function of the nervous system.

Major changes in key personnel and in organizational structure have occurred during the past year. Both a new Scientific Director and a new Clinical Director from outside the Institute were appointed. In addition, the Assistant Intramural Research Director has been promoted to the post of Laboratory Director. To strengthen management of the Intramural research effort, complete line responsibility and authority for the Program's seventeen Laboratories and Branches has been divided between the Laboratory and Clinical Directors. Two of these laboratories, Neuroimmunology and Neuropharmacology, were created during the past year to fill important voids in our research efforts. The Electroencephalographic Branch has been reorganized and expanded to include a section on Functional Neurosurgery and is now designated the Clinical Neurosciences Branch. The Laboratory of Biophysics has been reorganized into three sections. Two of these, the Section on Neural Membranes and the Section on Neural Systems, will be located in Woods Hole, Massachusetts, at the Marine Biological Laboratories. The third Section, Molecular Biophysics, will remain in Bethesda. Finally, administrative responsibility for the Guam Clinical Research Center was transferred from the Epidemiology Branch, C&FR, to the Office of the Intramural Director. It is expected that the foregoing changes will considerably broaden and strengthen the Institute's Intramural research programs.

Intramural resources have undergone a number of important modifications during the past year. There are now 182 Doctoral level scientists working within 24 program areas. Approximately 67% of these Doctorates are promising young scientists, who during training periods extending from one to four years, contribute vigor and innovative ideas to the research efforts of our tenured staff. The Intramural Program also continues to attract numerous doctoral level scientists as Visiting and Guest Workers; 50 are currently working in our laboratories and clinics. While the number of doctoral level scientists has increased by 11% during the past six years (due entirely to an expansion of non-tenured professional staff), attrition has operated to diminish the number of technical and clerical support personnel by 29%. The ratio of full time permanent support staff to doctoral level scientists has thus decreased from 1.6 to 1.0 during this period. The present ratio is at the lower margin of efficient operations. Space allocations during the past year to the Intramural Program increased by 13,610 sq. ft. mainly because of Building 36 laboratories formerly occupied by C&FR. The Intramural Other Objects Budget increased from 2.6 million to 5.5 million, while outside

research contracts guided by Intramural scientists grew from 2.4 to 3.3 million during this period.

Within the past year, the Intramural Program conducted 181 active projects. Fifty-one research projects were initiated, 15 completed, and 27 terminated. One-hundred-fifteen projects were in active cooperation with units in other NIH Institutes or with outside research institutions. Studies on infectious diseases, especially in virology, neurophysiology and neurochemistry, now command the largest personnel and budgetary allocation within the Intramural Program. The major disease-oriented projects utilizing the in-patient facilities of the NIH Clinical Center are those devoted to neuromuscular disorders, epilepsy, extrapyramidal dysfunction, peripheral neuropathies, and CNS tumors.

As judged by the number and quality of research publications as well as by the number of awards and honors conferred on Intramural staff members, our Program has enjoyed a remarkably successful year. During calendar year 1974, 169 major research reports appeared in 89 scientific journals, books or symposium proceedings. In addition, numerous lectures and seminars were presented by Intramural scientists before learned societies and University audiences in this country and abroad. Major honors and awards bestowed upon Intramural personnel during the past year included the election of Dr. Roscoe Brady to the National Academy of Sciences. Dr. Carleton Gajdusek, also a member of National Academy of Sciences, received the DHEW Distinguished Service Award and was invited to present this year's Dyer Lecture. In addition, numerous Intramural scientists were elected to the editorial boards of professional journals and to the scientific advisory boards of various non-profit and professional foundations and associations.

Program planning and evaluation activities have been considerably expanded and strengthened during the year. A computerized system to track Intramural personnel, space and financial resources by program area as well as by laboratory or branch has become fully operational. In addition, a computerized file of publication records and citations to these publications is now being established to allow for continuous comparison of certain parameters of resource inputs and research outputs within the Intramural Program. During the past year, the NINCDS Board of Scientific Counselors was expanded from 6 to 8 members adding other experts to this important Advisory panel. Moreover, the addition of at least 2 ad hoc members to the Board for each Laboratory reviewed has prompted more searching and knowledgeable evaluations. These changes have been well received by the Laboratory Chiefs and the Board of Scientific Counselors.

During the past year, laboratory research conducted by NINCDS Intramural scientists has yielded results of considerable importance to the field of neuroscience. Details of these findings are contained in the individual project and summary reports. Only selected examples, which seem especially critical to future progress, are summarized here.

Based on knowledge that defective virus particles can be used to rescue virus-infected animals from death, the Laboratory of Molecular Biology has made several important basic findings. Since defective virus particles harbor

potential dangers, a thorough understanding of the mechanisms of interference between viruses and defective particles is needed before vaccines can be prepared for human use. In working towards this understanding, the Molecular Biology Laboratory has shown that a nearly unlimited number of different defective virus particles can be produced. The Laboratory has also found that different parts of the virus genome can each serve as interfering RNA particles. Accordingly, it is possible that no particular RNA sequence is required for interference. If that were so, any RNA properly encased into a virus core with protein might be just as effective for interference as the ribonucleoprotein core of a defective virus. It is thus conceivable that superinfection by a large number of such particles containing harmless RNA might be a very effective means to abort virus infection. These findings may be an important step towards the development of an effective treatment or prevention of virus infection.

Other recent studies in this Laboratory have shown that greater amounts of certain vitamins are required for microbial differentiation than for microbial growth. This observation suggests that an increased amount of certain vitamins are needed during pregnancy for differentiation within the fetus than are required during adult life. Therapeutic implications include the possibility that adult daily doses of certain vitamins may not be adequate for women during pregnancy.

Intramural neuroanatomists have been able to clarify greatly the exact location and the mechanisms of synaptic transmission in the central and peripheral nervous system. Experiments using a new freeze-fracture technique have shown that local synaptic vesicles can be discharged only at specific points on the synaptic membrane and that these points are determined by specific structures within the membrane. Direct evidence has been found that synaptic vesicles discharge their contents by fusing with the synaptic membrane, a process well known in other secretory cells as exocytosis. One immediate practical aspect of this study is its definition of the normal structure of synapses in various functional states. This knowledge will permit separation of normal from pathological synapses using the electron microscope. In structural studies of epileptic brains, it should now be possible to distinguish normally active from pathologically active synapses. It should now also be possible to distinguish pathological states from changes resulting from increased or decreased activity at neuromuscular junctions. The new findings also indicate that different chemical types of synapse might be identified by the freeze-fracture technique and that this could contribute to the determination of the chemical organization of synapses in the central nervous system.

An Intramural neurophysiologist has developed a technique for simultaneously stimulating two sensory pathways (vestibular and visual) while recording intracellular potentials from elements of a well defined neural network. This technique will permit analysis of principles of information processing by the central nervous system and examination of the neural mechanisms of learning and behavior. The establishment of an Intramural research facility at the Marine Biological Laboratory at Woods Hole, Massachusetts, will increase the number of biological specimens with potential usefulness to this program.

The clinical branches continued studies spanning a broad array of applied neuroscience research.

In the field of neurochemistry, there has been sustained progress in enzyme replacement therapy for inherited neurological disease. Administration of glucocerebrosidase to patients with Gaucher's disease has been found to elicit a rapid and remarkably sustained decrease in the pathologically elevated concentration of glucocerebroside in blood. An important conceptual advance has been the realization that administration of exogenous enzyme leads to activation of the patient's mutated catalytically ineffective enzyme. This discovery strengthens the optimism currently being generated by neurochemical research on inborn errors of metabolism. Another important development has been the synthesis of a chromogenic analog of sphingomyelin which allows reliable diagnosis of Niemann-Pick disease and the identification of heterozygous carriers.

The Medical Neurology Branch has demonstrated the presence of a circulating serum factor which blocks alpha-bungarotoxin (a specific molecular probe which prevents binding of the acetylcholine molecule) at the neuromuscular junction of patients with myasthenia gravis. This histochemical evidence fits well with previously reported biochemical studies and may lead to important new therapeutic advances. The Medical Neurology Branch also has introduced a new technique for identifying denervation in patients with muscle disease. Detecting the spread of end-plate receptors during denervation to extrajunctional areas using alpha-bungarotoxin and immunoperoxidase serve as the basis for the new diagnostic test.

Another important development has been the first reproduction of biochemical and ultrastructural changes of a myopathy in tissue culture of muscle cells from a patient suffering from adult onset acid maltase deficiency.

The Surgical Neurology Branch, in collaboration with the Laboratory of Neural Control, has obtained encouraging results with an air supported microelectrode designed to record electrical activity from single neurons in man, in spite of the wide excursions of tissue induced by respiratory movement. In collaboration with the Armed Forces Radiobiology Research Institute, a totally reliable system for inflicting controlled head trauma in primates has now been developed and should form the basis for future quantitative studies of changes in metabolism and cerebral blood flow induced by head injury. It is anticipated that the acquisition of equipment for performing computerized axial tomography will add a new dimension to radiological research and diagnosis in the coming year. The Section on Neuroradiology has played a central role in the development of this technique which will improve neurological diagnostic services and provide a powerful new tool for future research.

The Infectious Diseases Branch has pursued its mission involving the study of perinatal, acute and chronic infectious diseases of the nervous system. Multiple Sclerosis and Subacute Sclerosing Panencephalitis are proving fertile areas for morphological study by electron microscopy, employing the techniques of freeze-fracturing and immunoperoxidase staining. Other work

emanating from this Branch has dealt with the availability of serologic tests for measles as aids to the diagnosis of Subacute Sclerosing Panencephalitis. Recent observations indicate the usefulness of the CSF rubeola complement-fixation test for the diagnosis of this disorder. Such a test would render biopsies rarely, if ever, necessary. Other Laboratory studies have shown that influenza virus inoculated into the monkey fetus results in hydrocephalus in at least one-half of the animals. These observations may have relevance to studies of children with congenital hydrocephalus.

The Laboratory of Experimental Neurology has continued to study pathways involved in generating focal seizures in young and adult primates, concluding that the propagation of focal paroxysmal activity shifts from subcortical to cortical regions with maturation. These investigations have been developed to include observations on regional cerebral blood flow and changes in cerebral catecholamine concentration during epileptic discharges.

The three new laboratories, established for studies in neuroimmunology, neuropharmacology and neuro-otolaryngology are currently having laboratory space renovated, installing equipment, recruiting personnel and embarking upon new research programs. Clinical studies of several direct acting dopamine receptor agonists have been undertaken by the Laboratory of Neuropharmacology. Such agents should, in theory, obviate certain limitations of L-dopa in the treatment of Parkinson's disease, since they do not require the integrity of presynaptic dopaminergic neurons for their function. A drug currently under study, bromocriptine, now appears to be at least equipotent to L-dopa in ameliorating the cardinal clinical features of this disorder.

A Neurovirology-immunology Study Group was convened to consider research priorities for the Guam Clinical Research Center. Recommendations of this group which will be acted upon during the coming year include: maintenance of a central tissue bank of frozen and fixed Guamanian tissue, expansion of the search for a possible C-type virus particle in Guamanian tissues, histocompatibility studies using HLA antigen typing, additional tissue culture studies, and expanded seroepidemiological studies. To facilitate implementation of these projects an experienced neuroimmunologist and a suitably trained technician have been assigned to the Guam based staff.

Annual Report of the Section on Technical Development

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders and Stroke

July 1, 1974 - June 30, 1975

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. 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 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, a medium size time-shared digital computer (SEL 810B) for real-time on-line applications, and by providing programming service and technical consultation.

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.).

INSTRUMENTATION

Following are selected instrumentation projects undertaken by the Section during the past year. These are chosen from a total of 201 projects, and are representative examples of electronic instrumentation.

(1) Patient Activity Monitoring System. The Section is developing a device which will continuously monitor the movement activity of a human subject and record the activity in a memory contained within the device. The device contains a movement transducer with an inherent threshold, which produces a binary output when the threshold is exceeded. These outputs, defined as units of activity, are counted. At pre-selected constant intervals this count is stored in memory and a new count initiated. The memory can store 256 counts, each of which may be as high as 4095 activity units. If the storage interval is 30 minutes, 5 days of data can be stored. The information is extracted from the device by a specially designed instrument containing a microcomputer. The data can then be printed and/or stored on magnetic tape for further analysis. The monitoring device will be produced in a five cubic inch package and can be worn on the body or any limb; if necessary, several devices can be worn by a single subject. The cost of each device will be less than $200 and the read-out instrument (only one is required) will be less than $500. It is expected that this system can be used in any research project where activity must be monitored (manic-depressives, hyperactive children, etc.).

(2) Amplitude/Time Window Discriminator System. The amplitude and time window discriminator developed last year has undergone extensive testing in five labs this year. The addition of time discrimination to standard amplitude discrimination has improved the processing accuracy of neurophysiological signals, while the multiplexed display of the signal and amplitude discrimination levels has made the instrument easier and faster to use. Additional functional units have been designed to support the discriminator, so that a typical system also includes a variable-gain, variable-bandwidth input amplifier, raster and intensity display generators, and a rate meter. Development of a histogram display unit with calculating capability is planned.

(3) High-Speed Microelectrode Amplifiers. Two special-purpose amplifiers were developed to increase the frequency response obtained from high-impedance recording microelectrodes. One design utilized a floating input-stage power supply that was bootstrapped at unity gain by a second-stage amplifier. This resulted in greater than a factor of ten decrease in amplifier input capacitance. For higher-capacitance microelectrodes, the second design utilized a multiple amplifier configuration to present a high input impedance at low frequencies, and a low impedance at higher frequencies. The low impedance improves the high frequency response by shunting the electrode capacitance. At these higher frequencies, the cell must supply current through the microelectrode but the need for capacitance neutralization is eliminated.

(4) Voltage Controlled Current Source and Recording Amplifier for

Micropipettes. The device which allows linear voltage control of current passed through a micropipette, originally designed and published by the Section, has been extensively redesigned to take advantage of advances in the electronics state-of-the-art. Low noise, low voltage drift amplifiers have been added, as have gain adjustments and a calibrated DC input which supplements the signal input. More than twenty of these devices have been built and delivered to investigators in the IRP.

(5) Multi-Channel Voltage-Controlled Current Sources for Drug Electrodes. The standard current source described above has been adopted to multibarrel micropipettes to be used in iontophoretic drug application studies. Use of high-voltage amplifiers gives the current sources a high voltage compliance to pass relatively large currents through very high resistance pipettes. Of primary importance in this application, is the ability of this design to provide a current monitor output that gives a true current reading even when the large voltage compliance of the circuit is exceeded. Both a five and a four channel instrument have been built, with the latter including a multiple trigger unit for pulsed applications.

(6) Selective Detection of Animals at a Specific Location. This system was designed to provide automatic detection of a specific animal at a feeding station. Each of five beagle puppies carries a low power, battery operated, crystal controlled transmitter weighing 5 grams and shaped as a 1.5cm. dia. cylinder, 3cm. long. Each transmitter operates on a separate carrier frequency in the 25 to 27 MHz band. Each frequency is monitored by a separate receiver, and activity on that frequency, indicating the presence of a specific puppy at the feeding station, causes a once per second pulse to appear on an event recorder, on the channel corresponding to that puppy.

(7) High Gain, Low Noise Amplifier. Operational amplifiers were selected to construct a three stage non-inverting amplifier which optimizes the trade-off between bandwidth and noise. The amplifier has a selectable gain of 10, 100 or 1000 and a bandwidth (1 db down) of at least 100 KHz at every setting. The noise at the output ranges from 6 millivolts for a gain of 10, to 120 millivolts for a gain of 1000. The main component of this noise is 60 Hz noise from the power supply.

(8) Micromarker. A device to mark the position of an intramuscular electrode for identification during biopsy examination was developed. The device is basically a current source which passes iron ions into the muscle. The rate of deposit, and hence the total accumulation, is controlled by the investigator.

(9) Parallel/Serial Converter. This instrument was designed to convert six decades of parallel BCD formatted data to serial form for output on a single polygraph channel. The output is coded by the pulse height in order to distinguish among a start pulse, a logical "1", and a logical "0". The system has both an automatic and a manual mode. In the automatic mode it can sample and convert the parallel input data at selectable intervals between thirty seconds and ten minutes. The unit can also be controlled externally from a front-panel switch.

(10) Real-Time Clock. This circuit was designed to be used as a peripheral device for a battery powered microprocessor system to control evoked response studies in a field location. The circuit contains a 1 KHz crystal controlled clock and 16 binary stages of division, allowing accurate timing of events up to 65 seconds with one millisecond resolution. The circuit was constructed of 17 CMOS integrated circuits, and uses less than five milliwatts of power in the active mode.

COMPUTER SERVICES

The Section on Technical Development undertook a systems study to determine the best means of utilizing recent advances in minicomputer technology and reductions in cost. The purpose of the study was to provide the laboratory scientist with a low cost, versatile system having real-time, on-line, data acquisition and processing capabilities and the ability to monitor and control an experimental apparatus. Two additional criteria were set. One, that the scientist may perform his experiment independent of any computing facility outside of his laboratory and to have a convenient means of relatively high speed, random access bulk storage for the results of a single experiment. Secondly, that the manufacturer supply an integrated package of hardware and software to minimize the time required for programming and interfacing experiments. This software must be oriented to the laboratory scientist.

The cost of minicomputers has dropped significantly, enough to put them within the budget of the individual laboratories, but the cost of peripherals such as line printers, magnetic tapes, disc storage devices and plotters remains high. These devices are not usually essential to the conduct of an experiment and may be used later, off-line. A cost effective means of providing these services is to locate them in a support facility in each of the areas of major use, the Clinical Center and Building 36. Each support computer will be provided with a full range of peripherals necessary for bulk storage and output of data, and will provide a means for extensive data processing. The individual laboratory computers will have a full range of scientific input and output devices such as analog-to-digital converters, digital-to-analog converters, a real-time clock, digital I/O and an oscilloscope controller. The laboratory computers will have sufficient memory to operate efficiently with higher level languages and will be provided with a low cost means of removable bulk storage which may be read by the support computer. The laboratory computers will also be connected to the support computer by a telephone linkage. All computers will be able to access the facilities of the Division of Computer Research and Technology by telephone, making their large computers available for sophisticated data processing. The resulting distributed network will offer a full range of computer capability to the scientist at lowest cost. The average cost for a laboratory computer will be less than $20,000.

By standardizing on one computer mainframe, STD will be able to maintain a library of modular routines specific to the needs of the IRP with a minimal investment of manpower. Another factor which was considered during the study was the availability of a line of microcomputers which are compatible

with, and programmable from, the support computers. Exploitation of the microcomputers should offer improved technological services in the future with large reductions in cost.

On the basis of this study, the Section has elected to standardize on the Digital Equipment Corporation's PDP-11 series. Three support computers, PDP-11/40, are being purchased for installation in Bldg. 36, Bldg. 10, and Poolesville. In addition, five PDP-11/10 computers are being purchased for use in individual laboratories in NIMH and NINCDS. Delivery is expected early in FY76.

The satellite network will phase out the existing multiprogramming facility in the Clinical Center by the end of FY76. The present two minicomputers (PDP-12 and Spear microLINC) operated by STD will be dedicated to specific laboratory applications and the Bldg. 36 support computer will replace the open shop facility presently provided by the PDP-12.

Computer Utilization

PDP-12

		Hours
NINCDS:	Laboratory of Neurophysiology	610
	Applied Neurological Branch (C&FR)	624
NIMH:	Laboratory of Neurophysiology	100
	Section on Technical Development	32
NICHD:	Behavioral Biology Branch	256
	TOTAL	1622

MicroLINC-300

NINCDS:	Laboratory of Experimental Neurology	40
	Laboratory of Biophysics	200
	Medical Neurology	200
NIMH:	Section on Technical Development	80
	TOTAL	520

SEL 810B

NIMH:	Laboratory of Psychology and Psychopathology	1250
	Adult Psychiatry Branch	1800
	TOTAL*	3050

*The SEL 810B is a time-shared computer. The real-time total is less than this figure.

ENGINEERING and FABRICATION

This table shows the distribution of the Section's workload among the various laboratories.

LABORATORY OR BRANCH	HOURS	PERCENT
Neuro-Otolaryngology, NINCDS	2319	7.94
Adult Psychiatry, NIMH	2297	7.87
Computer Services, NIMH & NINCDS	2026	6.94
Brain Evolution & Behavior, NIMH	2009	6.88
Behavioral Biology, NICHD	2002	6.86
Clinical Science, NIMH	1937	6.64
Biophysics, NINCDS	1899	6.50
Developmental Psychology, NIMH	1887	6.46
Neurophysiology, NINCDS	1857	6.36
Neuropharmacology, St. E's, NIMH	1718	5.89
Psychology and Psychopathology, NIMH	1145	3.92
Surgical Neurology, NINCDS	1007	3.45
Technical Development, NIMH/NINCDS	955	3.27
Clinical Psychobiology, NIMH	947	3.24
Molecular Biology, NINCDS	832	2.85
Neuropathology & Neuroanatomical Sciences, NINCDS	654	2.24
Neurophysiology, NIMH	519	1.78
Neurobiology, NIMH	505	1.73
Neurochemistry, NIMH	437	1.49
General & Comparative Biochemistry, NIMH	401	1.37
Perinatal Physiology. NINCDS	373	1.28
Medical Neurology, NINCDS	340	1.16
Experimental Neurology, NINCDS	322	1.10
Neurochemistry, NINCDS	301	1.03
Extramural Research, NIMH	178	.61
Socio-environmental Studies, NIMH	112	.38
Neural Control, NINCDS	109	.37
Cerebral Metabolism, NIMH	103	.35
NIMH (Total)	15,926	54.56
NINCDS (Total)	11,263	38.59
NICHD (Total)#	2,002	6.85
TOTAL*	29,191	100.00

*The time of the Section Chief is not included in the foregoing table.

#NICHD loans the Section one position, and is thus entitled to 2000 hours of service.

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Use one page for each separation.

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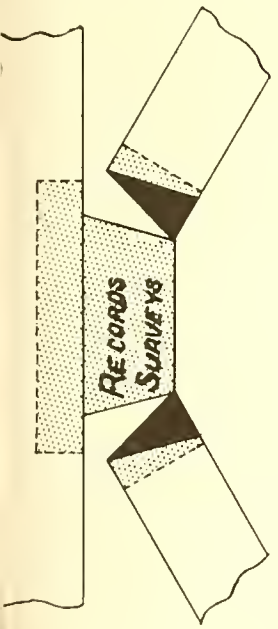
Cut off and discard all tabs except the one covered by tape.



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LAB. OF BIOPHYSICS
SURGICAL NEUROL. BRANCH
CNS STUDIES LAB.
CL. NEUROPHSY. BR.
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DEVELOP. & METAB. NEUROL. BR.
LAB. OF NEUROPATHOLOGY & NEUROANATOM. SC.
LAB. OF NEURAL CONTROL
OD, IR
LAB. OF NEUROPHYSIOLOGY
MEDICAL NEUROL. BRANCH

Project No. ZO1 NS 00201-20 ODIR
1. Office of the Director
2. NINCDS Guam Research Center
3. Agana, Guam

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Guam Research Center Studies

Previous Serial Number: NDS (CF) - 55 E 201

Principal Investigator: Thomas N. Chase, M.D.

Other Investigators: Frank H. Anderson, M.D.
Jacob A. Brody, M.D.
Kwang-Ming Chen, M.D.
Olivia Cruz, M.D.
Hideki Igisu, M.D.
Donald Koerner, M.D.
Yasuho Nagano, M.D.
Haruo Okazaki, M.D.
Yoshiro Yase, M.D.

Cooperating Units: Laboratory of Central Nervous System Studies, IR, NINCDS
Laboratory of Neuropharmacology, IR, NINCDS
Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts
Department of Neurology, Wakayama Medical College, Wakayama, Japan
Brain Research Institute, Niigata University, Niigata, Japan
Neurological Institute, Kyushu University, Fukuoka, Japan
The Mayo Clinic, Rochester, Minnesota
Guam Memorial Hospital, Agana, Guam
Medical Research Center, Brookhaven National Laboratory, Upton, New York
Trust Territory Health Office
Center for Demographic and Population Genetics, University of Texas, Houston, Texas

Man Years:

Total:	8
Professional:	3
Other:	5

Objectives: To study etiologic factors, pathogenetic mechanisms and therapeutic approaches to Guamanian amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia (PD).

Methods employed: Available epidemiological, neuropathological, neuropharmacologic, virologic and immunologic techniques are employed. Patients are referred to the Guam Research Center where they are followed and treated throughout their disease. Information is also gathered on patient families and control subjects. Autopsies are performed on almost all patients immediately after death and pathology specimens obtained for morphologic and biochemical study.

Major findings: 1. The computerized registry of new PD and ALS cases as well as controls continues to be maintained on Guam as well as in Bethesda. A retabulation of recent data by Dr. Jacob Brody suggests that the previously observed decline in the incidence of PD and ALS is no longer continuing. For the years 1970 through 1974, there actually appears to be an increase in ALS cases and deaths. For the same period, PD cases have also increased but deaths have decreased somewhat suggesting that the lives of PD patients may be prolonged, possibly due to treatment with L-dopa.

2. Arginine tolerance tests were performed on ALS and PD patients as well as on control subjects. Preliminary data suggest that PD patients not receiving L-dopa as well as those receiving this drug have a heightened blood glucose response to arginine infusion compared with age and sex matched controls. Control subjects included those with myotonic dystrophy and Charcot-Marie-Tooth disease. Final analysis of these data awaits study of several more control and untreated Parkinsonian subjects.

3. Studies have been carried out in collaboration with the Massachusetts General Hospital by Dr. Frank Anderson characterizing neurofibrillary tangles in PD and ALS patients as well as control subjects. In addition to the already reported higher incidence of neurofibrillary tangles in Guam PD patients, this study has shown a higher incidence of neurofibrillary tangles in a younger control population than previously reported anywhere in the world.

4. Studies carried out in collaboration with Dr. George Cotzias of the Brookhaven National Laboratories disclose that manganese levels in patients with ALS or PD were not distinguishable from those of non-Guamanian controls. These studies have now been terminated.

5. Studies of heavy metals, particularly manganese, in tissue from ALS and PD patients are now, however, being conducted by Dr. Yoshiro Yase of the Wakayama Medical College. Guam is known to have an unusually high amount of manganese in both water and soil.

6. A study of parathormone levels in ALS and PD patients and controls is now being conducted in collaboration with Dr. Fujita also of the Wakayama Medical College. Preliminary data suggest that basal parathormone levels are considerably higher in PD and ALS patients than in control subjects, and point to the need for calcium turnover studies.

7. A tissue bank of frozen and fixed brains, spinal cords, spinal fluid, sera and various internal organs is now being maintained in collaboration with the Laboratory of Central Nervous System Studies. The inventory is in the process of being computerized so that specimens will be more readily available to collaborating scientists.

8. Genetic analysis of family data on Guam ALS and PD cases carried out in cooperation with the Center for Demographic and Population Genetics, University of Texas at Houston, is now in its concluding phases. At present aggregate information on the village of Umatac consists of civil, church and NINCDS records. It is expected that in the next three months the first definable pedigree for Umatac will be completed. Umatac is of special interest genetically because it has the highest incidence of ALS and PD on Guam.

9. Virologic studies in collaboration with Dr. Michael Viola, University of Connecticut, and Dr. Lon White, Laboratory of Central Nervous System Studies, continue to explore the possibility that a C-type virus particle is present in Guamanian tissues. The major techniques being applied to this study are detection and characterization of the oncornovirus-like reverse transcriptase; and use of nucleic acid hybridization to identify viral RNA or DNA in brain cells. Additional material for this study is now being collected.

10. Studies continue on the long-term therapeutic and toxic effects of L-dopa in combination with a peripheral decarboxylase inhibitor in PD patients. In addition, the effects on longevity of this treatment are being evaluated.

Significance to biomedical research and the program of the Institute: Guam has the highest incidence in the world of motor neuron disease and the unique disease PD. The documentation of the epidemiological, clinical, and neuropathological aspects of ALS and PD have contributed to our knowledge of related central nervous system degenerative diseases. In fields in which there are no known causes and no known cures, data such as these provide one of the most likely avenues for development of concepts and facts which lead to prevention, causes and cures.

Proposed course: Major efforts will follow the recommendations of the neurovirology-immunology study group. Plans are being made to refurbish the laboratory on Guam for expanded investigations in the fields of tissue culture, histocompatibility studies using HLA antigen typing and other immunologic studies. Virologic studies, especially the search for a C-type virus particle, will be increased. A neurologist-immunologist and an immunologic support technician have been assigned to Guam for the coming year to implement these plans.

Keyword Descriptors: Amyotrophic lateral sclerosis, Guam, Parkinsonism and Dementia.

Honors and Awards: None

Publications:

Nemo, G., Brody, J.A. and Cruz, M.: Lymphocyte transformation studies of Guamanian amyotrophic lateral sclerosis and parkinsonism-dementia in patients. Neurology, 24:579-581, 1974.

Brody, J.A., Stanhope, J.M. and Kurland, L.T.: Patterns of amyotrophic lateral sclerosis and parkinsonism-dementia on Guam. Topics on Tropical Neurology, 4:45-70, 1975.

Reed, D.M. and Brody, J.A.: Amyotrophic lateral sclerosis and parkinsonism-dementia on Guam 1945-1972. I. Descriptive Epidemiology, Am. J. Epidemiol. 101:287-301, 1975.

Reed, D.M., Brody, J.A. and Holden, E.M.: Predicting the duration of Guam ALS. Neurology, 25:277-280, 1975.

Reed, D.M., Torres, J.M. and Brody, J.A.: Amyotrophic lateral sclerosis and parkinsonism-dementia on Guam - 1945-1972. II. Familial and genetic studies, Am. J. Epidemiol. 101:302-310, 1975.

Project No. Z01-NS-00913-14-ODIR
1. Office of the Director
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Single Cell Discharges from Various Nervous Structures and Their Functional Organization in Particular Reference to Somatosensory Activity in Man

Previous Serial Number: NDS(I)-62 SN/OC 913(c)

Principal Investigators: Choh-Luh Li, M.D., Ph.D., John Van Buren, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objective: To study the activity of single nerve cells in the cerebral cortex and subcortical nuclei in relation to motor and sensory functions.

Methods Employed: Two micro-electrodes are placed, one in the motor cortex and the other in the thalamus of patients undergoing operative procedures. The other parameters, e.g. EEG, EMG, are also measured in response to motor movement or sensory stimulation.

Major Findings: Some cells in the motor cortex were found to be active in close relation to motor movement, such as Parkinson tremors, voluntary flexors or extension of the opposite extremity. A few showed suggestive evidence that the activity is dependent upon the activity of the thalamic neurons.

Significance to Biomedical Research and the Program of the Institute: The program will add to our knowledge of normal and abnormal functions of the motor system in man.

Proposed Course of Project: This project has been terminated.

Keyword Descriptors: Neuronal discharges, Evoked responses.

Honors and Awards: None

Publications: None

Project No. Z01-NS-01417-09-ODIR
1. Office of the Director
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Changes in Physiological Properties of Brain Tissue at Low Temperatures and in Other Pathological Conditions

Previous Serial Number: NDS(I)-67 SN/OC 1417(c)

Principal Investigator: Choh-Luh Li, M.D., Ph.D.

Other Investigators: Joseph Fenstermacher, Ph.D., Barbro Johansson, M.D.

Cooperating Units: C-LCP

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objective: To determine the resistivity of nervous tissues under various neurosurgical conditions.

Methods Employed: Cats under Fluothane anesthesia, hypothermia, hypertension, etc. were used. In one experiment, a monkey was used who was subjected to electrical measurements for a period of two months. The method of measuring electrical resistance of the tissue was previously described (Li, et al. 1968). With this method, the results were applied to the Maxwell's Equation and the extracellular space of the tissue was calculated. The calculated extracellular space was then compared with the findings obtained by the concentration profiles of radioactively labeled compounds following subarachnoid perfusion. The brains of the animal after the experiment were fixed, sectioned and stained with Luxol Fast Blue-Nissl and Luxol Fast Blue-PAS stains for histological examination.

Major Findings: The extracellular space of the cat's cerebral cortex was found to be between 15 and 20% and was unchanged when the temperature of the cortex was decreased from 37 to 15° C. The rate of CSF formation was, however, found to be markedly reduced by hypothermia.

In another series of experiments, a sudden increase of systemic blood pressure by 90 mmHg was found to break down the blood brain barrier. With the

blood brain barrier broken down, the activity of the nerve cells was found to be changed or inhibited.

The results from experiments on brain edema show changes in the level of consciousness which are still being subjected to analysis.

Significance to Biomedical Research and the Program of the Institute: Change in extracellular space may be a measure of extracellular or intracellular edema of the cells. The latter, in turn, is a measure of the metabolic response of the nerve cells to various pathological or physiological conditions. It is hoped that the method herewith described will be applied to neurosurgical patients as a measure of the progress or improvement of the neurological conditions.

Proposed Course of the Project: This project has been terminated.

Keyword Descriptors: Neuronal resistivity. Hypothermia.

Honors and Awards: None

Publications: None

Project No. Z01-NS-01526-08-ODIR
1. Office of the Director
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Epileptic Neurons and Their Recurrent Axon Collaterals

Previous Serial Number: NDS(I)-68 SN/OC 1526(c)

Principal Investigator: Choh-Luh Li, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objective: To study the electrical properties of the individual nerve cells in the cerebral cortex under normal and induced pathological conditions.

Methods Employed: Glass micropipette electrodes were inserted into the cells in the motor cortex of anesthetized cats. The electrical activity of the cells were recorded; and, thereafter, electrical current of increasing intensity was applied. The electrical resistance of the cell was derived from the current-voltage ratio and the time constant of the cell membrane and capacitance were calculated. This was carried out before and after the application of strychnine to the exposed cortex.

In another series of experiments repetitive electrical stimulation was applied to the cortex through a gross electrode adjacent to the recording micropipette electrode, or to the individual cells through the recording micropipette electrode.

Major Findings: Some of the electrical properties of cortical neurons have been reported previously. The preliminary observations suggest that they are different from neurons under the effect of strychnine. For instance, the resistivity of normal cortical neurons measures $(9.17 \pm 2.68) \times 10^6$ ohms while that of the epileptic neuron was found to be in the order of 5 to 6 $\times 10^6$ ohms. Further, epileptiform activity of the cortex requires "synchronous" presynaptic bombardments and there is good evidence of negative feedback through the axon collaterals to the epileptic neurons.

Significance to Biomedical Research and the Program of the Institute: The electrical properties of the cell determine the cell's "natural" behavior as well as its response to the environment. If the electrical properties of the epileptic neurons are different it seems likely that any effective treatment will restore the normal electrical properties. Similarly, the electrical measurements may serve as a criterion of the effectiveness of the treatment of prognosis of the epileptic disorder.

Proposed Course of Project: This project has been terminated.

Keyword Descriptors: Epileptic neurons.

Honors and Awards: None

Publications: None

Project No. Z01-NS-01527-08-ODIR
1. Office of the Director
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Physiological Mechanism of Motor Function in the Cat

Previous Serial Number: NDS(I)-68 SN/OC 1527(c)

Principal Investigator: Choh-Luh Li, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objective: To study the functional organization of the pyramidal and extrapyramidal pathways.

Methods Employed: Intracellular and extracellular micropipette electrodes were used to record the activity of the nerve cells in the motor cortex of the cat and stimulation was applied to the basal nuclear structures. In the past year stimulation was applied primarily to the caudate nucleus and nucleus ventralis lateralis of the thalamus.

Major Findings: The discharges of cells in the motor cortex increase or decrease and the membrane potential increases or decreases in response to stimulation dependent upon the pre-stimulation activity of the cell and the duration of stimulation. The results of this experiment are in the process of analysis for publication.

Significance to Biomedical Research and the Program of the Institute: This study provides further information about the interaction of the pyramidal and extrapyramidal system at the central level. The present investigator is aware, however, that the regulatory effect of the extrapyramidal system on motor function may also be found in the cells of the motor cortex itself because only 4-5% of the cells in the motor cortex have descending axons in the medullary pyramid. The accumulated data may eventually shed some light on the understanding of motor function of dysfunction.

Proposed Course of Project: This project has been terminated.

Keyword Descriptors: Pyramidal and extrapyramidal pathways.

Honors and Awards: None

Publications: None

Project No. Z01-NS-02009-03-ODIR
1. Office of the Director
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Conditioning of Pain by Acupuncture

Previous Serial No.: NDS(I)-73 SN/OC 2009(c)

Principal Investigator: Choh-Luh Li, M.D., Ph.D.

Other Investigators: Herbert Lansdell, Ph.D.; Anthony Bak; Melvin Gravitz, Ph.D.; Ching-Yuan Ting (Chinese traditional Medical Doctor and Acupuncturist) and Dolores Blessing, R.N.

Cooperating Units: Laboratory of Neural Control, Laboratory of Neurophysiology, Veterinary Resources Branch, Division of Research Services.

Man Years:

Total:	0.8
Professional:	0.6
Other:	0.2

Project Description:

Objective: To investigate the efficacy of the so-called acupuncture-analgesia in experimentally produced pain. This serves as a first step for further study of the various therapeutic measures of pain in patients.

Methods Employed: Forty five normal volunteers were recruited; but, after a psychological screening, only 14 were accepted. They all had no history of chronic disease or psychiatric disorders, and were 27.6 ± 8.0 years old with an IQ of 118.9 ± 8.0. Each subject was assigned, according to a predetermined schedule, to the experimental sessions of acupuncture, placebo-acupuncture and hypnosis.

During each session, EEG, EKG and respiration were continuously recorded on a magnetic tape, and the recordings were subsequently analyzed with a computer. General physical examination and neurological examination were periodically made and blood samples periodically taken. A stimulating electrode, which was insulated except at the tip, was inserted near the supraorbital branch of the left trigeminal nerve. As the intensity of the stimulating current gradually increased, levels of minimal tingling sensation, minimal pain sensation and maximal or intolerable pain sensation were determined. In a given subject in a given session these three levels of

sensation were used as controls in reference to the levels obtained under acupuncture, placebo-acupuncture or hypnosis.

In the acupuncture session, the needle or needles were applied by a professional acupuncturist and the needle or needles were continuously manipulated according to the "classical instruction." No electrical stimulation was applied to the needle or needles. In all instances, sensations of distension, heaviness, soreness, and numbness were produced by the acupuncture needle or needles. This continued for 27.6 ± 5.7 minutes. Thereafter, stimulation of the supraorbital nerve was repeated and the three levels of sensation, i.e. minimum non-painful sensation, minimum painful sensation and maximum or intolerable painful sensation, were determined. In 6 of the 14 subjects, one acupuncture needle was applied to the dorsum of the right hand between the first and second metacarpals. In 4 subjects, three needles were applied, one to the right and one to the left hand over the dorsum between the first and second metacarpals, and one to a point on the face just inferior to the left zygoma. In the last 4 subjects, two more needles were added, one to the right and one to the left dorsum of the left feet between the first and second metatarsals. All these points were, according to the "classical instruction" and the experience of the acupuncturist, related to facial pain.

In the placebo-acupuncture sessions, the same experimental procedures were repeated, except that in all cases the subject received only one needle at a point on the right hand 3-4 cm away from the classical acupuncture point and the needle was applied and manipulated by one who was not trained in acupuncture.

In other sessions, hypnosis was induced based on traditional methods of eye fixation, arm levitation and suggestions for relaxation. In all instances, the subject was hypnotizable and exhibited trance behavior with an induction period of 27.1 ± 4.8 minutes.

There were four blood samples taken from the subject in each session and values were obtained for hemoglobin, hematocrit, white blood cell counts, platelet counts, sedimentation rate, prothrombin time, thrombin time, partial thromboplastin time, Na, K, chloride, bicarbonate, glucose, alkaline phosphatase, serum glutamic oxyloacetic transaminase, serum glutamic pyruvic transaminase, lactic dehydrogenase and cortisol.

Major Findings: Acupuncture and placebo-acupuncture were found to be ineffective in alleviating pain sensation or in elevating the levels of tolerance to pain, whereas hypnosis consistently raised the sensory levels of the experimental subjects. There were also no consistent changes in EEG, EKG, blood pressure, pulse rate, respiratory rate, and hematologic values under and following the treatment of acupuncture, placebo-acupuncture and hypnosis.

Significance to Biomedical Research and the Program of the Institute: In recent years, acupuncture has aroused a great deal of interest or controversy. In the U.S. National Capital alone, there have been at one time 24 acupuncture clinics or centers, which are, in a sense, for commercial practice rather than for the patient's welfare. Indeed, no one knows how and why acupuncture works, if it really works. It appears that biomedical research investigators in the National Institutes of Health have the obligation to evaluate the efficacy and usefulness of this procedure and eventually come up with an answer to the question of how and why acupuncture works, if it works at all.

Proposed Course of Project: As it was stated earlier, experimental pain is different from pain of pathological origin especially pain in many chronic diseases. In patients, the subjective response to pain varies with the psychological state and other characteristics, such as age, sex and race. It was reported that in the United States, low back pain alone causes approximately 7,000,000 people to be confined to bed every day. As a result, there is a loss of 200,000,000 man-days of work every year. For headache, people have spent $300,000,000 every year. These statistical figures do not include patients with causalgia, phantom limb pains, thalamic pains and cancer pains. As a medical doctor and a research investigator of the physiology of the nervous system, I have been frequently asked by many patients, general practitioners and outsiders if there is a better management of pain.

Unfortunately, up to this day, the mechanisms of pain sensation are still poorly understood and most of the available treatment, both medical and surgical, are empirical. It is the wish of this investigator that a research program of "Pain and its Management" be established in our Neurological Institute. If acceptable, this investigator would like to extend the present study to a long term (3-5 years) program which would consist of clinical research and basic research of pain.

In clinical research, patients with pain in NIH Clinical Center, George Washington University Hospital and D.C. Veterans Hospital will be investigated. A standardized rating scale of pain will be studied with the collaboration of our clinical psychologist and psychiatrist. For these patients, various treatments (pharmacological and non-pharmacological) will be tried. In addition, this clinical research unit will communicate with other Pain Clinics in Johns Hopkins University Hospital, University of Washington School of Medicine and Yale Medical School Hospital so that an exchange of information can be achieved. This information consists of not only the epidemiology of pain but also various physiological data and various treatments of different kinds of pain in different kinds of patients. The data will be submitted to the Office of Biometry and analyzed by its members who have the expertise and resources in statistics and computers. Finally, some better understanding and treatment of pain will be obtained.

In basic research of pain, the present study in our laboratory of the C-fibers and A-delta fibers and their activity in reference to the activity of other myelinated fibers of the laboratory animals at different levels of the central nervous system will be continued. In addition, the C-fiber responses recorded from different levels of the nervous system will be studied under various pharmaceutical agents.

Keyword Descriptors: Pain. Normal Volunteers. Acupuncture-analgesia. Hypno-analgesia.

Honors and Awards: Appointed Clinical Professor of Neurological Surgery by the George Washington University.

Publications: None

Project No. Z01-NS-02010-03-ODIR
1. Office of the Director
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Neurophysiological Mechanisms of Pain

Previous Serial Number: NDS(I)-73 SN/OC 2010(c)

Principal Investigator: Choh-Luh Li, M.D., Ph.D.

Other Investigators: Anthony Bak

Cooperating Units: Laboratory of Neurophysiology, NINCDS

Man Years:

Total:	0.8
Professional:	0.6
Other:	0.2

Project Description:

Objectives: To study the physiological mechanisms of pain and changes in pain responses to other sensory inputs and to pharmacological agents.

Methods Employed: The saphenous nerve of the cat is stimulated through a function generator with Haver-sine-wave electrical pulses of varying intensities. This differentiates the C component and A-delta component from the other components of the responses initiated by the mixed nerve. The C and possibly the A-delta component is to be recorded with extracellular and intracellular electrodes in the central nervous system at different levels. Interaction of these responses with other responses initiated from the mixed sensory nerve fibers is studied. These responses are also studied under the influence of various analgesic agents.

Major Findings: No conclusive statements can be made at this time.

Significance to Biomedical Research and the Program of the Institute: Needless to say, pain has a history as long as the human race, yet its mechanisms remain to be understood. Similarly, there are at least 154 pharmaceutical products which are known to alleviate pain, but their site of action is generally unknown. For example, it is still debatable whether sodium salicylate acts on the receptors, peripheral nerves or on the midbrain reticular formation even though the perivascular nerve fibers were claimed to

be the site of action. The present investigation may contribute to our knowledge of pain and therapy of pain.

Proposed Course of Project: The present study is to be continued with particular emphasis on the cell activity and the electrical properties of the cell membranes in response to pain and analgesic agents.

Keyword Descriptors: Pain

Honors and Awards: None

Publications: None

Serial No: Z01 NS 01789-06 ODIR
1. Office of the Director
of Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Physical Senses

Previous Serial Number: NDS(I)-70-ODIR 1789

Principal Investigator: Edith L. R. Corliss, Physicist

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.3
Professional: 0.3
Other: 0.0

Project Description:

Objectives: Preparation of a publication summarizing and interpreting experimental results on the properties of the normal human senses of sight, touch and hearing. These intact human senses are to be considered quantitatively somewhat by analogy to the description and evaluation of measuring instruments, i.e., which mechanisms are optimized, what resolution limits are obtained, over what dynamic range do they function, what response laws do they follow?

Major Findings: In order to obtain and interpret current experimental data on the performance characteristics of the intact human senses of sight, touch, and hearing a survey of the current literature has continued.

Primary effort this year has been devoted to the subject of hearing, in an effort to present written material on hearing in a form suitable for free-standing publication. Several papers have been prepared for publication in archival journals, and are in various stages of the editorial review process. They are: "A Test of Riesz Hypothesis", cleared by NIH and NBS, is being revised after review by Acoustical Society of America; and "Speech Recognition" and "Channel Capacity of the Ear as a Function of Frequency" being reviewed by both NIH and NBS. The drafting of illustrations, which has been a problem, has been solved thus far by having them made at the National Bureau of Standards.

Preparation of sections on the general evolution of the senses and the evolution of the sense of hearing has provided evidence that there is a clear distinction between the chemical senses - which seem to be derived from the need for food - and the physical senses, which seem to derive from the alarm and escape mechanisms in primitive creatures. This is seen most directly from the effects of sensory overload: nausea, in the chemical senses; and pain, in the physical senses. Also, a physical force applied to any of the sensory end organs will evoke a response of light, touch, or hearing, but will not evoke a sense of taste or smell; on the other hand, simple chemical stimulation, short of overt irritation, does not evoke a sensation of sight, touch or hearing. Moreover, there is well-defined evidence that the tactile sense is more primitive than either sight or hearing. Stimulation of the lateral-line organ in fish or reptiles, which although it lies along the skin is presumably the precursor of the ear in more advanced creatures, produces behavior that indicates the animal localizes the stimulus outside its body. This shows that the stimulus has been processed by triangulation with some memory and with input from the contralateral side. Whereas some experiments by Bekesy on the sense of touch have shown that it is possible to interpolate between coincident tactile stimuli applied to one side of the body, interaction with the contralateral side has not been demonstrated. In the primitive creatures, stimulation of nerve cells near the lateral line organ evokes only a surface reaction. Clearly, the lateral line must differ from the tactile receptors by being coupled directly through the central nervous system.

Proposed Course of the Project: The present contract between the NIH and the NBS terminates with this fiscal year. Some degree of continuation at the National Bureau of Standards is being considered.

Honors and Awards: None.

Publications: None.

Abstract for Research Narrative: A review of the evolution of the senses shows a clear distinction between the chemical senses -- presumably occupied with the acquisition of food -- and the physical senses, which seem to derive from the needs for alarm and escape. The most direct evidence comes from the effects of sensory overload: nausea, for taste and smell; and pain, for sight, touch and hearing. A physical force applied to the sensory end organs will evoke a perception of light, touch, or hearing from the physical end organs, but will not evoke a sense of taste or smell at the tongue or nose. On the other hand, simple chemical stimulation, short of overt irritation, does not evoke a sensation of sight, touch, or hearing. Moreover it appears that the tactile sense is the most primitive of all senses. Stimulation of the lateral-line organ in fish and primitive reptiles produces a response from the contralateral organ and a crossover through the central nervous system. Stimulation of tactile neurons adjacent to the lateral line produces only a surface reaction in primitive

creatures. Whereas Bekesy has shown that a person will interpolate between coincident tactile stimuli applied to one side of the body, interaction with the contralateral side has not been demonstrated.

HOW TO USE
THESE SEPARATORS

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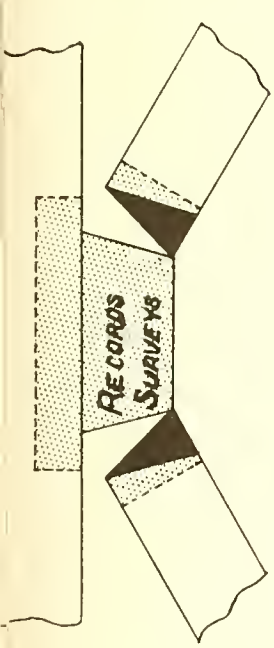

TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

LAB. OF BIOPHYSICS

SURGICAL NEUROL. BRANCH

CNS STUDIES LAB.

CL. EEG & NEUROPHSY.BR.

DEVELOP. & METAB.NEUROL.BR.

LAB. OF NEUROPATHOLOGY & NEUROANATOM. SC.

LAB. OF NEURAL CONTROL

LAB. OF NEUROPHYSIOLOGY

MEDICAL NEUROL. BRANCH

Annual Report
July 1, 1974 through June 30, 1975
Medical Neurology Branch, IR
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.

For the clinical investigations, 332 patients were admitted for a total of 6,853 patient days, and there were 964 outpatient visits. There were about 450 human muscle biopsies processed histochemically. Neurologic consultations were provided on 498 patients of other departments in the Clinical Center, with performance of indicated biopsies and electromyograms. In the past year, 16 papers were published, 16 are in press, and 32 were presented to meetings. One senior staff member has devoted full time to serving as Acting Assistant Scientific Director, IR, NINCDS.

The one-year approved residency training program in neurology has continued. Approximately 14 neurologists and other physicians and 11 technicians came this past year as guest workers to learn clinical research techniques in neurology, especially in neuromuscular diseases and the application of enzyme histochemistry thereto. The annual 3-day course on neuromuscular diseases in Houston, in collaboration with the Veterans Administration and Baylor, has been organized and most of the lectures given by our present and former staff.

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 Society/Association/ Foundation.

The collaborative PL-480 research program in neuromuscular disease with the Department of Neurology, Warsaw Medical Academy has been transferred to the Department of Neurology, Columbia. A collaborative program on neuromuscular diseases with Hopital Salpetriere and INSERM, Paris, is being developed. A formal understanding of collaboration in receptor research has been made with the Weitzman Institute, Israel.

Six detailed chapters for Shy's Neurology have been written and are in press: "Motor Neuron Disorders", "Central Core Disease and Focal Loss of Cross-Striations", "Rod Diseases", "Muscle Fiber Hypotrophies", "Introduction to the Myopathies", and "Introduction to Disorders of the Lower Motor Neuron".

"Muscular Dystrophies" and Other Non-Inflammatory Myopathies: Our histochemically-based hypothesis that the Duchenne type of muscular dystrophy (DMD) might be caused by ischemia mechanisms on the arterial side of the intramuscular vascular tree is now being explored by other groups. Having demonstrated in our model (rat aorta ligation followed by vasoactive amine) that following each dose of serotonin or norepinephrine the serum "muscle enzymes" rise, as in DMD, we have used that parameter to screen for prophalactic drugs which then might be useful clinically to prevent ischemic damage in muscle. We have reported that three such agents prevent serum rise of muscle enzymes in the animal model reflecting prevention of muscle damage -- directly dose-related in five of the combinations but a duel effect in one. With the model we have developed a new, rapid and simple radioisotopic method, based on uptake of Tc-diphosphonate, of quantitating active skeletal muscle damage in experimental animals -- it correlates very well with other more arduously determined parameters of muscle damage, loss of muscle K^+ and elevation of plasma creatine-phosphokinase (with AFRRI). We are using this tracer clinically and can identify active muscle damage in several types of myopathy including Duchenne muscular dystrophy and dermatomyositis. In muscles of human limbs amputated because of peripheral vascular disease, the pattern which we have recently found of grouping of necrotic or regenerating fibers identical to the pattern which we find in early Duchenne muscular dystrophy and experimental ischemic myopathy is at least harmonious with our ischemia hypothesis for Duchenne dystrophy (with VAH, D.C.). We have reported that infusions of serotonin into rat nerve-muscle preparations decrease the force of the evoked twitch, an experiment supporting the possibility that serotonin may play a role in some neuromuscular disorders.

Polymyositis/Dermatomyositis Complex (PM/DM): The mechanism of muscle damage in this disease complex is thought to be dysimmune, but more precise details remain unknown. Our previous report of immunoglobulin complexes deposited in blood vessels in 83% of the childhood cases and 29% of adult cases supported our earlier hypothesis that an aspect of muscle damage may be vascular. While disorders of both immunoglobulins (B-lymphocytes) and cellular immunity (T-lymphocytes) might be occurring in all cases of PM/DM, perhaps the former (as intravascular immune complexes) are more muscle-damaging in the childhood form and latter in the adult form. The immunologic abnormalities of PM/DM could in turn be caused by a viral infection; however, our attempts to "rescue" a virus from PM/DM patients' muscle thus far have been negative (with ID-NINCDS, and NCI). Moderate to massive subcutaneous calcification is a common and disabling complication of DM. We have found new ways to detect it in its early stages, isotopically with Tc-diphosphorate (with NM, CC) and by xerography. Unfortunately, therapeutic doses of diphosphonate have not been beneficial. We have demonstrated that a new histochemical finding -- alkaline phosphatase staining in the connective tissue -- is highly characteristic of PM/DM (and active myositis ossificans) as opposed to all other myopathies and thus can be used as a diagnostic point strongly suggestive of

PM/DM; this is especially useful in the childhood form, which can lack evidence of inflammation in the biopsy. The method of treatment we introduced to this disease 7 1/2 years ago, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred), has continued to prove to be, in our hands (about 50 cases) and others, the single best available treatment for children and adults (without or with cancer). It has the greatest therapeutic benefit, fewest side-effects, and is easiest to manage. However, because not all patients respond, we are seeking details of the patients' immunologic response to prednisone as well as predictive parameters thereof. By using T- vs. B-lymphocyte cell markers, T- and B-lymphocyte mitogens, and T-lymphocyte cytotoxicity on tissue-cultured chromium-labeled muscle fibers, we have established in DM/PM patients that while HSDAD-Pred is clinically cumulatively effective for months and longer, its measurable effect on the peripheral circulating lymphocytes, using currently available techniques, lasts less then 24 hrs; and these effects are more profound on the T-lymphocytes. We are correlating these data with concurrent blood levels of prednisone and prednisilone (with P,CC). In some prednisone-non-responders we have, on an occasional-case basis, successfully used azathiaprine (3 mg/kg) combined with the LT-HSDAD-Pred, and have now begun a double-blind trial to more clearly establish the efficacy of azathiaprine.

Periodic Paralysis (PP): In the hypokalemia form of PP, the treatment we introduced, long-term acetazolamide (Diamox), has continued to be the best prophylactic agent both for preventing attacks and improving inter-attack weakness, and it is now in the textbooks as such. Two of our patients have been treated successfully for more than 9 years. The mechanism of acetazolamide benefit in hypokalemic PP remains unknown, since muscle does not contain carbonic anhydrase. Renal calculi in one of 26 patients, possibly but not definitely related to the acetazolamide, have been the only suspected side-effect.

Other Myopathies: In adult-onset acid maltase deficiency we have provided the first demonstration of "reincarnation" of a muscle biochemical defect in cultured muscle fibers -- in fact, the muscle biopsy and the muscle cultured from it (with NYU) were identical by histology (vacuoles), histochemistry (acid phosphatase high in the vacuoles) electronmicroscopy (glycogen accumulated in lysosomes), and biochemistry (with Columbia) (5-10% acid maltase, elevated neutral maltase and acid phosphatase, normal enzyme kinetics). Also reincarnated in cultured muscle fibers were the ultrastructurally characteristic "cabbage bodies" of a patient with a chronic myopathy (the biochemical defect is not yet known) (with NYU). Some ultrastructural aspects of the mitochondrial abnormalities of "ragged-red" muscle fibers have been reincarnated in cultures from several patients with ragged-red fibers; this was the first morphologic defect reiterated in culture (with NYU). We have confirmed in another patient our previous finding that muscle fibers cultured from myophosphorylase deficiency disease recover some phosphorylase enzyme activity (with NYU).

In two cases of late-onset rod disease intranuclear rods, identical histochemically and ultrastructurally to the myofibrillar rods in the cytoplasm, were reported. Two possible pathokinetic mechanisms were suggested: (a) an origin from a previously unsuspected (or unemphasized) actin (or tropomyosin)-

like, plus-or-minus alpha-actinin-like, contractile protein common to both nuclear and cytoplasmic sites, or (b) origin from cytoplasmic contractile protein imported into the nucleus and crystallized there into rods. Either would represent a totally new concept regarding pathokinetic mechanisms, and mechanisms of nuclear defect possibly applicable to other diseases. In one of the patients an associated unusual serum paraprotein, IgG/lambda myeloma protein, was found. A new mid-childhood-onset form of rod disease was found with muscle biopsy abnormalities like those of adult-onset rod disease (but without nuclear rods).

A new clinically delineated "levitated arms syndrome" has been described in 2 cases and the cause identified as muscle fibrosis resulting from iatrogenic intramuscular injections into the deltoids, especially of pentazocine (Talwin). A third case had levitated arms and levitated legs (evident when sitting) from pentazocine injections into deltoid and rectus femoris muscles. Two were successfully treated by surgical transection of fibrous bands. These cases demonstrate a preventable and treatable iatrogenic disease.

Selective atrophy of the type II (glycolytic-rich, oxidative poor) muscle fibers, especially the subtype IIB fibers, has been shown to be the basis of cachectic atrophy accompanying cancer and other cachecting disorders. Possible hypothetical mechanisms have been formulated, i.e., a "Sparafucile" factor directly or indirectly resulting from the cancer indiscriminately assassinating the type II fibers vs. a muscle fiber martyrdom mechanism of protein catabolism to supply energy substrate, via alanine and glyconeogenesis, to cells more vital to the organism. The possible neurogenic vs. myogenic pathogenesis of the type II atrophy has been analysed. Evaluation of the mechanism of type II fiber atrophy in cancer patients is important because this "remote effect" muscle weakness is often the most crippling aspect of cancer -- if the mechanism becomes known it might be treatable independently of treatment and response of the cancer itself, and improvement of the muscle weakness and wasting could even make the patient better able to withstand the rigors of direct anti-cancer therapy.

A new neuromuscular disorder, striped loss of mitochondria, has been found in a family with dominantly inherited distal muscle weakness. A new type of mitochondrial abnormality, light-cored dense particles, has been found in skeletal muscle fibers of a patient with cardiac "assymetric septal hypertrophy". A new combined syndrome of muscle and erythrocyte phosphofructokinase deficiency and hypolipoproteinemia has been identified. Acupuncture myopathy has been reported as a new and increasingly prevalent histologic abnormality, induced as a side-effect of acupuncture, complicating the interpretation of subsequent muscle biopsies from acupuncture sites.

Neuromuscular Junction Abnormalities: Confirmed and adopted by most other physicians has been the treatment we introduced to myasthenia gravis (MG), LT-HSDAD-Pred. In our own series it continues to be extremely beneficial in the majority of cases, 27 of 30, and for as long as 9 years in a child and 5 years in an adult. Our 3 non-responders were females in the menstruating age group. We have recently modified the treatment slightly by giving, to patients not simultaneously taking anticholinesterase drugs, the single-dose 100 mg prednisone daily for the initial 2-3 weeks before converting to the

alternate-day schedule, apparently resulting in more rapid improvement. For clarity of the initial study, anticholinesterase drugs were stopped prior to LT-HSDAD-Pred treatment and have not needed to be resumed in the majority of patients. Although we suspected from a few initial clinical observations in MG patients an adverse interaction between corticosteroid and anticholinesterase drugs and in an animal nerve-muscle test preparation demonstrated that such can occur, we now find that low doses of one can be combined with the other advantageously -- but that patients taking both drugs sometimes seem to have a more "brittle" myasthenia and must be watched carefully. However, because neither anticholinesterase nor prednisone treatment is either curative or completely preventative, information on the pathokinesis is needed (v.i.). Lactate infusion has been reported to be a new provocative and apparently specific test for MG patients; as a corollary, endogenously produced lactate has been postulated to be a factor contributing to the excessive fatigability of myasthenics as well as to their occasionally observed "Mary Walker phenomenon". Contrary to the usually considered short action, 5-10 min., of i.v. edrophonium, we have documented by detailed chemical and electromyographic testing improvement in clinical strength and neuromuscular transmission lasting 1-2 hrs. in several MG patients. This has practical importance since repeated edrophonium tests are commonly used, without EMG monitoring, for adjusting dosage of other anticholinesterases.

A new factor, apparently an immunoglobulin G, has been found in the sera of MG patients which blocks binding of alpha-bungarotoxin (αBT) to acetylcholine (ACh) receptor, presumably because the factor itself is binding there (with NIHL). In 41% of the 89 MG patients the titer is high enough to block receptors at the normal neuromuscular junction (NMJ), and in those plus another 31% (total of 72%) it blocks binding of αBT to extrajunctional receptors in denervated muscle fibers, the latter apparently provides a more sensitive assay. All 9 MG patients with thymoma had the factor and all normal and non-MG-non-thymoma disease controls) lacked it. Half of the MG patients with the αBT blocking factor also had antimuscle antibody (antimuscle-I-band-reticulum antibody, which is an immunoglobulin G (with Bioscience)), and none had the latter without the former -- this strongly suggests that the two activities may be due to one antibody and that αBT blocking is a more sensitive assay for it than the antimuscle antibody assay. Since our ultrastructural localization in normal human NMJs shows αBT binding mainly at the tops and shoulders of the crests of the postjunctional sarcolemmal folds of the muscle fibers, but slightly also on the axonal membrane prejunctionally, it is presumably at those sites that the αBT blocking factor of MG sera is binding. Althoug this blocking would thereby be in the appropriate locus to cause the weakness characteristic of the disease, and often fatal, conclusive proof will require further studies, which we are currently doing. We are also investigating which cells (presumably B-lymphocytes) make the factor, why, and how can its production or presumed detrimental action be prevented. Counts of T-lymphocytes and B-lymphocytes in fresh thymus tissue removed from MG patients do not confirm the increased percent of the latter that was reported by others. Another immunologic abnormality, anti-native-DNA antibody, has been found in the sera of a significant portion of MG patients (with NIAMDD). From the basic point of view, we have reported that in mammalian muscle, as in tissues of lower species, the acetylcholine receptor and acetylcholinesterase are in separate

fractions of the sarcolemmal membrane preparation in vitro and therefore probably in somewhat different positions in the neuromuscular junction membrane in vivo.

The suprasteranal approach to thymectomy (which obviates splitting the sternum or ribs), highly recommended by one group, was, in our hands satisfactory in two patients but unsatisfactory in two others because about half of the thymus could not be removed -- total removal of which would have been, and at a second operation was, simple by the partial sternal-splitting approach. The latter approach remains our preference. We have demonstrated once again and re-emphasized that "out-of-control" MG patients can be remarkably improved by treating co-existant medical problems such as chronic respiratory infections, urinary tract infections, and anemias. Such possibly co-existing ameloriative problems must be sought in each MG patient by detailed general medical investigation. The remarkable ancillary benefit that broad-aspect nursing can provide to an MG patient has been emphasized, and specific details of that multidimensional nursing care have been published, in conjunction with a member of our nursing staff, based on our own experience. Our NIH Clinical Staff Conference summarizing the pertinent ultrastructural, histochemical, physiological, clinical diagnostic, therapeutic, respiratory-care and nursing-care aspects of MG has been published (with NHLI and CC Nursing).

Polyneuropathy (Peripheral Neuropathy) (PN): Our treatment of some cases of idiopathic PN with LT-HSDAD-Pred continues to be very successful and necessary, e.g., for as long as 10 years in an adult and 7 1/2 years in a child. Correlative studies indicate that patients most likely to respond are dysschwannian in type (slow nerve conduction times), relapsing, with elevated CSF protein, but even some patients with a dysneuronal type of PN, non-relapsing, or normal CSF have responded.

With muscle biopsy histochemistry we have demonstrated that idiopathic amyloidosis is often the cause of a sensory-greater-than-motor neuropathy beginning in adult patients (often ones undiagnosed in other centers). In our 10 cases of non-familial amyloid polyneuropathy, the onset was later adulthood (mean age 54), 8 were male, and 8 of the 10 had evidence of plasma cell dyscrasia as multiple myeloma, and/or serum and/or urine "paraprotein" immunoglobulin fragments. We have proposed that the neuropathy is due to a systemic metabolic abnormality, possibly related to an abnormal protein, rather than to focal amyloid deposits of immunoglobulin fragments. Treatment of 6 amyloid patients with melphalan, an "anti-myeloma" agent, has not been of obvious value.

A new principle/model for inducing experimental allergic neuropathy (EAN) in animals has been introduced. It involves immunizing the animals with soluble nerve protein (in contrast to previously used lipid-associated protein of myelin). Not only does this represent a new potential model of some human dysimmune peripheral neuropathies but it also represents a new approach to studying certain dysimmune disorders of the CNS, such as multiple sclerosis and parainfectious encephalopathies. Since the animals also have an abnormality of neuromuscular transmission, the model may also have some relationship to myasthenia gravis.

In studying the role of transported axoplasmic protein in the trophic maintenance of the neuromuscular junction (NMJ) and of the innervated muscle fibers, our autoradiographic studies (with Johns Hopkins) demonstrated a rapid anterograde transportation to the axon synaptic terminal at the NMJ and accumulation there of large amounts of protein synthesized in the lower motor neuron soma from amino acid precursor injected into the ventral horn less than 24 hrs. earlier. Thus we have demonstrated these rapidly transported axonal proteins are in the geographic position to have trophic influence on muscle and we propose that they do so, an hypothesis we are studying further. Autoradiographic studies have also shown: (a) direct evidence for retrograde intra-axonal transport of tetanus toxin, and (b) that fast anterograde axonal transport apparently contributes to motor nerve regeneration in experimentally sectioned nerves.

Motor Neuron Diseases: In amyotrophic lateral sclerosis (ALS) numerous different approaches by ourselves and others have failed to disclose the cause or treatment. Our studies continue to seek those goals. Last year we reported that among 16 patients with primary hyperparathyroidism, proximal neuromuscular weakness and histochemically-identified muscle fiber atrophy attributable to lower motor neuron involvement was present in nearly all, and that it sometimes presented an ALS-like syndrome -- the neuromuscular involvements reverted to or toward normal following surgical treatment of the hyperparathyroidism (with NIAMDD). We have now reported in 6 cases of secondary hyperparathyroidism a similar proximal neuromuscular weakness and also concluded it to be caused by lower motor neuron involvement (with NIAMDD). The secondary hyperparathyroidism is less easily treatable, but with medical treatment -- 20,25 dihydrotachysterol (DHT) and phosphate-binding in the gut -- the neuromuscular function can be distinctly improved.

To study the possible role of abnormality of parathyroid function and/or calcium metabolism in ordinary ALS, we have done radioisotopic calcium retention tests (N=80) on ALS patients and disease-controls: we have abnormally low calcium retention in 63% of ALS, 60% of polyneuropathy, and only 35% of myopathy patients (with NNMC). So far, treatment with DHT has been found to reverse the calcium-retention defect in some ALS patients but not result in clinical improvement in any. We are also studying the CSF levels of cyclic-AMP and cyclic-GMP in this group of patients (with NNMC). Very preliminary data indicate no clear disease-related differences. Although glucagon infusion increases cyclic-AMP in the serum 50-75 times basal levels, it does not alter CSF levels, suggesting that the CSF levels reflect only metabolism within the CNS of the cyclic nucleotide.

In progress is viral-antibody profiling of CSF from ALS patients, including those with a late-post-polio progressive muscular atrophy syndrome, that includes those of polio 1, 2, 3, mumps, measles, rubella, coxsackie, and influenza A and B (with ID, NINCDS). We are also conducting a morphologic search for virus after culturing of tissue samples from ALS patients.

In our report of fatal progressive muscular atrophy and lymphoma in sisters, 17 and 20 years of age respectively, the possible cause of both diseases by a single virus was raised (with NCI).

Previously, we have raised one possibility that ALS might in some way involve defective glycogen metabolism of the lower motor neuron soma, based on our histochemistry of anterior horn neurons in the cat which showed a very rich machinery for anerobic glycolysis and a relatively poor one for oxidative metabolism in alpha-motor neurons in comparison with the other neurons of the anterior and posterior horns. Such involvement could ultimately be due to a general metabolic or viral mechanism as discussed above.

A new technique, the staining of extrajunctional acetylcholine receptor molecules located diffusely in the sarcolemma with an immunoperoxidase technique utilizing binding of alpha-bungarotoxin to those receptors, was introduced which demonstrates denervated muscle fibers of experimental animals and in human denervating diseases (with NIHL). This technique will enhance identification and "dating" of denervated fibers. We are now using this technique to look for evidence, in the form of extrajunctional receptors, of defective motor neuron influence on muscle fibers in several diseases we have previously postulated possibly to be on such a basis rather than being myopathic -- they include central core disease, type I fiber hypotrophy with and without central nuclei, myotonic atrophy, myotonia congenita, some cases of benign congenital hypotonia, some of type II fiber atrophy, and congenital and adult-onset rod diseases. The denervated fibers in ALS-patient biopsies with demonstrable extrajunctional receptors, in turn were used as the more sensitive assay for finding the blocking factor in sera of myasthenia gravis patients. Human and rat skeletal muscle grown in tissue culture without innervation (pre-innervated muscle) (with NYU) also was shown to have diffuse extrajunctional ACh-receptors, and such fibers resemble those positive fibers in the early infantile cases of infantile spinal muscular atrophy.

We have recently published a statement of our opinion that some or many in the group of patients generally called "idiopathic scoliosis" are probably suffering from one of a number of neuromuscular disorders affecting paraspinal muscles. Certainly in our experience we have found that a number of flagrant and subtle, common and rare, neuromuscular diseases can cause scoliosis -- and we have urged that these should be sought with detailed histochemical studies of paraspinal muscles in all cases of supposedly "idiopathic scoliosis".

Ophthalmo-neurology: In our series of 36 patients we have demonstrated histochemically and electronmicroscopically that the commonest cause of progressive external ophthalmoplegia (after myasthenia gravis and myotonic atrophy are excluded) is the disorder characterized by "ragged-red" muscle fibers in limb muscles, whether or not the limbs themselves are weak. Other causes we have found, as manifested by limb muscle pathology, are, (i) a vacuolar oculo-cranio-somatic neuromuscular disease, (ii) some of the cases of type I fiber hypotrophy with central nuclei, (iii) rare cases of lower motor neuron disorder, (iv) rare cases of morphologically nonspecific myopathy, and (v) rare cases of type II fiber smallness. Ophthalmo-neurologic abnormalities in ataxias, v.i.

Central Nervous System Disorders:

Spinocerebellar ataxias -- 7 of 19 patients with spinocerebellar degeneration were reported to have defective oxidation of pyruvate in skeletal muscle slices, while succinate oxidation was normal. In three of those patients (from 2 unrelated families) with Friedreich's ataxia, serially cultured skin fibroblasts were reported to oxidize 1-^{14}C-pyruvate and 2-^{14}C-pyruvate at less than half the rate of (but U-^{14}-glutamate at rates comparable to) those found in normal fibroblasts. This appears to be a genetically determined metabolic defect, and one that might help lead to the basic biochemical abnormality of at least some cases of spinocerebellar ataxia.

In a study of slow eye movements in patients with spinocerebellar degeneration, it was found that patients made abnormally slow refixational eye movements by the saccadic system (i.e., they were slow saccades) rather than by the voluntary pursuit system. This led to the proposal of a new conceptual scheme of how both normal and defective saccadic eye movements might be generated. Study of a group of related patients with familial late-onset cerebellar ataxia revealed new information about non-visual control of eye position, since the striking abnormality was a defective smooth pursuit and fixation system. The patients showed evidence of various non-visual mechanisms of maintaining eye position that have not been previously delineated.
A study demonstrated that rebound nystagmus occurs in normal individuals if fixation is eliminated and only becomes clinically apparent in patients with spinocerebellar degeneration because of a coexisting defect of visually-mediated fixation mechanisms; thus rebound nystagmus can be interpreted as a manifestation of one of the brain's compensatory mechanisms for maintaining eye position when visual systems are ineffective. A unique combination of ophthalmoplegia and dissociated nystagmus was identified in patients with abetalipoproteinemia (Bassen-Kornsweig syndrome) (with NEI).

Progressive spastic paraplegia -- identified were two unrelated patients with a syndrome of chronic adrenal insufficiency from infancy and juvenile onset of progressive spastic paraplegia and "onion-bulb" peripheral neuropathy, with normal intelligence, (with NIAMDD).

Methodology and Basic Cellular Mechanisms: Many of these points are covered in the preceding categories; ones which are not are as follows:

Tissue culture -- (with NYU) a new program was developed, and published, for investigating adult human muscle grown aneurally in tissue culture. This included new techniques of: (a) tissue culture for obtaining luxurious growth of muscle fibers, e.g., explant-reexplantation technique and culture methods per se, and (b) preparation for histochemistry ("sandwich" preparation) and electronmicroscopy (drilling). A new drill and drilling technique was published that allow precise plucking after EM-histochemical staining and plastic embedding of the fibers in the desired state of development and showing the desired pathologic change (since all fibers are not equally affected, even in the original biopsy) for electronmicroscopy, rather than having to "look for a needle in a haystack". In this way we demonstrated good maturation of muscle fibers cultured aneurally, which showed lack of differentiation of them into different histochemical fiber types when cultured aneurally. We also utilized these techniques for growing and studying by electronmicroscopy, histochemistry, and biochemistry the abnormal

muscle from human diseases, as discussed above (v.s.) (with NYU). In tissue cultures of "normal" chick embryo skeletal muscle we were able to induce certain bizarre changes of mitochondrial morphology by pulses of dinitrophenol (DNP), but did not produce all the mitochondrial changes occurring in "ragged red" fibers of certain human neuromuscular diseases (with NYU). In the course of that study we found that DNP markedly promoted the detectability of avian leucosis/sarcoma (ALS) virus in all such "normal" chick muscle cultures as evident morphologically (C-particles) and by complement fixation avian leucosis (COFAL) titers (with NYU). This demonstrated that the "normal" chick embryo muscle cultures so frequently used for physiologic, biochemical, immunologic and developmental studies are actually virally contaminated test objects. The question of whether that ALS virus has a "normal" role in development of "normal" chick muscle in vivo or in vitro is raised by this study. Our finding the C-particles exclusively in dilations attributed to T-tubules raises the intriguing possiblity that T-tubules can be the aqueducts of virus infestation of or shedding from muscle fibers. The question of viruses harbored in mitochondria (as "mitochondriophages", analogous to bacteriophages) is raised by the C-particles being provoked by DNP, an uncoupler of mitochondria oxidative phosphorylation.

Histochemistry -- The systematic approach in application of a rapid short (5-stain) and long (18-stain) battery of histochemical reactions to fresh-frozen sections of human muscle biopsies we have developed is being followed in nearly all centers for neuromuscular research throughout the world. This includes basic stains developed (e.g., our modified trichrome), our system of fiber nomenclature, and our new concepts in the analytical approach to neuromuscular pathology (e.g., selective vs. non-selective fiber-type involvement; total-unit vs. partial-unit involvement; complete vs. incomplete defect of neuron-to-muscle influence; fiber-type grouping; fiber-type hypotrophy; and motor-unit hypoplasia). A number of investigators and technicians come each year to learn this approach. Many outside biopsies are sent to us for histochemical processing and/or interpretation.

At the light-microscopic level the immunohistochemical staining of alpha-bungarotoxin (αBT) binding to ACh receptors has been introduced as a new method to delineate muscle fibers with incomplete motor neuron influence -- these include (a) denervated fibers (as in ALS and polyneuropathies), (b) pre-innervated fibers (as in tissue cultured fibers of chick rat and human muscle, and possibly also in very young cases of infantile spinal muscular atrophy), and (c) regenerating-degenerating ("regen-degen") muscle fibers (as in active myopathies, e.g., polymyosiits/dermatomyositis) (with NIHL). The regen-degen fibers thus are probably ones separated from their original motor neuron influence and not yet subjugated by a new neural influence.

Use of the non-specific esterase has been introduced as a new method for highlighting the slightly to moderately atrophic, "rongulated" or angular, denervated muscle fibers -- it is extremely useful in the histochemical diagnosis of minimal denervation.

Last year we formulized the nomenclature we have been using for many years for human muscle fiber histochemical types, based on two fiber types, I and II, and that nomenclature was adopted without change and published by the Research Group for the World Federation of Neurology, as well as ourselves. Now we have histochemically demonstrated two distinct subtypes of the type I fibers, and shown selective involvement of one subtype in certain human neuromuscular diseases (with NYU).

Electronmicroscopy (EM) -- The technique, recently introduced by others, for staining calcium at the EM level by antimony is being used to study (a) normal distribution of calcium in muscle fibers, (b) increase in myopathic disorders, especially in parallel with radioactive diphosphonate localization by total tissue counting and autoradiography, and (c) possible decrease in denervated muscle, according to our preliminary studies. Details of the immunoperoxidase localization ultrastructurally of α-bungarotoxin to acetylcholine-receptors is discussed above (v.i.). Cytochrome-oxidase-stained mitochondria showed that nearly all of the intramitochondrial crystals of "ragged-red fibers" lack that enzyme stainability. The new abnormality of "zipper tubules", identified histochemically in type I fibers, has been elucidated by EM.

Biochemistry -- Emphasis has been placed on studying mammalian sarcolemmal membranes. Not only have pure fractions of rat sarcolemma been obtained, but methods have been developed that provide quantities of sarcolemmal membrane from human muscle obtained from amputation and radical mastectomy. In these membrane fractions and subfractions methods have been established for studying acetylcholine receptor, acetylcholinesterase, Na^+-K^+ ATPase (Na^+-stimulated phosphorylation), adenylate cyclase, divalent cation (viz., Ca^{++}) binding/transport, and Ca^{++}-stimulated ATPase. Some of these have been studied in sarcoplasmic reticulum fractions as well. Elucidated have been detailed properties of (a) the adenylate cyclase (i.e., fraction localization, kinetics, catecholamine activation, guanylyl-inidodiphosphate activation, insulin and glucagon inhibition, and response to denervation); (b) the sarcolemmal protein phosphorylation (i.e., Na^+ enhancement blocked by K^+, phosphorprotein state suggesting an acylphosphate bond, high turnover rate suggesting it is a functional intermediate of Na^+K^+ATPase, and molecular weight); the Ca^{++} uptake and release by human sarcoplasmic reticulum (SR) (i.e., by use of specific antibodies made against SR which block the Ca^{++} uptake and inhibit adenyl cyclase but do not affect ATPase activity, suggesting different localization of these functions within the SR); and (d) physicochemical properties of ACh-receptor cf. acetylcholinesterase. These assays will now be used to seek biochemical defects of sarcolemmal or sarcoplasmic reticulum function in muscle biopsies from patients with various neuromuscular disorders.

Electromyography (EMG) -- Open-biopsy electromyography (to correlate EMG and histochemical findings from the same field of muscle fibers), and extracellular micromarking (to give a 50-100 μM dia. spot in histochemical sections), two techniques developed by us and reported previously, are being used to directly correlate EMG-recorded spontaneous activity and α-bungarotoxin-positivity of muscle fibers in patients with ALS and other denervating disorders, as well as in the polymyositis/dermatomyositis complex. With the open-biopsy EMG technique last year we reported that the pattern of brief-duration small-amplitude overly-abundant potentials (BSAPs) on voluntary effort can sometimes come from

regions showing no myopathy in the usual sense but, instead, consisting of type I fiber atrophy, type I fiber hypotrophy with or without central nuclei, type II fiber paucity, both-fiber atrophy of myasthenia gravis, and even "ordinary denervation". From this, and on theoretical grounds, we have published a critique of EMG and emphasized that the former and widely used designation of "myopathic EMG" for the BSAP pattern is erroneous, since according to our theoretical formulation it could also be due to (a) fractionation of the motor unit on an "ordinary" neuropathic basis or to (b) type-I-fiber (or both-fiber) smallness on a proposed subtle neurogenic defect-of-trophic-factor basis. This formulation alters the entire concept of the pathogenesis of a number of diseases which are considered "myopathic" primarily on the basis of their EMG pattern (e.g., myotonic atrophy ("myotonic dystrophy"), myotonia congenita, paramyotonia congenita, central core disease, benign congenital hypotonia with I-fiber predominance, I-fiber hypotrophy, congenital rod disease, some cases of II-fiber atrophy, myasthenia gravis, the facilitating myasthenic syndrome, and possibly some cases of facio-scapulo-humoral syndrome). Even though we are writing and speaking that there is nonesuch animal as a diagnostic "myopathic EMG" many persons are still erroneously (in our view) using that term.

Autoradiography -- (v.s.)

Basic neurophysiology -- Analysis of the rapid events associated with muscle fiber contraction and elasticity, using our specially designed equipment, has added new details on the basic functional properties of muscle of animals, as a model for human muscle function.

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Principal Investigator: W. King Engel, M.D.

Other Investigators: Steven P. Ringel, M.D.
Adam N. Bender, M.D.
N. Bojji Reddy, M.D.
Bernard M. Patten, M.D.
Valerie Askanas, M.D., Ph.D.

Cooperating Units: Department of Neurology, Baylor College of Medicine, Houston, Texas
Muscle Research Laboratory, Institute for Rehabilitation Medicine, New York University School of Medicine, New York, N.Y.
Muscular Dystrophy Associations of America

Man Years:

Total:	3.5
Professional:	2.0
Other:	1.5

Project Description:

Objectives: (1) To study the cellular and subcellular localization of a variety of histochemical reactions in normal human skeletal muscle, neurons and peripheral nerves and to see how they are altered in various neuromuscular and other neurologic diseases. (2) To develop new histochemical techniques and to analyse mechanisms of the reactions. (3) To correlate histochemically defined types of muscle fibers with physiologic, ultrastructural, and biochemical properties. (4) To identify selective or non-selective involvement of a particular histochemical fiber type in the various neuromuscular diseases.

Methods Employed: About 20 histochemical reactions are being done routinely on muscle and neural tissue - they have been outlined in previous reports. New specially developed or adapted reactions include ones at the electronmicroscopic level for calcium, acetylcholine receptor, cytochrome oxidase, acid phosphatase, and acetylcholinesterase (see our Electronmicroscopy Project).

Patient Material: Biopsies obtained for diagnostic purposes were studied histochemically from Medical Neurology Branch patients as well as from consultation patients; patient biopsy material was also submitted by outside physicians and investigators. Some muscle biopsies were grown in tissue culture prior to histochemical study (see our Tissue Culture Project).

Major Findings: In general, the systematic approach for application of a rapid short (5-stain) and long (18-stain) battery of histochemical reactions to fresh-frozen sections of human muscle biopsies we have developed is being emulated in virtually all centers for neuromuscular research throughout the world. This includes basic stains developed (e.g., our modified trichrome), our system of fiber nomenclature, our new concepts in the analytical approach to neuromuscular pathology (e.g., selective vs. non-selective fiber-type involvement; total-unit vs. partial-unit involvement; complete vs. incomplete defect of neuron-to-muscle influence; fiber-type grouping; fiber-type hypotrophy; and motor-unit hypoplasia); and introduction of new, more appropriate descriptive terms (e.g., fiber smallness or largeness). A number of investigators and technicians come each year to learn this approach. Many outside biopsies are sent to us for histochemical processing and/or interpretation.

Last year we formulized the nomenclature we have been using for many years for human muscle fiber histochemical types, based on two fiber types, I and II, and that nomenclature was adopted without change and published by the Research Group for the World Federation of Neurology, as well as ourselves. Now we have histochemically demonstrated within the group of type I fibers (identified with regular 9.4 and acid-reversed ATPase reactions) two distinct subtypes of the human type I fibers. We have also shown selective involvement of one type-I-fiber subtype in certain human neuromuscular diseases, i.e., type I fiber hypotrophy with central nuclei and as subtype groups in chronic infantile spinal muscular atrophy (with NYU). In contrast to subtype Ib fibers, subtype Ia fibers show slightly darker NADH-TR, NADPH-TR, SDH-TR non-specific esterase and oil-red-O-for-lipid staining, but slightly lighter staining for menadione-mediated alpha-glycerophosphate-TR, phosphorylase and glycogen.

At the light-microscopic level the immunohistochemical staining of alpha-bungarotoxin (αBT) binding to ACh receptors has been introduced as a new method to delineate muscle fibers with incomplete motor neuron influence -- these include (a) denervated fibers (as in ALS and polyneuropathies), (b) pre-innervated fibers (as in tissue cultured fibers of chick rat and human muscle, and possibly also in very young cases of infantile spinal muscular atrophy), and (c) regenerating-degenerating ("regen-degen") muscle fibers (as in active myopathies, e.g., polymyositis/dermatomyositis) (with NIHL). The regen-degen fibers thus are probably ones separated from their original motor neuron influence and not yet subjugated by a new neural influence.

Use of the non-specific esterase has been introduced as a new method for highlighting the slightly to moderately atrophic, "rongulated" or angular, denervated muscle fibers -- it is extremely useful in the histochemical diagnosis of minimal denervation.

The segmental histochemical changes over a 6-month period during denervation and reinnervation of partially denervated cat muscle were studied after single spinal root section (L-6 or L-7). Atrophic changes in the form of small angular fibers with strong DPNH staining were marked by the first month. Less pronounced atrophy was also present throughout the later months with the appearance of intermediate size fibers of rounded shape and normal DPNH staining. The denervation atrophy was multi-fascicular but often regional in distribution. Central fiber changes resembling targetoids were noted in severely denervated muscles during the first three months. "Central paleness" for all stains was seen in some large fibers throughout all stages of observation. Histochemical type grouping was present in all animals sacrificed after three months. In the early stages, type grouping and fiber atrophy were present together. Type grouping was often observed in a regional pattern, with a wide variation in the size of the groups. Myopathic features in the form of phagocytosis, interstitial and perivascular inflammation were seen in the severely denervated muscles during the early stages.

In a biochemical-histochemical correlative study, it was shown by biochemical assay of Ca^{++} activated ATPase activities of isolated myofibrillar, mitochondrial, and sarcoplasmic reticulum (SR) fractions of guinea pig "red" (type-I-fiber) vs. "white" (type-II-fiber) muscle, under exactly the same conditions as employed histochemically (pH 9.4), without or with various degrees of acid pre-incubation, that our previous histochemical impressions were confirmed regarding: (a) true reversal of relative amounts of myofibrillar ATPase in type I vs. II fibers, and (b) the additional contribution of the intermyofibrillar membranous components (mitochondria and SR) in creating the greater overall histochemical darkness of type I cf. II fibers. The myofibrillar component, since it contains about 15-fold more protein than the SR and mitochondrial components combined, is likely to be quantitatively the major determinant of type II muscle fiber histochemical coloration in regular (non-preincubated) and acid-preincubated ATPase reactions.

Alkaline-phosphatase-positive muscle fibers, an empiric abnormality in certain human muscle diseases, were produced experimentally.

Numerous interrelated studies between this Project and other Medical Neurology Branch Projects were recorded under those other projects, for example:

a. Reincarnation of the same histochemical (as well as electron-microscopic, and in some cases biochemical) abnormalities in muscle fibers grown in tissue culture as in the original biopsies, e.g., vacuoles of glycogen storage in adult-onset acid maltase deficiency, acid-phosphatase-positive vacuoles in "cabbage-body myopathy", and some mitochondrial abnormalities in ragged-red fibers -- Tissue Culture and EM Projects.

b. Demonstration of the return of phosphorylase in muscle fibers cultured from a phosphorylase-deficiency patient whose original biopsy lacked any muscle-fiber phosphorylase -- Tissue Culture Project.

c. Introduction of a new light and electronmicroscopic technique, utilizing an immunoperoxidase technique with alpha-bungarotoxin, to stain acetylcholine receptor (AChR) at the normal neuromuscular junction and to show differences of receptor in the sarcolemmal membrane of animal and human denervated fibers (v.s.); the latter was used to identify in sera of myasthenia gravis patients a new factor capable of blocking the AChR (as evident by its blocking alpha-bungarotoxin binding to AChR) -- Myasthenia Gravis Project.

d. "Rugged-red" muscle fibers were found in the skeletal muscle of a patient with assymetric septal hypertrophy of the heart, which led to delineation by electronmicroscopy of light-cored dense particles in mitochondria -- Myopathy Project.

e. Demonstration and summarization of the muscle fiber changes occurring in myasthenia gravis -- Myasthenia Gravis Project.

f. Identifying a particular pattern of muscle fiber damage that provided the stimulus for the ischemic myopathy hypothesis of Duchenne muscular dystrophy, and now showing similar changes in amputated limbs in peripheral vascular disease. Also a basis of determining muscle fiber damage in our various animal models -- Myopathy Project.

g. Identification of iatrogenic and experimental-animal "needle myopathy", which provided the basis of our formulation of the newly considered entity "'Acupuncture Myopathy' (Remembrance of Things Passed)" -- Myopathy Project.

h. Providing the basis of diagnosis of patients with 3 categories of disease, rod (nemaline disease), central core disease, and muscle fiber hypotrophy, about each of which a detailed chapter was written for a forthcoming book -- Electronmicroscopy Project.

i. Our original technique of "Open-biopsy Electromyography" for direct correlation of electromyographic changes (e.g., fibrillations and positive sharp waves) and histochemical data from the same fibers, in patients with various neuromuscular disorders -- Electrophysiology Project.

j. Histochemistry of normal human and animal muscle cells and neurons in tissue culture -- Tissue Culture Project.

k. Histochemical basis for suggesting that a number of erstwhile myopathic diseases may be neurogenic, e.g., myotonia congenita, paramyotonia congenita, myotonic atrophy ("dystrophy"), type I fiber hypotrophy with or without central nuclei, central core disease, and the type I fiber predominance form of the benign congenital hypotonia syndrome -- ALS Project.

l. Histochemistry integrated with electromyography was the basis of our new concept of "hypoplastic motor units" in certain neuromuscular disorders, such as benign-congenital-hypotonia-with-type-I-fiber-predominance and central-core disease -- ALS Project.

m. Identifying new diseases, such as focal-loss-of-cross-striations, striped loss of mitochondria, and central-cores-with-central-hot-spots -- ALS and Myopathy Projects.

n. Identifying type II fiber atrophy and evidence of subtle denervation in patients with primary and secondary hyperparathyroidism -- ALS Project.

o. Identifying type-II fiber muscle atrophy, and evidence of neuropathy in some of the cases, associated with a distant neoplasm -- ALS Project.

p. Diagnosing amyloid with crystal violet stain of fresh-frozen sections in muscle biopsies of patients in whom the diagnosis previously was missed by others -- ALS Project.

q. Finding rods in nuclei in two cases of adult-onset rod disease, the ultrastructure of which was detailed by electronmicroscopy -- Electronmicroscopy Project.

r. Identifying the nearly-diagnostic alkaline-phosphatase-positive staining in connective tissue in the dermatomyositis/polymyositis complex -- Myopathy Project.

s. Demonstrated, on the basis of histochemical findings in limb muscle biopsies, that a variety of different neuromuscular diseases can cause the common clinical syndrome of scoliosis; and conversely, these findings provided the basis for our published proposal that all cases of so-called "idiopathic scoliosis" deserve to be studied by histochemical evaluation of muscle biopsies to seek a neuromuscular disorder as the basis. We propose that many, if not most or all, cases of "idiopathic scoliosis" will be found to be based on a subtle neuromuscular disease -- ALS Project.

t. Identification of a new childhood-onset form of rod disease with histochemical changes like adult-onset rod disease and not like the congenital form -- Electronmicroscopy Project.

Significance to Bio-Medical Research and the Program of the Institute: We have firmly established over the past 10 years (see first paragraph above, under "Major Findings") that histochemistry of muscle biopsies is the single most important laboratory evaluation of human neuromuscular disease, and that it is also extremely useful in (a) screening for correlating with electron-microscopic studies, (b) correlation with electromyographic findings recorded

from the same fibers, (c) evaluating growth in tissue culture of human muscle biopsies from patients with various neuromuscular diseases, (d) correlating with other studies on the same biopsy, such as biochemistry, immunology and autoradiography and (e) evaluating animal models. This approach has provided insight into the possibility that certain diseases heretofore called "myopathies" may actually be caused by a form of neuronal abnormality, perhaps related to a defect of neuronal trophic function. This type of analysis (a) helps establish whether the basic pathogenesis and etiology are related to properties reflecting the metabolic "sameness" or the "differentness" of the lower motor neurons or muscle fibers, and (b) directs subsequent investigative studies toward analysing those two groups of properties of muscle and nerve cells. Obviously, knowing whether a certain neuromuscular disease is due to nerve cell or muscle cell abnormality may be critical to understanding its cause and treatment.

Proposed Course of Project: Further correlations of the results from light microscopy with those of electronmicroscopy, tissue culture, physiology, biochemistry, autoradiography and immunology will be made in human neuromuscular diseases and certain normal animal studies. Other animal models of human neuromuscular disorders will be produced and studied.

Keyword Descriptors: acetylcholine receptor, muscle histochemistry, neuromuscular diseases, muscle fiber subtypes, ATPase, muscle

Honors and Awards: None

Publications:

Askanas, V. and Engel, W. K.: Distinct subtypes of type I fibers of human skeletal muscle. Neurology, 1975, in press.

Askanas, V. and Engel, W. K.: Distinct histochemical subtypes of type I fibers of human skeletal muscle. Trans. Am. Neurol. Assoc., 1975, in press.

Ringel, S. P., Bender, A. N., Festoff, B. W., Engel, W. K., Vogel, Z. rat skeletal muscle fibers: Ultrastructural demonstration and clinical application. Nature, 1975, in press.

Engel, W. K.: Rods in abundant nuclei of two cases of adult-onset rod disease (AROD). A proposed pathokinesis for the disease. IIIrd Int. Cong. on Muscle Diseases, Sept. 15-21, 1974, Newcastle upon Tyne, Great Britain, Excerpta Medica Int. Cong. Series No. 334, p. 15.

Engel, W. K.: Recent advances in neuromuscular diseases. Proc. of the Children's Hospital, Buenos Aires, 1975, in press.

Serial No. Z01 NS 00917-14 MN
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemistry Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Barry W. Festoff, M.D.
N. Bojji Reddy, M.D.

Other Investigators: R. A. Pieter Kark, M.D.
John P. Blass, M.D.
Eugene C. Weinbach, M.D.
Salvatore DiMauro, M.D.
Valerie Askanas, M.D.

Cooperating Units: U.C.L.A., School of Medicine, Los Angeles, Calif.
Physiology and Biochemistry Section, LPD, NIAID
Columbia University, College of Physicians and Surgeons, New York, N.Y.
Muscle Research Laboratory, I.R.M., New York University School of Medicine, New York, N.Y.

Man Years:

Total:	4.0
Professional:	2.2
Other:	1.8

Project Description:

Objectives: (1) To seek and analyse biochemical abnormalities of neuromuscular and other human neurologic diseases; (2) to evaluate biochemical properties, and possible defects thereof, in specific subcellular components after their isolations and purifications, with special emphasis on characterizing the structural and functional components of the sarcolemmal membrane complex of mammalian (including human) skeletal muscle; (3) to elaborate biochemical differences and similarities between "red" and "white" muscle of animals and between type I (similar to "red") and type II (similar to "white") individual muscle fibers of humans and animals.

Methods Employed: Blood, spinal fluid, muscle biopsy, and cultured fibroblast samples of patients were studied with a variety of techniques, as was rat muscle:

a. Adapted to mammalian muscle have been a number of techniques of isolation and purification of rat and human sarcolemmal (SL) sarcoplasmic reticulum (SR) membranes.

b. Various membrane-located enzymes studied are mentioned below in each section.

c. As an extension of "a" (above), the mammalian (rat) nicotinic cholinergic receptor and specific acetylcholinesterase have been isolated from the same muscle preparation and each profiled by various physio-chemical techniques.

d. ^{125}I-alpha-bungarotoxin was used to identify the nicotinic cholinergic receptor.

e. Virtually fiber-type-pure soleus (type I, red) and extensor digitorum longus (EDL) (type II, white) guinea pig muscle was fractionated into sarcoplasmic reticulum (SR) mitochondria, myofibrils, actomyosin, and myosin according to established procedures. These fractions were assayed at pH 9.4 (with calcium as the activating factor) for their ATPase activity without and with EDTA pretreatment at varying pHs (4-10.5) in media exactly corresponding to that used for histochemistry, in order to correlate biochemical subcellular localization with that of histochemistry.

f. Standard isotope techniques for oxidation of succinate, beta-OH-butyrate, palmitate, and pyruvate have been adapted to tissue slices of small biopsy specimens of human muscle.

g. Standard oxygen electrode techniques for the oxidation by pure mitochondrial fractions, isolated from human muscle biopsies and cultured human skin fibroblasts, of succinate, beta-OH-butyrate, palmitate, pyruvate, and NADH.

h. Biochemical assays (Dr. DiMauro) for acid and neutral maltases (both with natural and artificial substrates) phosphorylase, acid phosphatase glycogen and total protein have been minaturized and used successfully with our tissue-cultured human muscle grown by Dr. Askanas.

i. Lactate, pyruvate, and creatine phosphokinase (CPK) assays of blood and spinal fluid.

Patient Material: Selected Medical Neurology Branch patients with neurological diseases, utilizing portions of specimens obtained for diagnostic purposes.

Major Findings: (1) Characterization of Mammalian Sarcolemma ACh-Receptor vs. ACh Esterase -- Markers for "end-plate" and general membrane function have been identified on density gradient fractions of "crude" sarcolemmal vesicles. These markers have been purified relative to the homogenate of muscle from which they are derived. Acetylcholinesterase (AChE) and

acetylcholinergic receptor (as defined by binding of alpha-bungarotoxin, specific for nicotinic receptors), NaKATPase, adenylate cyclase, Ca^{++} binding and uptake, and insulin binding have been assayed in the fractions of discontinuous gradients monitored by electronmicroscopy. AChE, NaKATPase and adenylate cyclase preferentially distribute in lighter density fractions while the receptor activity is highest in the mid-to-heavy density bands. Publication of this study represented the first demonstration in mammalian muscle of the molecular distinction between AChE and the ACh-receptor. These results are consistent with the concept that AChE may be superficially bound in the intersynaptic matrix or "fuzz" while the receptor is a component of the true plasma membrane. Our next studies directed at a further molecular "dissection" of the sarcolemma are in progress. The functional nature of the carbohydrate-rich "fuzz" or basement membrane, the turnover of AChE and receptor in states of denervation and re-innervation are continuing.

(2) Adenyl cyclase (AC) -- An investigation of subcellular distribution of this enzyme in rat and human skeletal muscle revealed that it is associated with SL, SR and mitochondrial fractions. The general properties of AC of all the three fractions of rat and human were found to be similar except that the activity levels are considerably lower in human compared to that of rat. Extensive kinetic analysis of AC of human SL was carried out and the optimal conditions for enzyme activity were worked out. Besides fluoride, catecholamines, viz., isoproterenol and epinephrine, at very low concentrations (10^{-5}M) enhanced the basal activity by 2 to 3 fold. The catecholamine stimulation was effectively blocked by propanolol, a beta blocker. The catecholamine stimulation was further enhanced by GTP while other nucleotide triphosphates were less effective. Guanylyl-imidodiphosphate (GMP PNP) an analogue of GPT, was found to be a more potent activator of AC than GTP. The AMP-PNP stimulated the basal as well as the catecholamine sensitive AC. The nucleotides are believed to stimulate AC by binding to "regulatory" sites of the enzyme. This has been the first demonstration of nucleotide effect on this enzyme in skeletal muscle. These results suggest typical beta adrenergic characteristics of muscle AC. On the other hand, polypeptide hormones like insulin and glucagon were found to inhibit basal AC. Since insulin dramatically inhibits this cyclase system and it is postulated, may be involved in the regulation of glucose transport-linked alteration in cation permeability by possibly de-phosphomylating the NaKATPase intermediate discussed above. In this regard the study of myotonic dystrophy should be doubly rewarding because of the co-existence of insulin resistance and putative alteration in ion transport in skeletal muscle.

Immunochemical characterization of AC of SL was carried out. The antisera produced in rabbits against human SL inhibited the fluoride and catecholamine stimulated AC.

Preliminary results on the AC activity in fast (EDL) and slow (soleus) muscles of rat suggest that the slow muscle enzyme is more responsive to catecholamine stimulation. Denervation experiments on rat gastrocnemius muscle revealed that this enzyme activity is enhanced during early periods (one week) of denervation but progressively declines during 2 to 4 weeks of denervation. Further work is needed to ascertain the possible neural control of this enzyme activity in skeletal muscle.

(3) Phosphorylation -- The properties of rat muscle SL membrane protein phosphorylation was extensively characterized. The phosphorylation was specifically enhanced by Na^+ while K^+ inhibited Na^+ activation. Chemical properties of phosphoprotein suggested an acylphosphate bond. The phosphoprotein showed a high rate of turnover suggesting that it is a functional intermediate of membrane bound (Na+K)ATPase. The molecular weight of the phosphoryl acceptor protein was estimated to be 102,000 daltons on SDS-gel electrophoresis. Similar results were also obtained with human preparations.

(4) Calcium Uptake of Sarcoplasmic Reticulum and Sarcolemma -- Routine assays for Ca^{++} binding/transport in sarcolemmal and sarcoplasmic reticulum membranes have been established. The properties of Ca^{++}-uptake and release of human SR and its interrelationship with Ca^{++}-stimulated ATPase are being investigated utilizing the antibodies produced against SR. It was found that antisera effectively blocked the Ca^{++}-uptake but did not affect the ATPase activity. Furthermore the antisera shifted the Ca^{++} dependency curve for ATPase activity to the left. A potent anti-SR antibody was recovered from immunized rabbits. Studies to date reveal that this antibody inhibits Ca^{++}-uptake (85%) and adenylate cyclase (90%) but stimulates Ca^{++}-stimulated ATPase ("Ca^{++}-pump") activity of the SR. These effects are not complement-dependent as shown by appropriate heating experiments. Binding to SR components by antibody have been demonstrated in conventional double diffusion discs (after detergent solubilizing SR) as well as in a novel method of separating SR proteins by SDS electrophoresis and then reacting whole gel with antibody. These studies may shed light on the orientation of these important molecules in the SR membranes, *in vivo*. Immunohistochemical studies are planned using techniques developed for other antigens. The system may be adapted for exploring the presence of anti-SR antibodies in human disorders such as polymyositis, muscular dystrophy, etc. The effects of a number of agents which may alter these events such as cAMP, cGMP, caffeine, quinidine, and the new anti-spastic drug, dantrolene sodium (Dantrium) will be evaluated.

(5) Rapid Technique of Calcium Uptake and Stimulation of ATPase -- In conjunction with SR studies, the development of a rapid technique for simultaneously measuring $^{45}Ca^{++}$-uptake and Ca^{++}-stimulated ATP(^{32}P)-ase activities in the same samples. This technique is simple, highly reproducible, and extremely valuable when limited amounts of membrane are available (as, for example, from biopsy material).

(6) Human Muscle Source -- Protocol designed and implemented to obtain large quantities of human leg and pectoral muscles from amputation and radical mastectomy operations respectively, which are performed at the Clinical Center. Histochemical check of all muscles obtained for presence of neuromuscular disturbance has been performed. Several samples were obtained from patients with striking type II (glycolytic) fiber atrophy. Membranes obtained from these patients represent an ideal source to study membrane functions in disorders affecting only one of the major types of fibers present in human muscle. Studies are at present preliminary in this regard.

(7) Biochemical-Histochemical Correlations of Muscle ATPase -- In a biochemical-histochemical correlative study, it was shown by biochemical assay of Ca^{++}-activated ATPase activities of isolated myofibrillar, mitochondrial, and sarcoplasmic reticulum (SR) fractions of guinea pig "red" (type-I-fiber) vs. "white" (type-II-fiber) muscle, under exactly the same conditions as employed histochemically (pH 9.4), without or with various degrees of acid pre-incubation, that our previous histochemical impressions were confirmed regarding: (a) true reversal of relative amounts of myofibrillar ATPase in type I vs. II fibers, and (b) the additional contribution of the intermyofibrillar membranous components (mitochondria and SR) in creating the greater overall histochemical darkness of type I cf. II fibers. The myofibrillar component, since it contains about 15-fold more protein than the SR and mitochondrial components combined, is likely to be quantitatively the major determinant of type II muscle fiber histochemical coloration in regular (non-preincubated) and acid-preincubated ATPase reactions.

(8) Oxidative Metabolism in Small Samples of Normal and Diseased Human Muscle -- In slices of human muscle biopsies we previously reported that muscle from more than half the patients with spinocerebellar denervation and 1/3 of cases with idiopathic or familial neuropathy oxidized pyruvate at a rate more than 5 SE below the control mean. We have now published that in the cultured skin fibroblasts of patients from two families with spinocerebellar degeneration (Friedreich's ataxia type) that same biochemical defect is demonstrable. This has now been confirmed by another group (Andre Barbeau, personal communication) (see our ALS-Spinocerebellar Degeneration Project).

(9) In human muscle fibers grown in tissue culture, biochemical assays have provided the first demonstration of:

a. Reincarnation of the acid maltase deficiency in cultured skeletal muscle fibers, with normal neutral maltase and somewhat elevated acid phosphatase (in parallel with reincarnation in the cultured muscle fibers of the histochemical and electronmicroscopic changes characteristic of the original biopsy) (see our Tissue Culture Project).

b. In parallel with reincarnation in the cultured fibers of the histochemical and electronmicroscopic changes of multilaminar "cabbage bodies" as were characteristic of the original biopsy of a patient with young-adult-onset "vacuolar" myopathy, reiteration in culture of the same biochemical changes, namely, normal neutral maltase, slightly elevated glycogen, and elevated (1 1/2 times normal) neutral maltase and acid phosphatase) (see our Tissue Culture Project).

Significance to Bio-Medical Research and the Program of the Institute: Elucidation of biochemical abnormalities, particularly in the realm of enzymes and other proteins, is important to the understanding of neurologic and neuromuscular diseases, and in seeking means of therapy. Putting the various abnormalities in proper perspective regarding pathogenesis and disease specificity is often neglected, but it is vital to planning future investiga-

tive and therapeutic procedures. Both the properties of the general sarcolemmal membrane and the specialized properties of the neuromuscular junctional regions of the sarcolemma hold great promise as a model for excitable membranes, as well as for the elucidation of basic mechanisms in various neuromuscular diseases. The same is true of the sarcoplasmic reticulum membranes. Having comparative studies of biochemical differences between type I and type II skeletal muscle fibers will help provide a basis for understanding the pathogenesis of the many neuromuscular disorders preferentially affecting one of these fiber types, as detailed in the Histochemistry Project. The precise localization and characterization of normal biochemical properties to each type of subcellular element of the muscle fiber can serve as the basis for seeking biochemical defects thereof in various human neuromuscular diseases. The spinocerebellar ataxias, usually hereditary, have long been resistant to yielding their secrets to scientific analysis, but a beginning is now being made in their biochemical analysis.

Proposed Course of Project: Insofar as personnel and space permit, the above techniques will be further perfected and applied to muscle and/or nerve from a variety of human neuromuscular and central nervous system disorders to seek further details of biochemical defects therein. Biochemical differences and similarities of normal human type I and type II fibers will be explored further. Various aspects of the membrane studies to be studied are mentioned in several sections of "Major Findings".

Keyword Descriptors: sarcolemma, sarcoplasmic reticulum, protein kinase, adenylcyclase, sarcolemmal calcium transport, ATPase, sarcolemmal ATPase, sarcoplasmic reticulum

Honors and Awards: None

Publications:

Festoff, B. W. and Engel, W. K.: In vitro analysis of the general properties and junctional receptor characteristics of skeletal muscle membranes. Isolation, purification, and partial characterization of sarcolemmal fragments. Proc. Natl. Acad. Sci. U.S.A. 71:2435-2439, 1974.

Reddy, N. B. and Engel, W. K.: Biochemical characterization of the histochemical "reversed adenosine triphosphatase" in subcellular fractions of muscle fibers. J. Histochem. Cytochem. 22:292-293, 1974.

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1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Myopathies

Previous Serial Number: Same

Principal Investigator: W. King Engel, M.D.

Other Investigators:
Valerie Askanas, M.D., Ph.D.
Barry A. Siegel, M.D.
S. Charles Bean, M.D.
Roger W. Kula, M.D.
Barry E. Levin, M.D.
Salvatore DiMauro, M.D.
John L. Sever, M.D.
James Hawley, M.D.

Cooperating Units:
Muscle Research Laboratory, I.R.M., New York University School of Medicine, New York, N.Y.
Armed Forces Radiobiology Research Institute, Bethesda, Maryland
V.A. Hospital, White River Junction, Vermont
Columbia University, College of Physicians & Surgeons, New York, New York
Infectious Diseases Branch, NINCDS
Veterans Administration Hospital, Washington, D.C.

Man Years:

Total:	3.6
Professional:	2.6
Other:	1.0

Project Description:

Objectives: To more fully elaborate the clinical, histochemical, biochemical, ultrastructural and immunologic abnormalities of patients with the various myopathies. To further sub-classify patients in each category using those parameters. To seek pathogenic mechanisms, using a variety of different techniques including tissue culture and the ones listed above, applied to the patient's body fluids and tissues, especially to the muscle biopsy specimens. To treat myopathic disorders by different methods in order to learn which is most effective within each disease category. To produce animal models of pathogenic phenomena.

Methods Employed: Most of the details of techniques applied to human material are described in the various methodological projects of this Branch. For comparison with Duchenne muscular dystrophy, non-structural ischemic lesions of muscle were produced in animals, the rise of serum "muscle enzymes" followed, and preventative drugs sought. Damage of muscle fibers was sought using radioactive Tc-diphosphonate, which binds to calcium, and with an electronmicroscopic stain of calcium.

Patient Material: 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: We have previously proposed a new hypothesis, based on muscle histochemical studies, concerning the pathogenesis of Duchenne's progressive pseudohypertrophic muscular dystrophy (DMD). Because the earliest histochemical change is a grouped pattern of degenerating and regenerating muscle fibers, it was hypothesized that the disease is caused by an abnormality of small arterial blood vessels on either a structural or functional basis. That principle was established and the hypothesis shown to be possible by our production of identical early and late muscle lesions after single and repeated embolization of rabbit arteries (structural arterial occlusion). We then reported production of the same early and late Duchenne-like muscle lesions with non-structural, presumably functional, lesions of intramuscular blood vessels by combining a subthreshold vascular factor (aortic ligation) and a subthreshold dose of a vasoactive amine (either serotonin [5-hydroxytryptamine] or norepinephrine), thereby establishing the possibility of a functional abnormality of blood vessels and/or vasoactive substances affecting them being the basis of Duchenne dystrophy (thus like small "strokes" or "transient-ischemic-attacks" of muscle). We then reported that in this model, following each dose of serotonin or norepinephrine in the previously ligated animal there was a striking rise of "muscle enzymes" (CPK, SGOT, SGPT, LDH) in the serum (peak about 12 hours), which returned to normal by 3-5 days, after a single dose, while repeated doses of serotonin caused repeated rises of those enzymes. This demonstrated a further parallel of the model with human DMD and also provided a convenient index with which various therapeutic drugs could be quantitatively evaluated. We have now published a report on the identification of 3 drugs, pretreatment with which has completely blocked the serotonin-provoked muscle damage in this model, and two which prevent the norepinephrine-induced muscle damage, all having typical dose-response curves. One agent has had paradoxical effects, blocking in low doses and enhancing in high doses the muscle-damaging effect of norepinephrine.

With the aorta-ligation-plus-vasoactive-amine model we have developed a new, rapid and simple radioisotopic method, based on uptake of Tc-diphosphonate, of quantitating active skeletal muscle damage in experimental animal -- it correlates very well with other more arduously determined parameters of muscle damage, loss of muscle K^+ and elevation of plasma creatinephosphokinase (with Dr. B. Siegel, AFRRI). We are using this tracer clinically and can identify active muscle damage in several types of myopathy including Duchenne muscular dystrophy and dermatomyositis.

In muscles of human limbs amputated because of peripheral vascular disease, the pattern which we have recently found of grouping of necrotic or regenerating fibers identical to the pattern which we find in early Duchenne muscular dystrophy and experimental ischemic myopathy is at least harmonious with our ischemia hypothesis for Duchenne dystrophy (with Dr. J. Hawley, VAH, D.C.).

We have reported that infusions of serotonin into rat nerve-muscle preparations decrease the force of the evoked twitch, an experiment supporting the possibility that serotonin may play a role in some neuromuscular disorders.

In adult-onset acid maltase deficiency we have provided the first demonstration of "reincarnation" of a muscle biochemical defect in cultured muscle fibers -- in fact, the muscle biopsy and the muscle cultured from it (by Dr. Valerie Askanas, NYU) were identical by histology (vacuoles), histochemistry (acid phosphatase high in the vacuoles) electronmicroscopy (glycogen accumulated in lysosomes), and biochemistry (by Dr. S. DiMauro, Columbia) (5-10% acid maltase, elevated neutral maltase and acid phosphatase, normal enzyme kinetics. (See our Tissue Culture Project for details of all these tissue culture studies of myopathies.)

Also reincarnated in cultured muscle fibers were the ultrastructurally characteristic "cabbage bodies" of a patient with a chronic myopathy (the biochemical defect is not yet known) (with Dr. V. Askanas, NYU).

For the human muscle tissue culture studies including evaluation of the cultures with histochemical, electronmicroscopic, immunologic and biochemical methods, new techniques have had to be worked out (with Dr. V. Askanas).

We have confirmed in another patient our previous finding that muscle fibers cultured from myophosphorylase deficiency disease recover approximately normal phosphorylase enzyme activity (with Dr. V. Askanas, NYU).

In the polymyositis/dermatomyositis complex (PM/DM), the mechanism of muscle damage is thought to be dysimmune, but more precise details remain unknown. Our previous report of immunoglobulin complexes deposited in blood vessels in 83% of the childhood cases and 29% of adult cases supported our earlier hypothesis that an aspect of muscle damage may be vascular. While disorders of both immunoglobulins (B-lymphocytes) and cellular immunity (T-lymphocytes) might be occurring in all cases of PM/DM, perhaps the former (as intravascular immune complexes) are more muscle-damaging in the childhood form and latter in the adult form.

The immunologic abnormalities of PM/DM could in turn be caused by a viral infection; however, our attempts to "rescue" a virus from PM/DM patients' muscle thus far have been negative (with ID, NINCDS, and NCI). Moderate to massive subcutaneous calcification is a common and disabling complication of DM. We have found new ways to detect it in its early stages, isotopically with Tc-diphosphonate (with NM, CC) and by xerography. Unfortunately, therapeutic doses of diphosphonate have not been beneficial.

The method of treatment we introduced to patients of all ages with the PM/DM complex 7 1/2 years ago, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred), has now proven in our hands to be the single best available treatment, both for children and adults (without or with cancer). It has greatest therapeutic benefit, fewest side-effects, and is easiest to manage. Our experience now includes about 50 carefully studied adults and children, many of them considered therapeutic failures on other regimens. However, not all respond, but in 4 very sick patients we have had subsequent remarkable improvement when azathiaprine was judiciously added to the alternate-day prednisone program. We have now begun a double-blind trial to more clearly establish the efficacy of azathiaprine.

Since either type of immunological abnormality proposed in childhood or adult PM/DM could in turn be caused by a viral infection and/or an associated cancer, or the cancer and the "immuno-myopathy" could both be viral-induced, we have attempted to recover or "rescue" a virus from muscle biopsies of PM/DM patients, in collaboration with 2 different laboratories, but have so far failed to reveal a transmissible agent (with NCI and ID, NINCDS).

Because not all PM/DM patients treated with LT-HSDAD-Pred respond, we are seeking details of the patients' immunologic response to prednisone as well as predictive parameters thereof. By using T- vs. B-lymphocyte cell markers, T- and B-lymphocyte mitogens, and T-lymphocyte cytotoxicity on tissue-cultured chromium-labeled muscle fibers, we have established in DM/PM patients that while 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 these effects are more profound on the T-lymphocytes. We are correlating these data with concurrent blood levels of prednisone and prednisilone (with P, CC).

We have demonstrated that a new histochemical finding -- alkaline phosphatase staining in the connective tissue -- is highly characteristic of essentially all active cases of PM/DM as opposed to all other myopathies (except active myositis ossificans and rare cases of extremely active ordinary myopathy) and thus can be used as a diagnostic point strongly suggestive of PM/DM; this is especially useful in the childhood form, which can lack evidence of inflammation in the biopsy. This was given statistical support by our detailed review of 50 PM/DM cases in comparison with 300 cases of other neuromuscular diseases, including 25 cases of Duchenne muscular dystrophy.

In two cases of late-onset rod disease intranuclear rods, identical histochemically and ultrastructurally to the myofibrillar rods in the cytoplasm, were reported (see our Electronmicroscopy Project). Two possible pathokinetic mechanisms were suggested: (a) an origin from a previously unsuspected (or unemphasized) actin (or tropomyosin)-like, plus-or-minus alpha-actinin-like, contractile protein common to both nuclear and cytoplasmic sites, or (b) origin from cytoplasmic contractile protein imported into the nucleus and crystallized there into rods. Either would represent a totally new concept regarding pathokinetic mechanisms, and mechanisms of nuclear defect possibly applicable to other diseases. In one of the patients an associated unusual serum para-

protein, IgG/lambda myeloma protein, was found. A new mid-childhood-onset form of rod disease was found with muscle biopsy abnormalities like those of adult-onset rod disease (but without nuclear rods).

A new clinically delineated "levitated arms syndrome" has been identified in 3 cases and the cause discerned as repeated iatrogenic intramuscular injections into the deltoids; two were very successfully treated by surgical transection of the fibrous bands. The usual offending drug was pentazozine (Talwin). One patient had, in addition to "levitated arms", "levitated legs" (evident when sitting) due to pentazozine injections into the rectus femoris muscles. These cases demonstrate a preventable and treatable iatrogenic disease.

We have previously introduced the term for a characteristic type of muscle fiber abnormality, "ragged-red fibers", consisting of fibers with large collections of enlarged mitochondria identified histochemically and confirmed by electronmicroscopy. Ultrastructurally the mitochondria have bizarre whorls of cristae and intra-cristal semi-crystalline inclusions. Ragged-red fibers in limb muscle are often seen in a myopathy accompanying progressive external ophthalmoplegia, as we previously reported. This year we added to the group of a few other diseases known to be associated with "ragged-red fibers" (some with lactic acidosis and some without) one presenting as cardiac involvement with idiopathic septal hypertrophy. Study of the more accessible limb muscle in such patients may help the understanding of cardiac involvement if it is caused by the same biochemical defect. With electronmicroscopic-histochemistry, we have just determined that nearly all of the intracristal semi-crystalline structures of the mitochondria of ragged-red fibers lack cytochrome oxidase (see our Electronmicroscopy Project). Under our Tissue Culture Project we described our studies growing in tissue culture (in collaboration with Dr. Valerie Askanas, NYU) muscle fibers from biopsies of patients with ragged-red fibers and our findings that some ultrastructural aspects of the mitochondrial abnormalities of "ragged-red" muscle fibers have been reincarnated in cultures from several patients with ragged-red fibers; this was the first morphologic defect reiterated in culture (with Dr. V. Askanas, NYU).

In our series of 36 patients we have demonstrated histochemically and electronmicroscopically that the commonest cause of progressive external ophthalmoplegia (after myasthenia gravis and myotonic atrophy are excluded) is the disorder characterized by "ragged-red" muscle fibers in limb muscles, whether or not the limbs themselves are weak. Other causes we have found, as manifested by limb muscle pathology, are, (i) a vacuolar oculo-cranio-somatic neuromuscular disease, (ii) some of the cases of type I fiber hypotrophy with central nuclei, (iii) rare cases of lower motor neuron disorder, (iv) rare cases of morphologically nonspecific myopathy, and (v) rare cases of type II fiber smallness.

Selective atrophy of the type II (glycolytic-rich, oxidative-poor) muscle fibers, especially the subtype IIB fibers, has been shown to be the basis of cachectic atrophy accompanying cancer and other cachecting disorders. Possible hypothetical mechanisms have been formulated, i.e., a "Sparafucile" factor directly or indirectly resulting from the cancer indiscriminately assassinating

the type II fibers vs. a muscle fiber martyrdom mechanism of protein catabolism to supply energy substrate, via alanine and glyconeogenesis, to cells more vital to the organism. The possible neurogenic vs. myogenic pathogenesis of the type II atrophy has been analysed. Evaluation of the mechanism of type II fiber atrophy in cancer patients is important because this "remote effect" muscle weakness is often the most crippling aspect of cancer -- if the mechanism becomes known it might be treatable independently of treatment and response of the cancer itself, and improvement of the muscle weakness and wasting could even make the patient better able to withstand the rigors of direct anti-cancer therapy.

A new neuromuscular disorder, striped loss of mitochondria, has been found in a family with dominantly inherited distal muscle weakness. A new type of mitochondrial abnormality, light-cored dense particles, has been found in skeletal muscle fibers of a patient with cardiac "assymetric septal hypertrophy".

A new combined syndrome of muscle and erythrocyte phosphofructokinase deficiency and hypolipoproteinemia has been identified.

Acupuncture myopathy has been reported as a new and increasingly prevalent histologic abnormality, induced as a side-effect of acupuncture, complicating the interpretation of subsequent muscle biopsies from acupuncture sites.

As described in our Electrophysiology Project, we have specifically criticized the term "myopathic EMG" which, unfortunately in our view, is still widely used even in the other major centers of human neuromuscular research. Our detailed critique has been published.

The principal investigator organized the scientific program of the third 3-day symposium on Neuromuscular Diseases in Houston, March 1975, under the sponsorship of the Veterans Administration and Baylor Medical School. It was attended by more than 400 physicians, as were the first two symposia, and the principal speakers were recorded on video tape for general distribution. Virtually all of the speakers were current or former members of the Medical Neurology Branch.

In myotonic atrophy (myotonic "dystrophy"), several lines of evidence continue to support our hypothesis that muscle involvement in this disease is not myopathic, but due mainly to an altered nerve influence on muscle (and therefore should not be listed in the dystrophy category). We propose that it involves a fractionation of the motor units, not loss of whole motor units as another has proposed. We have not been able to confirm the abnormality of sarcolemmal membrane protein kinase reported by others.

In myotonia congenita and paramyotonia congenita, also thought by others to be myopathies, we have now postulated a major neurogenic aspect to the pathogenesis on the basis of histochemical evidence, a hypothesis with which other findings are harmonious. Other erstwhile "myopathies" now suspected of being mainly or partly neurogenic are detailed in our Amyotrophic Lateral Sclerosis Project.

Our other studies bearing on this project but reported under some of our other projects include:

1. Biochemical evaluation of human and animal muscle fiber sarcolemmal and sarcoplasmic reticulum membranes (our Biochemistry Project).

2. Localization of calcium at the ultrastructural level in damaged muscle fibers (our Electronmicroscopy Project).

3. Identifying sub-typing of type-I muscle fibers and selective involvement of one subtype in some neuromuscular diseases (our Histochemistry Project).

4. Demonstration of apparently widespread infection of chick embryo muscle in culture with avian leucosis/sarcoma virus, showing that this favorite preparation of experimentalists is a contaminated test object (our Tissue Culture Project).

Significance to Bio-Medical Research and the Program of the Institute: The myopathies represent a group of disorders rather similar clinically, the detailed differences of which have only begun to be worked out in the recent few years. One of the major methods for distinguishing these disorders is by histochemistry of the muscle biopsy. Other methods include electronmicroscopy, biochemistry, and immunology. Hence, the distinguishing of various new forms of disease represents a step toward the overall understanding of this group of disorders. We are now determining whether tissue culture of the abnormal muscle will aid in the diagnosis or understanding of the cause. It is only when the detailed differences of the various forms of myopathy are analyzed that the pathogenesis, etiology, and treatment can be understood. In fact, we are now proposing that some erstwhile "myopathies" (such as myotonic "dystrophy") are the result of abnormal neural influence on muscle. Identification of characteristic histochemical and biochemical defects (such as the inability to utilize long-chain fatty acids and phosphorylase deficiency) in the myopathies has provided leads to further explore the pathogenesis as a possible treatment. We are seeking others. Reproduction of several diseases in muscle fibers cultured from biopsies of the patients both proves a myopathic nature of the disease and represents a new tool available for studying the abnormalities in cells living away from the complex environment of the patient.

Specific or semi-specific structural changes of certain elements, such as mitochondria, especially when coupled with a metabolic defect, such as lactic acidosis, provide leads toward finding a specific metabolic defect.

Our histologically based hypothesis of ischemia as the cause of Duchenne muscular dystrophy raises for consideration the possible use of drugs to increase the blood flow through perfusion vessels of muscle. The finding of drugs useful in our model for preventing muscle damage further raises the possibility of their use in Duchenne dystrophy patients.

Radioactive diphosphonate localization potentially represents a new way of identifying muscle damage in patients and carriers of muscle disease.

The beneficial response obtained in the therapy of polymyositis/dermatomyositis demonstrates two additional regimens available in the management of such disorders, high-single-dose alternate-day prednisone without or with azathiaprine. Our proposals that childhood and adult forms of polymyositis/dermatomyositis have different pathogenesis might help us to more clearly define the mechanism of each and lead to better, more accurate treatment of each.

Our identification of the iatrogenic cause of the "arm levitation" and "leg levitation" syndromes can result in its prevention if physicians throughout the country are aware of the cause, and our demonstration of a treatment will be of benefit to other patients so afflicted.

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

Keyword Descriptors: myopathy, Duchenne muscular dystrophy, muscular dystrophy, experimental myopathy, diphosphonate, muscle calcium, polymyositis, dermatomyositis, muscle mitochondria abnormalities, ragged-red muscle fibers, pentazozine, muscle fibrosis - iatrogenic, alternate day prednisone treatment of dermatomyositis/polymyositis, lymphocytes - dermatomyositis, progressive external ophthalmoplegia, acid maltase deficiency, vacuolar myopathy, cancer atrophy of muscle, serotonin, acupuncture myopathy, norepinephrine, levitated arms (and legs) syndrome, rod (nemaline) disease, muscle phosphorylase deficiency in abnormal muscle, alkaline phosphatase, peripheral vascular disease

Honors and Awards: None

Publications:

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Serial No. Z01 NS 01037-13 MN
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Tissue Culture Applied to the Study of Human Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Valerie Askanas, M.D., Ph.D.

Other Investigators: None

Cooperating Unit: Muscle Research Laboratory, Institute for Rehabilitation Medicine, New York University School of Medicine, New York, N.Y.

Man Years:

Total:	4.4
Professional:	1.4
Other:	3.0

Project Description:

Objectives: To study the growth characteristics, histochemical and biochemical reactivities, immunologic properties, and electronmicroscopy and autoradiographic details of human and animal skeletal muscle and animal neurons grown in tissue culture. To reiterate abnormalities of human cells (muscle, schwann cells, fibroblasts) in culture, or to induce those abnormalities in cultured normal human or animal tissues. To compare the growth and histochemical characteristics of "red" vs. "white" muscle in culture. To study interactions between lower motor neurons and muscle fibers in culture. To seek growth-inhibitory or other "toxic" aspects in body fluids of our patients, using cultured neurons or muscle cells as test objects. The tissue is from either normal or abnormal biopsied adult or child human tissue, primarily muscle, or from normal chick, mouse or rat embryos.

Methods Employed: Standard tissue culture techniques have been modified to obtain good growth from human cells and other technical modifications have been made which have permitted us to apply to the cultured cells other investigative techniques, e.g., histochemistry, electronmicroscopy, biochemistry and immunology. Soon we will be using autoradiography. The cultures of human muscle from our patients has been done in collaboration with New York University (v.i. for details).

Patient Material: A portion of the muscle biopsy from certain adult and child patients with neuromuscular disease is available for culture purposes.

Major Findings: Although tissue culture techniques have been used extensively in studying the development of muscle from animal embryos, human skeletal muscle has been difficult to culture. We previously developed and published a system in which we achieve reproducible growth in Maximow slides from 80% of explants of muscle biopsies of children and adults.

Now our human muscle biopsies are able to be grown (aneurally) more conveniently and more luxuriously, quantitatively and qualitatively, in plastic petri dishes in the laboratory of Dr. Askanas. We have worked out the methods of preparing the specimens in our tissue culture laboratory and sending them to Dr. Askanas. To the cultured fibers she has applied the histochemical reactions of NADH-TR, phosphorylase, modified trichrome, hematoxylin-eosin, and three different ATPase reactions to both longitudinal whole-mounts and cryostat cross-sections, the latter for the first time to human cultured muscle, using the new "sandwich technique" she developed for cultured animal muscle. Our published study has shown that the aneurally cultured human fibers do not on their own differentiate into distinct fiber types (presumably motor neuron trophic influence, different patterns of activity, or both are needed for fiber type differentiation). For electronmicroscopy (see our EM Project), the cultures are histochemical-EM prestained, embedded, cell-selected, and blocked in New York, with the sectioning and electronmicroscopy then being done in our EM laboratory. Although the logistics of this system have been fairly well worked out, more precise informational feed-back control will be achieved when the sectioning can be done there and the sections brought here by Dr. Askanas for her study by EM with us -- and this will ensure even better correlation of EM and histochemical data from the cultured fibers with those of the original biopsy specimen of that patient as studied by her in our laboratories here. Ideally, we would like Dr. Askanas to be working entirely in our Branch.

Because abnormalities in human disease often affect only an occasional fiber or part of a fiber we have developed, with Dr. Askanas, a new technique for embedding, prestaining the whole culture for EM-histochemistry and for cell identification, and quickly and precisely plucking (on the basis of selection by light-microscopy) those individual muscle fibers of greatest potential EM interest, i.e., those in the desired state of development and/or showing the desired pathologic change, since all fibers are not equally affected even in the original biopsy. This avoids having to "look for a needle in a haystack". For this we have invented a drill and drilling technique. Details of all these technical advances have been published.

The first focus of the culturing of human muscle fibers was to seek whether biopsies from patients with "ragged-red" and "rugged-red" fibers (ones with abnormal bizarre mitochondria, see our EM Project) reproduce that change when growing in vitro, free of other body influences. To date, we have demonstrated good growth of such biopsies in culture, have obtained fibers with mitochondria nicely stained by the EM cytochrome oxidase method and have

found certain abnormalities of mitochondria reproduced (viz., enlarged mitochondria, abnormal cristae, amorphous intramitochondrial material), but so far no distinct intramitochondrial semi-crystalline structures or light-cored dense particles have been found. Still, this was the first morphologic defect at least partially reiterated in culture. In tissue cultures of "normal" chick embryo skeletal muscle we were able to induce certain bizarre changes of mitochondrial morphology by pulses of dinitrophenol (DNP), but did not produce all the mitochondrial changes occurring in "ragged red" fibers of certain human neuromuscular diseases (with Dr. V. Askanas, NYU).

In adult-onset acid maltase deficiency we have provided the first demonstration of "reincarnation" of a muscle biochemical defect in cultured muscle fibers -- in fact, the muscle biopsy and the muscle cultured from it (by Dr. V. Askanas) were identical by histology (vacuoles), histochemistry (acid phosphatase high in the vacuoles) electronmicroscopy (glycogen accumulated in lysosomes), and biochemistry (by Dr. S. DiMauro, Columbia) (5-10% acid maltase, elevated neutral maltase and acid phosphatase, normal enzyme kinetics). Also reincarnated in cultured muscle fibers were the ultrastructurally characteristic "cabbage bodies" of a patient with a chronic myopathy (the biochemical defect is not yet known) (with Dr. V. Askanas). Most recently incarnated in tissue cultured muscle is the biochemical defect of chronic infantile acid maltase deficiency (with Dr. V. Askanas). We have confirmed in another patient our previous finding that muscle fibers cultured from myophosphorylase deficiency disease recover some phosphorylase enzyme activity (with Dr. V. Askanas).

We found that DNP markedly promoted the detectability of avian leucosis/sarcoma (ALS) virus in all such "normal" chick muscle cultures as evident morphologically (C-particles) and by complement fixation avian leucosis (COFAL) titers (with Dr. V. Askanas). This demonstrated that the "normal" chick embryo muscle cultures so frequently used for physiologic, biochemical, immunologic and developmental studies are actually virally contaminated test objects. The question of whether that ALS virus has a "normal" role in development of "normal" chick muscle *in vivo* or *in vitro* is raised by this study. Our finding the C-particles exclusively in dilations attributed to T-tubules raises the intriguing possibility that T-tubules can be the aqueducts of virus infestation of or shedding from muscle fibers. The question of viruses harbored in mitochondria (as "mitochondriophages", analogous to bacteriophages) is raised by the C-particles being provoked by DNP, an uncoupler of mitochondria oxidative phosphorylation.

Tissue-cultured muscle fibers of chick embryo, newborn rat, and adult human biopsies all grown by Dr. V. Askanas, have been stained with alpha-bungarotoxin immunoperoxidase technique to localize acetylcholine-receptor (AChR). In all, we have found AChR diffusely throughout the sarcolemmal membrane of these regenerating "pre-innervated" muscle fibers. Clearly evident "hot-spots" of AChR were in the chick-embryo fibers and absent from the rat fibers; the human fibers had hotter zones but not small hot-spots as in the chick embryo fibers.

Significance to Bio-Medical Research and the Program of the Institute: In addition to determining the histochemical properties of normal muscle and its growth characteristics in vitro, the studies of spontaneous or induced pathologic changes in human muscle in vitro will be used to seek disturbed growth patterns, and morphologic and biochemical abnormalities, in order to glean clues to their pathogenesis and possible treatment. More particularly, ways of improving deficient growth will be sought. Agents found to improve deficient growth in vitro will then be tested for their usefulness in the patient with muscle disease in respect to promoting growth of abnormal muscle or retarding its degeneration.

Proposed Course of Project: Studies of normal and pathologic human muscle in tissue culture will be extended and new variations applied.

Keyword Descriptors: tissue culture, cultured human muscle, acid maltase deficiency, vacuolar myopathy

Honors and Awards: None

Publications:

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Serial No. Z01 NS 01039-13 MN
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Amyotrophic Lateral Sclerosis (ALS) and Other Lower Motor Neuron Diseases, Including Peripheral Neuropathies and Spinocerebellar Ataxias

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Benjamin R. Brooks, M.D.

Other Investigators: Bernard M. Patten, M.D.
Larry E. Mallette, M.D.
Gerald D. Aurbach, M.D.
Justino F. Campa, M.D.
R. A. Pieter Kark, M.D.
John P. Blass, M.D.
John W. Griffin, M.D.
Donald Price, M.D.
Steven P. Ringel, M.D.
Jerome E. Kurent, M.D.
John L. Sever, M.D.
David S. Zee, M.D.
David G. Cogan, M.D.
Robert D. Yee, M.D.
Jonas Sode, M.D.

Cooperating Units: Department of Neurology, Baylor College of Medicine, Houston, Texas
Metabolic Diseases Branch, NIAMDD
Department of Neurology, University of Virginia School of Medicine, Charlottesville, Virginia
Departments of Neurology & Biochemistry, U.C.L.A., School of Medicine, Los Angeles, California
Muscular Dystrophy Associations of America
Infectious Diseases Branch, NINCDS
Department of Neuropathology, Johns Hopkins School of Medicine, Baltimore, Maryland
Clinical Branch, NEI
Department of Endocrinology, NNMC, Bethesda, Md.

Man Years:

Total:	2.8
Professional:	2.6
Other:	0.2

Project Description:

Objectives: In ALS and other diseases affecting the lower motor neurons, 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) specific or symptomatic therapeutic response; (d) new methods of analysing the abnormalities; and (e) animal models of the human pathophysiologic states.

Methods Employed: The techniques of histochemistry, biochemistry, electronmicroscopy, immunology, tissue culture, electromyography and autoradiography were as detailed in other project reports on those methods. Additional methods were as follows: double-blind, single-blind and non-blinded therapeutic trials, the efficacy of which is judged by clinical testing, functional evaluation, and serial quantitative evaluation of muscle function using an apparatus designed by us for quantitating isometric muscle tension; biochemical assays of several parameters of oxidative enzyme metabolism (with U.C.L.A.); obtaining parameters of plasma cell dysfunction; measuring retention of Ca^{45} taken up from the intestine; axoplasmic transport was evaluated autoradiographically in rats after H^3-leucine was injected into the region of the anterior horn of the spinal cord; the soluble fraction of nerve served as a new type of antigen for inducing experimental allergic neuritis; localization of alpha-bungarotoxin to acetylcholine receptor was as described in the Myasthenia Gravis Project.

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:

MOTOR NEURON DISORDERS

In amyotrophic lateral sclerosis (ALS) numerous different approaches by ourselves and others have failed to disclose the cause or treatment. Our studies continue to seek those goals.

Last year we reported that among 16 patients with primary hyperparathyroidism, proximal neuromuscular weakness and histochemically-identified muscle fiber atrophy attributable to lower motor neuron involvement was present in nearly all, and that it sometimes presented an ALS-like syndrome -- the neuromuscular involvements reverted to or toward normal following surgical treatment of the hyperparathyroidism (with NIAMDD). We have now reported in 6 cases of secondary hyperparathyroidism a similar proximal neuromuscular weakness and also concluded it to be probably caused by lower motor neuron involvement (type-II fiber atrophy with some small angular fibers positive with DPNH-tetrazolium reductase and non-specific esterase staining) (with NIAMDD). The secondary hyperparathyroidism is less easily treatable, but with medical treatment -- 20,25 dihydrotachysterol (DHT) and phosphate-binding in the gut -- the neuromuscular function can be distinctly improved.

To study the possible role of abnormality of parathyroid function and/or calcium metabolism in ordinary ALS, we have done radioisotopic calcium retention tests (N=80) on ALS patients and disease-controls: we have abnormally low calcium retention in 63% of ALS, 60% of polyneuropathy, and only 35% of myopathy patients (with NNMC). So far, treatment with DHT has been found to reverse the calcium-retention defect in some ALS patients but not result in clinical improvement in any.

We are also studying the CSF levels of cyclic-AMP and cyclic-GMP in this group of patients (with NNMC). Very preliminary data indicate no clear disease-related differences. Although glucagon infusion increases cyclic-AMP in the serum 50-75 times basal levels, it does not alter CSF levels, suggesting that the CSF levels reflect only metabolism within the CNS of the cyclic nucleotide.

In progress is viral-antibody profiling of CSF from ALS patients, including those with a late-post-polio progressive muscular atrophy syndrome, that includes those of polio 1, 2, 3, mumps, measles, rubella, coxsackie, and influenza A and B (with ID, NINCDS). We are also conducting a morphologic search for virus after culturing of tissue samples from ALS patients.

In our report of fatal progressive muscular atrophy and lymphoma in sisters, 17 and 20 years of age respectively, the possible cause of both diseases by a single virus was raised (with NCI).

Previously, we have raised one possibility that ALS might in some way involve defective glycogen metabolism of the lower motor neuron soma, based on our histochemistry of anterior horn neurons in the cat which showed a very rich machinery for anerobic glycolysis (e.g., phosphorylase enzyme and glycogen content) and a relatively poor one for oxidative metabolism (e.g., succinate dehydrogenase) in alpha-motor neurons in comparison with the other neurons of the anterior and posterior horns. Since human motor neurons, too, are rich in phosphorylase, one theoretical possibility is that ALS might be related to a primary or secondary abnormality of glycogen metabolism (it is known that lower motor neurons are involved in the glycogenesis of infantile acid maltase deficiency). Such involvement could ultimately be due to a general metabolic or viral mechanism, as discussed above.

A new technique, the staining of extrajunctional acetylcholine receptor molecules located diffusely in the sarcolemma with an immunoperoxidase technique utilizing binding of alpha-bungarotoxin to those receptors, was introduced which demonstrates denervated muscle fibers of experimental animals and in human denervating diseases (with NIHL). This technique will enhance identification and "dating" of denervated fibers. We are now using this technique to look for evidence, in the form of extrajunctional receptors, of defective motor neuron influence on muscle fibers in several diseases we have previously postulated possibly to be on such a basis rather than being myopathic -- they include central core disease, type I fiber hypotrophy with and without central nuclei, myotonic atrophy, myotonia congenita, some cases of bening congenital hypotonia, some of type II fiber atrophy, and congenital and adult-onset rod diseases. The denervated fibers in ALS-patient biopsies with demonstrable extrajunctional receptors, in turn were used as the more sensitive assay for finding the blocking factor in sera of myasthenia gravis

patients. Human and rat skeletal muscle grown in tissue culture without innervation (pre-innervated muscle) (with NYU) also was shown to have diffuse extrajunctional ACh-receptors, and such fibers resemble those positive fibers in the early infantile cases of infantile spinal muscular atrophy.

New hypotheses on the pathogenesis of some neuromuscular disorders that we reported previously have continued to be our "first choice" explanation for those diseases -- specifically that some erstwhile "myopathic" disorders are actually caused by a lower motor neuron abnormality. It is proposed that in spite of a "myopathic" (which we consider often to be a misnomer) EMG (a "BSAP" EMG by our recently reported designation) certain disorders are not myopathic but rather neurogenic (our new approach to EMG studies is described in the Electromyography and Clinical Neurophysiology Project). As part of this formulation, details have been proposed regarding how a motor neuron might be defective in a variety of qualitative or quantitative ways with a variety of time courses toward death. Each of these could give a different clinical disorder. Diseases newly hypothesized to be possibly neurogenic are: some forms of type II muscle fiber atrophy (cachectic and disuse atrophy, cortico-steroid atrophy ["myopathy"]), myotonic atrophy (myotonic "dystrophy"), myotonia congenita and paramyotonia congenita, type I fiber hypotrophy with central nuclei, central core disease, benign congenital hypotonia (with type I fiber predominance), and facioscapulohumeral syndrome (some cases), and perhaps (or only in a small part) thyrotoxic atrophy ("myopathy"), some cases of facioscapulohumeral disease, focal loss of cross-striation, and late-onset and congenital rod diseases too.

Further evidence was adduced by ourselves and also by others to support our original hypothesis that central core disease is more likely to be an abnormality of the neural influence on muscle than a myopathic disorder as originally proposed by others. An updated full review of central core disease has been written in a book chapter; also covered were full details of the entity we originally described as "focal loss of cross-striations", one in which we have postulated a lower motor neuron defect in spite of the BSAP EMG.

On the basis of histochemistry and electronmicroscopy, a new disorder was reported; a new form of central core disease in which there is a "hot spot" of enzymatic activity and lipid droplets in the center of the core.

A new form of the benign congenital hypotonia syndrome was described, consisting of marked type I muscle fiber predominance -- this was proposed to be caused by an in utero time-limited neuropathy affecting the type I motor neurons resulting in "motor unit hypoplasia" of type I and possibly some type II units (most of the type II units are proposed to be either seriously affected or absent to account for the severe type II fiber paucity).

A new clinico-pathologic syndrome of type I muscle fiber smallness without central nuclei has been found in a child with progressive weakness; it was proposed to be of neural origin.

The new concept of congenitally "hypoplastic" motor units was introduced and reported to explain diseases of congenital non- (or only slowly) pro-

gressive muscle weakness associated with type I muscle fiber predominance (probably actually on the basis of type II fiber paucity) and a BSAP EMG pattern. Since the type I motor units fire earliest in EMG recording of voluntary units and in the diseases considered the units are measured as BSAP, one must harmonize that finding with the apparent structural normality of the I fibers which are present in moderate or marked predominance. Our concept is that existing type I units are "hypoplastic" (fewer than normal number of fibers per motor unit) and that type II units are, to account for paucity of II muscle fibers, either non-existent or severely hypoplastic -- and that these changes are caused by an *in utero* time limited disorder of the lower motor neurons.

The generalized muscle cachectic atrophy and weakness, which is a common and often the most crippling manifestation of early focal cancer as well as of hypercorticosteroidism (Cushing's syndrome and iatrogenic) and of disuse, histochemically is selective atrophy of type II muscle fibers. We have previously reported a new hypothesis to the effect that this type II atrophy, at least in *some cases* (other cases might be myopathic, as discussed in our Myopathy Project), may be a pan-neuropathy of all lower motor neurons to which the type II muscle fibers are more susceptible, and might not be a myopathy as is usually stated. That proposal was supported by our report that the ultrastructural aspects of type II atrophy are qualitatively indistinguishable from typical denervation atrophy, but milder in degree. We have shown that usually there is no alteration of the EMG pattern in type II atrophy, either early or when the patient is severely wasted and weak, probably because the early-firing motor units usually measured during voluntary effort are the type I units (see our Electromyography and Clinical Neurophysiology Project). We have found (with NCI) in patients with non-neurologic, localized (non-metastatic) cancer, before there is clinical evidence of anorexia, weight loss, or decreased daily activity, significant type II fiber atrophy by muscle biopsy. The mechanism of the atrophy in such early-cancer patients is unknown, but at least some of it seems to be on the basis of defective lower motor neuron influence on muscle. This is especially likely to be so in biopsies with II-fiber atrophy in which there are scattered small angular fibers excessively dark with NADH-TR and non-specific esterase stainings (i.e., ones like those in ordinary denervation). We plan to turn our attention to ways to prevent or treat type II atrophy, which would significantly benefit cancer patients even if the basic neoplasm could not be excised.

The trophic influence of the lower motor neuron on schwann cells and muscle cells is also being studied by autoradiography combined with electron-microscopy in animal models (see our Autoradiography Project). This should tell us about normal trophic mechanisms and help us consider derargements thereof in various neuromuscular diseases and peripheral neuropathies.

We have recently published a statement of our opinion that some or many in the group of patients generally called "idiopathic scoliosis" are probably suffering from one of a number of neuromuscular disorders affecting paraspinal muscles. Certainly in our experience we have found that a number of flagrant and subtle, common and rare, neuromuscular diseases can cause scoliosis -- and we have urged that these should be sought with detailed histochemical studies of paraspinal muscles in all cases of supposedly "idiopathic scoliosis".

Reviewed in our other project reports are other topics related to ALS and denervating diseases, e.g.:

1. Detailed timed stages of muscle fiber histochemical changes with denervation and reinnervation following single lumbar or sural root section were delineated (with U. Va.) (our Histochemistry Project).

2. Non-specific esterase was introduced as a new histochemical way to highlight denervated fibers, enhancing diagnostic studies (our Histochemistry Project).

3. Open biopsy electromyography (EMG), combined with micromarking, both techniques introduced by us, are being used for histochemical-EMG correlation of fibrillations and positive sharp waves in ALS and other denervated patients (our ALS Project).

PERIPHERAL NEUROPATHIES

Our treatment of a number of cases of idiopathic peripheral neuropathy with long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred) continues to be very successful and necessary, e.g., for as long as 10 years in an adult and 7 1/2 years in a child. Correlative studies indicate that the patients most likely to respond are dysschwannian in type (slow nerve conduction times), relapsing, and with elevated CSF protein; but even some patients with a dysneuronal type of peripheral neuropathy, non-relapsing, or normal CSF have responded (see our Myopathy Project for details of the LT-HSDAD Program).

With muscle biopsy histochemistry we have demonstrated that idiopathic amyloidosis is often the cause of a sensory-greater-than-motor neuropathy beginning in adult patients (often ones undiagnosed in other centers). In our 10 cases of non-familial amyloid polyneuropathy, the onset was later adulthood (mean age 54), 8 were male, and 8 of the 10 had evidence of plasma cell dyscrasia as multiple myeloma, and/or serum and/or urine "paraprotein" immunoglobulin fragments. We have proposed that the neuropathy is due to a systemic metabolic abnormality, possibly related to an abnormal protein, rather than to focal amyloid deposits of immunoglobulin fragments. Treatment of 6 amyloid patients with melphalan, an "anti-myeloma" agent, has not been of obvious value.

A new principle/model for inducing experimental allergic neuropathy (EAN) in animals has been introduced. It involves immunizing the animals with soluble nerve protein (in contrast to previously used lipid-associated protein of myelin). Not only does this represent a new potential model of some human dysimmune peripheral neuropathies but it also represents a new approach to studying certain dysimmune disorders of the CNS, such as multiple sclerosis and parainfectious encephalopathies. Since the animals also have an abnormality of neuromuscular transmission, the model may also have some relationship to myasthenia gravis.

In studying the role of transported axoplasmic protein in the trophic maintenance of the neuromuscular junction (NMJ) and of the innervated muscle fibers, our autoradiographic studies (with Johns Hopkins) demonstrated a rapid anterograde transportation to the axon synaptic terminal at the NMJ and accumulation there of large amounts of protein synthesized in the lower motor neuron soma from amino acid precursor injected into the ventral horn less than 24 hrs. earlier. Thus we have demonstrated these rapidly transported axonal proteins are in the geographic position to have trophic influence on muscle and we propose that they do so, an hypothesis we are studying further. Autoradiographic studies have also shown: (a) direct evidence for retrograde intraaxonal transport of tetanus toxin, and (b) that fast anterograde axonal transport apparently contributes to motor nerve regeneration in experimentally sectioned nerves.

Progressive spastic paraplegia -- identified were two unrelated patients with a syndrome of chronic adrenal insufficiency from infancy and juvenile onset of progressive spastic paraplegia and "onion-bulb" peripheral neuropathy, with normal intelligence, (with NIAMDD).

SPINOCEREBELLAR ATAXIAS

In only a very few of the various types of hereditary spinocerebellar degeneration is a metabolic abnormality known. Previously we have reported pyruvate decarboxylase deficiency in a patient with intermittent cerebellar ataxia (who also had excessive lipid droplets in his skeletal muscle fibers). We also have reported previously, using a system we developed for applying radioisotopic biochemical assays to small samples of biopsied human muscle, that the muscle biopsy slices from 7 of 19 patients with spinocerebellar degeneration oxidized pyruvate at a rate more than 6 SE below the control mean while succinate oxidation was normal; the oxidation rates were not related to the severity of any neuropathic changes in the muscle. In our current publication we have shown in two unrelated patients with Friedreich's ataxia, in whom we had previously shown impaired oxidation of pyruvate by their muscle slices, that cultured skin fibroblasts oxidize 1-^{14}C-pyruvate and 2-^{14}C-pyruvate at less than half the rate of (but U-^{14}C-glutamate at rates comparable to) those found in normal fibroblasts (see our Biochemistry Project). This defect of pyruvate oxidation in some patients with Friedreich's ataxia has now been confirmed by another group (Andre Barbeau, personal communication).

In a study of slow eye movements in patients with spinocerebellar degeneration, it was found that patients made abnormally slow refixational eye movements by the saccadic system (i.e., they were slow saccades) rather than by the voluntary pursuit system. This led to the proposal of a new conceptual scheme of how both normal and defective saccadic eye movements might be generated.

Study of a group of related patients with familial late-onset cerebellar ataxia revealed new information about non-visual control of eye position,

since the striking abnormality was a defective smooth pursuit and fixation system. The patients showed evidence of various non-visual mechanisms of maintaining eye position that have not been previously delineated.

A study demonstrated that rebound nystagmus occurs in normal individuals if fixation is eliminated and only becomes clinically apparent in patients with spinocerebellar degeneration because of a coexisting defect of visually-mediated fixation mechanisms; thus rebound nystagmus can be interpreted as a manifestation of one of the brain's compensatory mechanisms for maintaining eye position when visual systems are ineffective.

A unique combination of ophthalmoplegia and dissociated nystagmus was identified in patients with abetalipoproteinemia (Bassen-Kornsweig syndrome) (with NEI).

Significance to Bio-Medical Research and the Program of the Institute: New details have been accumulated on the morphologic and biochemical substrata of at least some patients with yet-idiopathic forms of ALS, peripheral neuropathies, the spinocerebellar ataxias, and other diseases of the lower motor neuron, as well as on basic cellular mechanisms of the lower motor neuron. Identifying known and currently treatable underlying diseases (e.g., primary or secondary hyperparathyroidism) in some of these clinical syndromes provides such patients with cures and gives possible leads to understanding the more numerous yet-idiopathic cases. New aspects of the beneficial use of prednisone in some cases of apparently subtle dysimmune chronic neuropathy indicate additional patients should be tried on the drug on a prolonged program, every-other-day, even if they have been severely paralysed months or longer. A new outlook on the mechanisms of neuropathy in primary amyloidosis is presented.

If our proposals are correct that some erstwhile myopathic disorders are actually caused by abnormalities of the lower motor neuron, then all bio-chemical searches for the basic abnormality that are focused on the muscle represent explorations in the wrong forest -- we must direct our attention to the motor neuron rather than the muscle.

Fuller understanding of the mechanism by which a focal cancer produces generalized muscle weakness and wasting could lead to a means of treating that often-most-disabling aspect of cancer even when the basic neoplasm itself cannot be treated -- such would be of major benefit to a great number of cancer patients.

Proposed Course of Project: To more fully develop studies underway in the hope that identification of metabolic, immune, or infectious etiologies will lead to a means of treating and preventing these disorders. To further explore the interactions of motor neuron and muscle and disorders thereof. To undertake additional therapeutic trials. We are intrigued with the possibility of treating the cachectic muscle wasting aspects of cancer patients.

Keyword Descriptors: amyotrophic lateral sclerosis, peripheral neuropathy, spinocerebellar ataxia, amyloid neuropathy, motor neuron histochemistry, muscle atrophy in cancer, cachectic atrophy, motor unit, neuromuscular diseases, axoplasmic flow, alternate-day prednisone treatment of neuropathy, experimental allergic neuropathy, progressive spastic paraplegia, rebound nystagmus, open-biopsy electromyography, experimental denervation, secondary hyperparathyroidism, cyclic AMP, calcium absorption, pyruvate oxidation defect in Friedreich's ataxis, idiopathic scoliosis, extrajunctional acetylcholine receptors, slow saccades

Honors and Awards: None

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Serial No. Z01 NS 01189-07 MN
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Episodic Weakness

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.

Other Investigators: S. Charles Bean, M.D.

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To define more clearly and to treat those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis. 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: sarcolemma, T-system, sarcoplasmic reticulum, myofibrillar complex (i.e., the total excitation-contraction coupling mechanism). Studies are done with agents which are either provocative or therapeutic with respect to periodic paralysis syndromes, with a view to obtaining more information regarding abnormalities of pertinent metabolic pathways and methods of treatment.

Methods Employed: The techniques of clinical investigation (including electromyography and clinical biochemistry), muscle biopsy with samples for histochemical analysis, electronmicroscopy, and biochemical assays of tissue are delineated in our other projects. Provocation loading tests and therapeutic trials to raise or lower potassium or sodium were used. Acetazolamide was administered as a prophylactic agent for hypokalemic periodic paralysis.

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; or myotonic congenita. (Patients with myasthenia gravis are part of another Medical Neurology Branch project by that name.)

Major Findings: A new use for acetazolamide (Diamox), a carbonic anhydrase inhibitor, was introduced by us 9 years ago -- it is now widely accepted as the best prophylactic agent available in hypokalemic periodic paralysis (LoKPP). Our on-going follow-up discloses that it has thus far provided moderate to striking benefit for 24 of our 26 patients, two for over 9 years. It has also improved the moderate or severe persistent proximal weakness between attacks that afflicted 19 patients on conventional therapy. The mechanisms of acetazolamide action in this disease remains unknown, since muscle itself has no carbonic anhydrase. One patient has had a renal stone, of uncertain relation to the acetazolamide. All patients are being monitored for renal stones by x-rays twice yearly. No other side effects have been evident.

Paramyotonia congenita has chemical features of both hyperkalemic periodic paralysis (HiKPP) and also myotonia congenita, viz, episodic weakness associated with a raising serum K^+ and widespread myotonia respectively. Although both LoKPP and HiKPP traditionally have been thought of as primary diseases of muscle (i.e., myopathies), we have demonstrated that there is in both paramyotonia congenita and myotonia congenita histochemical evidence which we interpret as definite denervation in the muscle. Thus we have offered a new hypothesis that the muscle weakness and myotonia of paramyotonia congenita and myotonia congenita might be on the basis of muscle fiber abnormality induced by abnormality of the lower motor neuron (LMN). Two other alternatives are possible: the disorders are myopathies in which there is an aspect of non-receptiveness to LMN influences and/or that both LMN and muscle are involved concomitantly).

Significance to Bio-Medical Research and the Program of the Institute: Acetazolamide has proven to be the most effective single prophylactic agent available in the treatment of hypokalemic periodic paralysis. It provides new avenues for investigation of the basic pathophysiology in this disease. If the hypothesis of a neural basis of paramyotonia congenita and myotonia congenita is correct, that would change the direction of research into their cause and treatment.

Proposed Course of Project: To explore in more detail, with patients and animals, the mechanism of action of acetazolamide prophylaxis in hypokalemic periodic paralysis and the pathogenesis of the disease itself. To seek even better therapeutic agents. To seek further evidence for or against the hypothesized neural basis of paramyotonia congenita and myotonia congenita.

Keyword Descriptors: hypokalemic periodic paralysis, acetazolamide

Honors and Awards: None

Publications: None

Serial No. ZO1 NS 01190-11 MN
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Myasthenia Gravis (MG)

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Adam N. Bender, M.D.
Steven P. Ringel, M.D.

Other Investigators: John L. Trotter, M.D.
Jay D. Cook, M.D.
Richard B. Rosenbaum, M.D.
Benjamin R. Brooks, M.D.
Charles L. McIntosh, M.D.
Bernard M. Patten, M.D.
Michael L. Swerdlow, M.D.

Cooperating Units: Surgery Branch, IR, NHLI
Department of Neurology, Baylor College of Medicine, Houston, Texas
Muscular Dystrophy Associations of America
Department of Neurology, Montefiore Hospital, N.Y.

Man Years:

Total:	4.4
Professional:	3.4
Other:	1.0

Project Description:

Objectives: To apply clinical, immunologic, histochemical, pharmacologic, electrophysiologic and electronmicroscopic techniques to investigate the etiology and pathogenesis of myasthenia gravis. To seek new or improved methods of treatment.

Methods Employed: Clinical studies, including edrophonium, prostigmine, curare and lactate challenging tests, were done on patients with myasthenia gravis, on a group of patients with myasthenic syndrome in association with clinical entities with established denervation, and on a number of patients with a variety of other neuromuscular diseases. Competence of neuromuscular transmission was studied neurophysiologically in patients with myasthenia gravis and other neuromuscular disorders. Experience was extended and modified with a new treatment program introduced by us, consisting of long-term high-single-dose (100 mg for the average adult) alternate-day prednisone with the essential adjuncts of supplemental potassium, antacids, and low-sodium, low-carbohydrate and high-protein diet. Blocking effects of MG sera on the acetylcholine receptor were studied by testing the blocking of α-bungarotoxin binding there using an immunoperoxidase technique. Immunologic, autoradio-

graphic, histochemical and electronmicroscopic methods are listed under those projects.

Patient Material: Myasthenia gravis patients participated in the therapeutic trials. Sera, muscle, thymus and other tissue were obtained during diagnostic or therapeutic procedures from Medical Neurology Branch patients.

Major Findings: The new treatment that five years ago we introduced for adult myasthenia gravis (MG), namely high-single-dose alternate-day prednisone, has continued to give excellent results in 27 of 30 adult patients we have thus far treated, proving to be much more effective therapy than anti-cholinesterase drugs. It seems especially effective in older males, but both sexes of all age groups have responded. Our 3 non-responders were females in the menstruating age group. Side-effects of our long-term treatment have been present but relatively minimal, in the form of osteo-porosis of some older females, subclinical or clinical cataracts, and, in a rare patient, aggrevation of pre-existing diabetes mellitus or hyper-tension. Gastrointestinal ulcers or bleeding, even in patients with a previous ulcer history, have not occurred. Since it was potentially too hazardous to take critically ill myasthenia gravis patients off all other anti-myasthenia drugs and let them be nursed on a respirator for 1-2 months while they would have been on placebo, other evidence that this treatment is responsible for the benefit must be weighed. Favoring prednisone as the beneficial factor are: (a) the patients had become unresponsive to all their other treatments prior to being started on prednisone; (b) the upward inflection in their strength curve always began a few days to weeks after prednisone was started and was more-or-less continuously upward thereafter; (c) they did not improve when their anticholinesterases were stopped prior to prednisone; (d) a distinct benefit-worsening cycle related to on-day vs. off-day prednisone was demonstrated, especially early in the course of treatment, evident by clinical examination, respiratory function measurements, and EMG-measured synaptic efficiency; and (e) in some patients the myasthenic state returned as the prednisone dosage was lowered to a certain level and improved again with raising the dosage. This treatment program is still in a long-term investi-gation phase.

A male with juvenile myasthenia gravis has now been maintained at about 95% improvement for 8 years on corticosteroids, the last 5 1/2 years on the program new for this disease, high-single-dose alternate-day prednisone. A second juvenile myasthenic male has also shown beneficial response for 64 months, and a third male for 42 months. This is in contrast to the short-term response from the short courses of ACTH previously given for juvenile (or adult) myasthenia gravis. As yet, no juvenile has failed to respond to our program, although one atypical chronic infantile case has not had satisfactory response.

We have recently modified the treatment slightly by giving, to patients not simultaneously taking anticholinesterase drugs, the single-dose 100 mg prednisone daily for the initial 2-3 weeks before converting to the alternate-day schedule, apparently resulting in more rapid improvement. For clarity of the initial study, anticholinesterase drugs were stopped prior to LT-HSDAD-Pred treatment and have not needed to be resumed in the majority of patients. Although we suspected from a few initial clinical observations in MG patients an adverse interaction between corticosteroid and anticholinesterase drugs and in an animal nerve-muscle test preparation demonstrated that such can occur, we now find that low doses of one can be combined with the other advantageously -- but that patients taking both drugs sometimes seem to have a more "brittle" myasthenia and must be watched carefully. However, because neither anticholinesterase nor prednisone treatment is either curative or completely preventative, information on the pathokinesis is needed.

Response of lymphocytes during the 48-hour cycle to high-single-dose alternate-day prednisone, as well as blood levels of prednisone and prednisilone, are being studied in myasthenia gravis patients as in the dermatomyositis/polymyositis patients (see our Myopathy Project).

The suprasternal approach to thymectomy (which obviates splitting the sternum or ribs), highly recommended by one group, was, in our hands satisfactory in two patients but unsatisfactory in two others because about half of the thymus could not be removed -- total removal of which would have been, and at a second operation was, simple by the partial sternal-splitting approach. The latter approach remains our preference.

We have demonstrated once again and re-emphasized that "out-of-control" MG patients can be remarkably improved by treating co-existent medical problems such as chronic respiratory infections, urinary tract infections, and anemias. Such possibly co-existing ameloriative problems must be sought in each MG patient by detailed general medical investigation.

The remarkable ancillary benefit that broad-aspect nursing can provide to an MG patient has been emphasized, and specific details of that multi-dimensional nursing care have been published, in conjunction with Mary Thompson, R.N., based on our own experience.

A new factor, apparently an immunoglobulin G, has been found in the sera of MG patients which blocks binding of alpha-bungarotoxin (αBT) to acetylcholine (ACh) receptor, presumably because the factor itself is binding there (with NIHL). In 41% of the 89 MG patients the titer is high enough to block receptors at the normal neuromuscular junction (NMJ), and in those plus another 31% (total of 72%) it blocks binding of αBT to extra-junctional receptors in denervated muscle fibers, the latter apparently provides a more sensitive assay. All 9 MG patients with thymoma had the factor and all normal and non-MG-non-thymoma disease controls lacked it. Half of the MG patients with the αBT blocking factor also had antimuscle antibody (antimuscle-I-band-reticulum antibody, which is an immunoglobulin G

(with H. Smith, Bioscience)), and none had the latter without the former -- this strongly suggests that the two activities may be due to one antibody and that αBT blocking is a more sensitive assay for it than the antimuscle antibody assay. Since our ultrastructural localization in normal human NMJs shows αBT binding mainly at the tops and shoulders of the crests of the postjunctional sarcolemmal folds of the muscle fibers, but slightly also on the axonal membrane prejunctionally, it is presumably at those sites that the αBT blocking factor of MG sera is binding. Although this blocking would thereby be in the appropriate locus to cause the weakness characteristic of the disease, and often fatal, conclusive proof will require further studies, which we are currently doing. We are also investigating which cells (presumably B-lymphocytes) make the factor, why, and how can its production or presumed detrimental action be prevented.

Counts of T-lymphocytes and B-lymphocytes in fresh thymus tissue removed from MG patients do not confirm the increased percent of the latter that was reported by others.

Another immunologic abnormality, anti-native-DNA antibody, has been found in the sera of a significant portion of MG patients (with NIAMDD). Our staff presented an N.I.H. Clinical Center Conference on myasthenia gravis, summarizing the current status and our own studies regarding clinical, pharmacological, junctional-physiological, treatment and nursing aspects. This conference has been published and has also been put on video-tape for general distribution.

Lactate infusion has been reported to be a new provocative and apparently specific test for MG patients; as a corollary, endogenously produced lactate has been postulated to be a factor contributing to the excessive fatigability of myasthenics as well as to their occasionally observed "Mary Walker phenomenon".

Contrary to the usually considered short action, 5-10 min., of i.v. edrophonium, we have documented by detailed chemical and electromyographic testing improvement in clinical strength and neuromuscular transmission lasting 1-2 hrs. in several MG patients. This has practical importance since repeated edrophonium tests are commonly used, without EMG monitoring, for adjusting dosage of other anticholinesterases.

On the basis of our recent studies, our previous hypothesis (Engel, W.K. and Warmolts, J.R.: Ann. N. Y. Acad. Sci. 183:72-87, 1971) of the pathokinesis of myasthenia gravis now is modified as follows: (a) the detrimental factor acting at the neuromuscular junction may be an IgG produced by B-lymphocytes in nodes, spleen, or circulation, (b) any thymic influence on those B-lymphocytes, not on the neuromuscular system directly, and (c) the detrimental factor may be acting mainly at the neuromuscular junction predominantly postsynaptically but also to some extent presynaptically.

The basic molecular aspects of the acetylcholine-receptor and the specific acetylcholinesterase, both substances highly concentrated at the neuromuscular junction, have been analysed for the first time in mammalian

muscle by us and the data published. They were shown to be different molecules in distinctly different sarcolemmal membrane fractions and therefore probably in somewhat different positions in the neuromuscular junction membrane in vivo, the former probably in the plasmalemma and the latter possibly in the outer "fuzz" (see our Biochemistry Project). We now expect to be able to perform the same assays, for the first time, in human muscle.

A serum antibody in MG patients specifically against neuronal nuclei but not against nuclei of other cells (except spermatogonia) was reported by another group. We have now reported that our studies fail to reveal such a specific antineuronal antibody in sera of many myasthenics representing various ages and duration and severity of disease, making us skeptical of that other report.

Significance to Bio-Medical Research and the Program of the Institute: The response of myasthenic patients to alternate-day prednisone indicates that much of the abnormality is not irreversible, and it supports the idea that improvement can result even in the most severely affected and advanced stages of this disease. The demonstrated benefit of alternate-day prednisone in juvenile myasthenia gravis led us to apply this new program to adult myasthenia gravis patients. It has resulted in a remarkable new method of treatment which is now widely used throughout the world. Advantages of this prednisone program in the treatment of myasthenia gravis include its being a non-surgical means of providing lasting improvement by an oral medication not requiring administration at rigid intervals during the day, not producing an initial drug-induced worsening, while allowing continuous long-term use, and with its effect thought to be anti-pathogenic rather than anti-symptomatic. We now consider alternate-day prednisone the most effective medical treatment in myasthenia gravis and believe it offers the possibility of suppressing the disease effectively for a sufficiently long period for it to disappear completely. If this will occur, the disease would be cured in such a patient.

The ACh-receptor-blocking factor we have found may be the key to the cause of weakness in myasthenia gravis and if so represents a new potential form of treatment by specifically inhibiting the cells which produce it or specifically blocking its action on the neuromuscular junction.

Lactate infusion seems to be a new and relatively safe test for helping to diagnose myasthenia gravis. The other findings described herein help shed light on the pathogenesis of myasthenia gravis.

Proposed Course of Project: The various clinical and basic mechanistic aspects of our new alternate-day prednisone program will be investigated more extensively, and predictive parameters sought to help anticipate which patients will respond and how much drug they will need. Details of the production and action of the ACh-receptor blocking substance will be further sought. Other facets of the pathogenesis and etiology will be explored.

Serial No. Z01 NS 01190-11 MN

Keyword Descriptors: myasthenia gravis, alternate-day prednisone treatment, acetylcholine receptor, lactate infusion, edrophonium effect

Honors and Awards: None

Publications:

Engel, W. K., Festoff, B. W., Patten, B. M., Swerdlow, M. L., Newball, H. H., Thompson, M. D.: Myasthenia gravis. Ann. Intern. Med. 81: 225-246, 1974.

Whitaker, J. N. and Engel, W. K.: Tissue non-specificity of anti-nuclear antibodies in myasthenia gravis. Neurology 24: 1184, 1974.

Bender, A. N., Ringel, S. P., Engel, W. K., Daniels, M. P. and Vogel, Z.: Myasthenia gravis: A serum factor blocking acetylcholine receptors of the human neuromuscular junction. Lancet I: 607-609, 1975.

Patten, B. M., Oliver, K. L. and Engel, W. K.: Effect of lactate infusions on patients with myasthenia gravis. Neurology 24: 986-990, 1974.

Rosenbaum, R. B., Bender, A. N. and Engel, W. K.: Prolonged response to edrophonium in myasthenia gravis. Trans. Am. Neurol. Assoc., 1975, in press.

Oliver, K. L., Patten, B. M. and Engel, W. K.: Effect of lactate infusions on patients with myasthenia gravis. IIIrd Int. Cong. on Muscle Diseases, Sept. 15-21, 1974, Newcastle upon Tyne, Great Britain, Excerpta Medica Int. Cong. Series No. 334, p. 68.

Serial No. Z01 NS 01191-11 MN
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunological Abnormalities of Neurologic Diseases

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Jay D. Cook, M.D.
John L. Trotter, M.D.

Other Investigators: Adam N. Bender, M.D.
Steven P. Ringel, M.D.
John N. Whitaker, M.D.
Warren Strober, M.D.

Cooperating Units: Department of Neurology, V.A. Hospital, Memphis, Tennessee
Metabolism Branch, NCI
Muscular Dystrophy Associations of America

Man Years:

Total:	4.1
Professional:	2.5
Other:	1.6

Project Description:

Objectives: To apply a variety of basic immunological techniques to the study of human diseases affecting the neuromuscular system and sometimes the central nervous system.

Methods Employed: Various basic immunochemical and immunocellular techniques were employed for studying both immunoglobulin and cellular aspects of altered immune states.

Patient Material: Specimens of peripheral blood, urine, cerebrospinal fluid (CSF), bone marrow, muscle, peripheral nerve, thymus and other tissue was obtained as part of diagnostic or therapeutic procedures from patients with neurological disease admitted to the Medical Neurology Branch.

Major Findings: Our immunology laboratory has been re-established on a modest basis, with capabilities in several sophisticated basic techniques, using highly purified immunoreagents we have made ourselves (with NCI).

In the polymyositis/dermatomyositis complex (PM/DM), the mechanism of muscle damage is thought to be "dysimmune", but more precise details remain unknown. Our previous report of immunoglobulin complexes deposited in blood vessels in 83% of the childhood cases and 29% of adult cases supported our earlier hypothesis that an aspect of muscle damage may be vascular. While disorders of both immunoglobulins (B-lymphocytes) and cellular immunity (T-lymphocytes) might be occurring in all cases of PM/DM, perhaps the former (as intravascular immune complexes) are more muscle-damaging in the childhood form and latter in the adult form. The immunologic abnormalities of PM/DM could in turn be caused by a viral infection; however, our attempts to "rescue" a virus from PM/DM patients' muscle thus far have been negative (with ID, NINCDS, and NCI).

The method of "immunosuppressive" treatment we introduced to the polymyositis/dermatomyositis disease complex 7 1/2 years ago, long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred), has continued to prove to be, in our hands (about 50 cases) and others, the single best available treatment for children and adults (without or with cancer). It has the greatest therapeutic benefit, fewest side-effects, and is easiest to manage. However, because not all patients respond, we are seeking details of the patients' immunologic response to prednisone as well as predictive parameters thereof. By using T- vs. B-lymphocyte cell markers, T- and B-lymphocyte mitogens, and T-lymphocyte cytotoxicity on tissue-cultured chromium-labeled muscle fibers, we have established in DM/PM patients that while 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 these effects are more profound on the T-lymphocytes. We are correlating these data with concurrent blood levels of prednisone and prednisilone (with P, CC). In some prednisone-non-responders we have, on an occasional-case basis, successfully used another "immunosuppressive" drug, azathiaprine (3 mg/kg), combined with the LT-HSDAD-Pred, and have now begun a double-blind trial to more clearly establish the efficacy of azathiaprine.

As part of the study in which immunoglobulin and complement levels were studied in a large group of our patients, immunoelectrophoretic determinations were done on all the patients admitted to the Medical Neurology Branch. In one patient with adult-onset rod neuromuscular disease, an unusual paraproteinemia was found, consisting of IgG/lambda myeloma protein. He appears to have had a monoclonal gammopathy for over a year, without detectable evidence of myeloma. The possible relationship between the paraprotein and the neuromuscular disease is being explored. In his biopsy, we recently found rods in the muscle nuclei that are ultrastructurally identical to those within myofibrils (see our Electronmicroscopy Project).

The site of the abnormality in myasthenia gravis causing the neuromuscular weakness is still undetermined. Our own evidence and that of others at one time made us feel that the neuron and presynaptic mechanisms were likely to underlie the myasthenia gravis defect. Because of that, the studies previously done by another worker, in which he found antineuronal binding of sera from patients with myasthenia gravis, seemed very important but needed to be confirmed and possibly extended. He suggested that the factor was neuron-specific, in its binding to nuclei of neurons only, except for spermatogonia binding too. In our indirect immunofluorescent study of 15 patients with myasthenia gravis, binding of antibodies to neurons (to their nuclei) of the IgG, IgA and IgM classes was found only in those patients who also had antinuclear antibody in these classes. Additionally, patients with systemic lupus erythematosus, who did not have myasthenia gravis but who did have antinuclear factor, had the same spectrum of staining patterns. Therefore, we concluded and reported that the neuronal nuclear binding really represents part of the antinuclear factor activity sometimes present in the sera of patients with myasthenia gravis and is neither neuronal-specific nor a phenomenon specific to that disease (see our Myasthenia Gravis Project).

Four immunologic studies are recorded in more detail under our Myasthenia Gravis (MG) Project: (a) In patients' muscle biopsies the immunoperoxidase technique was used in conjunction with alpha-bungarotoxin to localize at light and electronmicroscopic levels the acetylcholine receptor, at the normal neuromuscular junction and diffusely in the sarcolemma of denervated fibers. (b) That technique was also used to demonstrate a blocking factor (probably an IgG antibody) in the serum of myasthenia gravis (MG) patients (see our Myasthenia Gravis Project). (c) Further immunologic studies suggested that that blocking factor of MG sera was probably the same as antimuscle/antithymus antibody (see our Myasthenia Gravis Project).

Other immunologic studies recorded in more detail under other projects are: (a) In idiopathic amyloidosis, identification of plasma cell dyscrasia in 8 of 10 patients, in the form of serum and/or urine "paraprotein" immunoglobulin fragments and/or multiple myeloma (our ALS-Peripheral Neuropathy Project). (b) Introduction of a new "dysimmune" model of experimental allergic neuritis -- one in which the antigen is soluble nerve protein (rather than previously used lipid-associated protein of myelin) and in the affected animals there is a defect of neuromuscular transmission in addition to the neuritis from nerve roots to nerve endings in muscles (our ALS-Peripheral Neuropathy Project). (c) Detailed evaluation of various viral antibodies is being done with the sera of amyotrophic lateral sclerosis patients (our ALS Project).

Specimens collected from every Medical Neurology Branch patient and stored in our serum and spinal fluid bank have proven to be valuable in the study of a number of entities of possible viral and/or autoimmune etiology.

Significance to Bio-Medical Research and the Program of the Institute: Since myasthenia gravis, dermatomyositis/polymyositis, and some types of progressive or relapsing polyneuropathies are probably on a dysimmune basis, analysis of the details of the abnormal immune process is expected to help formulation of better methods of treatment and prevention.

Proposed Course of Project: Ideally, all the immunologic studies noted herein should be elucidated more fully with the best available immunological techniques. However, with our extremely limited resources we are restricted in what we can do.

Keyword Descriptors: lymphocytes, immunoglobulins, myasthenia gravis, dermatomyositis, polymyositis, idiopathic amyloidosis, relapsing polyneuropathy

Honors and Awards: None

Publications:

Cook, J. D., Trotter, J. L. and Engel, W. K.: The effects of high-single-dose alternate-day prednisone on the immunological system of patients with neuromuscular diseases. Trans. Am. Neurol. Assoc., 1975, in press.

Serial No. Z01 NS 01192-11 MN
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Electronmicroscopic Studies of Skeletal Muscle and Neurons

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Adam N. Bender, M.D.
Valerie Askanas, M.D., Ph.D.
Steven P. Ringel, M.D.

Other Investigators: John W. Griffin, M.D.

Cooperating Units: Muscle Research Laboratory, Institute for Rehabilitation Medicine, New York University School of Medicine, New York, N.Y.
Muscular Dystrophy Associations of America

Man Years:

Total:	3.6
Professional:	2.1
Other:	1.5

Project Description:

Objectives: To study the early subcellular changes of human muscle in diseases that are confined to muscle alone, as well as in conditions which produce secondary alterations in muscle, such as denervating and metabolic diseases. To study subcellular changes of human neurons and peripheral nerves in certain diseases.

Methods Employed: A portion of patient diagnostic biopsy muscle is placed in our specially-designed clamp to maintain resting length and is immediately fixed in cold buffered glutaraldehyde or osmium tetroxide for eventual araldite or epon embedding. Other fixative combinations and buffers (including cacodylate), and various electronmicroscopic (EM) staining procedures are used. EM histochemistry for acid phosphatase, cytochrome oxidase, butyric dehydrogenase, calcium and an ATPase have been done on human muscle, as has the EM immunohistochemical technique of using alpha-bungarotoxin to localize the acetylcholine receptor (AChR). Adjacent biopsy material is immediately frozen for cryostat sections and incubated in various histochemical media, as described in the Histochemistry Project. Histochemically

stained cryostat sections, ready within half an hour after the biopsy, are used to determine which of the biopsies contain light microscopic features of special interest and thereby guide choosing material appropriate for electron-microscopic study and for directing the search for ultrastructural abnormalities. Biopsy specimens of human nerve or brain tissue are fixed and embedded for electronmicroscopy and are similarly monitored by rapid enzyme histochemistry of adjacent tissue. Normal and abnormal human muscle grown in tissue is also studied by EM (for new techniques, v.i.). Skin samples and cultured fibroblasts from our patients are sometimes studied, as are mitochondrial preps of sarcolemmal membrane from our biochemical studies. In the animal models and tissue cultures studied in parallel to the human diseases, marker substances such as peroxidase, ferritin, ruthenium red, and lanthanum are sometimes used with electronmicroscopy.

Patient Material: Patients with various myopathies and neurogenic forms of muscular weakness are biopsied for diagnostic purposes, as are asymptomatic suspected carriers of progressive genetic myopathies. Muscle biopsies and nerve biopsies are the major tissues examined. Muscle from patients is sometimes grown in tissue culture for a few weeks before being studied by EM. Occasional brain biopsies are obtained from patients with certain forms of progressive degenerative disease. Other abnormal tissues from neurologic patients are sometimes examined, such as skin and cultured fibroblasts.

Major Findings: A new drill and drilling technique was published that allow precise plucking after EM-histochemical staining and plastic embedding of the fibers in the desired state of development and showing the desired pathologic change (since all fibers are not equally affected, even in the original biopsy) for electronmicroscopy, rather than having to "look for a needle in a haystack". In this way we demonstrated good maturation of muscle fibers cultured aneurally, which showed lack of differentiation of them into different histochemical fiber types when cultured aneurally (see our Tissue Culture Project). We also utilized these techniques for growing and studying by electronmicroscopy, histochemistry, and biochemistry the abnormal muscle from human diseases, e.g., acid maltase deficiency, vacuolar myopathy with "cabbage bodies", and diseases with ragged-red or rugged-red fibers (see our Tissue Culture Project).

Electronmicroscopy of muscle fibers cultured from our patients has been focused on the problem of seeking whether biopsies from patients with "ragged-red" and "rugged-red" fibers (ones with abnormal, bizarre mitochondria) reproduce that change when growing in vitro, free of other body influences. The biopsies are prepared in our tissue culture laboratory and sent to Dr. Valerie Askanas in New York for culturing and histochemical or EM staining, and then embedding, cell-selection and blocking, with the subsequent sectioning and electronmicroscopy then being done in our EM laboratory. This system has now been well worked out logistically, and it will be even more precisely coordinated when the sectioning can be done there and the sections brought here by Dr. Askanas for her to study by EM with us. And because abnormalities in human disease often affect only an occasional fiber or part of a fiber we have developed a new technique for embedding

whole cultures, prestaining for EM-histochemistry and for cell identification, and quickly and precisely getting out (on the basis of selection by light-microscopy) those individual muscle fibers of greatest potential EM interest. To date we have demonstrated good growth of ragged-red and rugged-red fiber biopsies in culture, and have obtained fibers with mitochondria nicely stained by the EM cytochrome oxidase method, and have found suggestive abnormalities of mitochondria. (Details of all these technical advances have been prepared for publication -- see also our Tissue Culture Project.)

In tissue cultures of "normal" chick embryo skeletal muscle we were able to induce certain electronmicroscopically evident bizarre changes of mitochondrial morphology by pulses of dinitrophenol (DNP), but did not produce all the mitochondrial changes occurring in "ragged-red" fibers of certain human neuromuscular diseases. In the course of that study we found that DNP markedly promoted the detectability of avian leucosis/sarcoma (ALS) virus in all such "normal" chick muscle cultures as evident morphologically (C-particles) and by complement fixation avian leucosis (COFAL) titers. This demonstrated that the"normal" chick embryo muscle cultures so frequently used for physiologic, biochemical, immunologic and developmental studies are actually virally contaminated test objects. The question of whether that ALS virus has a "normal" role in development of "normal" chick muscle in vivo or in vitro is raised by this study. Our finding the C-particles exclusively in dilations attributed to T-tubules raises the intriguing possibility that T-tubules can be the aqueducts of virus infestation of or shedding from muscle fibers. The question of viruses harbored in mitochondria (as "mitochondriophages", analogous to bacteriophages) is raised by the C-particles being provoked by DNP, an uncoupler of mitochondria oxidative phosphorylation (see our Tissue Culture Project).

Using our new immunoperoxidase technique with alpha-bungarotoxin to localize the acetylcholine receptor (AChR) at the light and electronmicroscopic levels, we have demonstrated : (a) localization in normal human NMJs shows alpha-bungarotoxin binding mainly at the tops and shoulders of the crests of the postjunctional sarcolemmal folds of the muscle fibers, but slightly also on the axonal membrane prejunctionally; (b) lack of AChR in the normal extrajunctional sarcolemma but its diffuse appearance there in muscle fibers denervated experimentally or in human diseases; (c) blockade of binding at junctional and extrajunctional sites caused by a factor in sera of the majority of myasthenia gravis patients (see our Amyotrophic Lateral Sclerosis and Myasthenia Gravis Projects).

"Ragged-red fibers" is now a generally used term we introduced to describe the histochemical appearance of abnormal muscle fibers which contain collections of large, bizarrely-structured mitochondria by EM. We have also introduced the name "rugged-red fibers" for ones containing many of the same mitochondrial abnormalities but less overall fiber abnormality. In one patient with rugged-red fibers, who also had mild muscle weakness and cardiac idiopathic septal hypertrophy (with NHLI), numerous light-cored dense particles were found in numerous structurally abnormal mitochondria. These particles resemble ones induced experimentally by calcium abnormalities and also have

some resemblance to viral particles (in which case they might be considered a sort of "mitochondriophage"). This study is in press.

Histochemical techniques for cytochrome oxidase, beta-hydroxybutyric dehydrogenase, acid phosphatase and calcium, as well as the immunoperoxidase-bungarotoxin technique for localizing acetylcholine receptor, at the EM level are established in the laboratory and are being used on the human muscle biopsies. The initial objective has been to delineate the bizarre mitochondrial changes present in "ragged-red fibers". We have shown that: (a) the intramitochondrial crystals are typically not stained by the ultrastructural cytochrome oxidase reaction; (b) mitochondria differ in the amounts of beta-hydroxybutyric acid dehydrogenase they contain; (c) lysosomes in acid maltase deficiency and other vacuolar myopathies have high acid phosphatase; (d) in necrotic, pre-necrotic, and regenerating-degenerating muscle fibers calcium accumulates in myofibrils, sarcoplasmic reticulum and mitochondria, apparently related to the stage and/or severity of muscle fiber damage.

In two cases of late-onset rod disease intranuclear rods, identical histochemically and ultrastructurally to the myofibrillar rods in the cytoplasm, were reported. Two possible pathokinetic mechanisms were suggested: (a) an origin from a previously unsuspected (or unemphasized) actin (or tropomyosin)-like, plus-or-minus alpha-actinin-like, contractile protein common to both nuclear and cytoplasmic sites, or (b) origin from cytoplasmic contractile protein imported into the nucleus and crystallized there into rods. Either would represent a totally new concept regarding pathokinetic mechanisms, and mechanisms of nuclear defect possibly applicable to other diseases.

The technique, recently introduced by others, for staining calcium at the EM level by antimony is being used to study (a) normal distribution of calcium in muscle fibers, (b) increase in myopathic disorders, especially in parallel with radioactive diphosphonate localization by total tissue counting and autoradiography, and (c) possible decrease in denervated muscle, according to our preliminary studies.

Study of proteins transported in the axoplasmic flow is being done with correlated autoradiographic and electronmicroscopy studies (see our Autoradiography Project).

A new structural abnormality of human muscle fibers has been identified by light-microscopic histochemistry and by EM. On the basis of its ultrastructural appearance it is tentatively called "zipper tubules" and exists only in the type I fibers.

By electronmicroscopy, combined with histochemistry, 3 more cases of what we originally described as focal loss of cross-striations have been identified. The original case has been described in full EM and histochemical detail in a chapter in a book in press. The new cases (brothers) we are calling "striped loss of mitochondria".

Electronmicroscopy has been used to delineate muscle cell abnormalities discussed in some of our other projects. For example, chapters of a book are in press containing detailed EM studies of central core disease, rod (nemaline) disease and type I fiber hypotrophy with central nuclei.

An EM search for viral particles in polymyositis and other sporadic neuromuscular diseases is being conducted, in an attempt to confirm one report by another group. To date, "myxovirus-like" structures have been found in some cases of polymyositis, but their significance remains unknown, since other investigators are finding them in various other diseases.

The degree of "purity" of cell fractions, including preps of mitochondria and of sarcolemmal and sarcoplasmic reticulum membranes, used in our biochemical studies (see our Biochemistry Project) have been monitored by electronmicroscopy.

EM autoradiographic study of transfer of "putative trophic molecules" from lower motor neuron axon to schwann cell or to muscle fiber is in progress (see our Autoradiography Project).

Significance to Bio-Medical Research and the Program of the Institute: Analysis of the changes in ultrastructure of the neuromuscular disorders is in its childhood. Identification of new forms of disease still occur rapidly in this stage. However, one must be careful not to name a condition on the basis of limited, though careful, EM or histochemical studies. Therefore, a broad experience with as many well-studied cases as possible is necessary, as is close correlation of EM with histochemistry. It appears that certain morphologic changes can indicate suspected metabolic defects, as in semi-crystalline bodies, perhaps the light-cored dense particles, and other changes of mitochondria. Certain other ultrastructural changes, e.g., accumulation of lipid lysosomes, or glycogen in fibers provide clues as to which metabolic pathways are likely to be the site of a presumed enzyme defect. These ultrastructural changes will help guide formulation of pathogenesis and treatment of the neuromuscular and neurologic disorders. Experimental production of identical defects provides a new tool for analysing these changes. Similar expectations pertain to the other human neuromuscular disorders being studied, as well as the neuronal abnormalities. Perpetuation of, or production of, characteristic ultrastructural abnormalities of human muscle cells in tissue culture should help clarify disease mechanisms. Electronmicroscopy is also indispensable for the detailed morphologic analysis of topics listed under our other projects, e.g., the viral-etiology hypothesis of polymyositis and dermatomyositis, monitoring purity of cell fractions studied biochemically, and localization substances by EM-autoradiography.

Proposed Course of Project: Further studies that combine histochemical, immunologic, autoradiographic, viral, and tissue culture techniques with electronmicroscopy applied to human neurologic diseases and animal models thereof have been planned, so as to supplement the morphologic approach with pertinent studies of cellular dynamics.

Keyword Descriptors: muscle ultrastructural histochemistry, muscle ultrastructure, muscle mitochondria, muscle tissue culture, muscle - virus, avian leukosis virus, acetyocholine receptor, muscle nuclei - rods, α-actinin, tropomyosin

Honors and Awards: None

Publications:

Bender, A. N. and Engel, W. K.: Light-cored dense particles in mitochondria of a patient with skeletal muscle and myocardial disease. J. Neuropathol. Exp. Neurol., 1975, in press.

Engel, W. K. and Oberc, M. A.: Abundant nuclear rods in adult-onset rod disease. J. Neuropathol. Exp. Neurol. 34:119-132, 1975.

Engel, W. K.: Central core disease and focal loss of cross-striations. In Shy, G. M., Goldensohn, E. S. and Appel, S. H. (Eds.): The Cellular and Molecular Basis of Neurologic Disease, Philadelphia, Lea and Febiger, 1975, in press.

Engel, W. K.: Muscle fiber hypotrophy with central nuclei. In Shy, G. M., Goldensohn, E. S. and Appel, S. H. (Eds.): The Cellular and Molecular Basis of Neurologic Disease. Philadelphia, Lea Febiger, 1975, in press.

Serial No. ZO1 NS 01193-11 MN
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Radioautography Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
John W. Griffin, M.D.

Other Investigators: Donald Price, M.D.

Cooperating Units: Department of Neuropathology, Johns Hopkins School of Medicine, Baltimore, Maryland

Man Years:

Total:	1.8
Professional:	1.2
Other:	0.6

Project Description:

Objectives: To apply radioautographic techniques to study structure and function in normal and abnormal neurons and muscle cells of man and of animal models of human disease.

Methods Employed: Standard radioautographic methods, primarily using emulsion-dipped slides. Various histochemical enzymatic counterstains for neuron, neuromuscular junction, or muscle fiber components are done after photographic development of the slides, such as esterase to show location of neuromuscular junctions. A number of samples are also prepared for electron-microscopic autoradiography. Application of isotope is by one of several methods, e.g.: injection of tritiated leucine into rat anterior horns to study axoplasmic flow and transfer; injection of labeled substances into muscle to test its uptake and retrograde transport in axons; "pulse-feeding" cultured human muscle or animal muscle or neurons various labeled substances.

Patient Material: Some human muscle is tissue cultured before auto-radiographic study.

Major Findings: Borrowed (from Johns Hopkins School of Medicine) were facilities for EM-autoradiographic studies of axon-to-schwann-cell and axon-to-muscle transfer of ^{3}H-leucine-labeled proteins. Rat lower motor neurons (LMNs) are labeled by stereotactic injection of ^{3}H-leucine into the anterior horns. The distribution of tritiated proteins at 6 and 48 hours and 5 and 7 days is evaluated by autoradiographic and scintillation counting techniques. Intraaxonal label was present in the sciatic nerves at all times studied. Within 24 hrs. or less there were large accumulations of labeled protein in axonal presynaptic endings at the neuromuscular junctions. Thus, it must have gotten there by fast axonal transport. There was only minimal, but some transfer of the labeled molecules to muscle. Thus we have demonstrated these rapidly transported axonal proteins are in the geographic position to have trophic influence on muscle and we propose that they do so, an hypothesis we are studying further. Autoradiographic studies have also shown: (a) direct evidence for retrograde intra-axonal transport of tetanus toxin, and (b) that fast anterograde axonal transport apparently contributes to motor nerve regeneration in experimentally sectioned nerves. We have demonstrated appearance of labeled protein in schwann cells of sciatic nerve after ^{3}H-leucine injection into the anterior horns. Studies designed to distinguish between axon-to-schwann-cell macromolecular transfer vs. re-utilization of labeled leucine are in progress -- if the former is true it will support our hypothesis of several years ago regarding a trophic influence of axon on schwann cells.

We are also developing techniques to study autoradiographically in vitro metabolic parameters of nerve fascicles taken during diagnostic nerve biopsy from patients with various abnormalities affecting their peripheral nerves.

Finally, we have developed plans to combine autoradiographic techniques with our cultures of human skeletal muscle from patients with various types of disease and also with our cultures of animal neurons and muscle. We are delayed in this by lack of any space and technical help of our own in autoradiography.

Significance to Bio-Medical Research and the Program of the Institute: (a) Details on the type of trophic control of axon on schwann cells and muscle fibers, and the molecules involved, if we can clarify them, may have broad implications for understanding various neuromuscular diseases and "peripheral neuropathies". (b) Autoradiographically obtained information on human normal and diseased muscle fiber metabolism and animal neuronal and muscle fiber metabolism will have important bearing on a number of human neuromuscular diseases.

Proposed Course of Project: Extend our preliminary studies to various human neuromuscular diseases in a more complete and systematic manner, and extend our preliminary animal model studies, as discussed above.

Keyword Descriptors: autoradiography, axonal transport, nerve regeneration, tetanus toxin, trophic factors

Honors and Awards: None

Publications:

Price, D. L., Griffin, J. W., Young, A., Peck, K. and Stocks, A.: Tetanus toxin: Direct evidence for retrograde intraaxonal transport. Science 188:945-947, 1975.

Griffin, J. W., Drachman, D. B. and Price, D. L.: Contribution of fast axonal transport to motor nerve regeneration. Neurology 25:366-367, 1975.

Price, D. L. Griffin, J., Stocks, A., Peck, K. and Young, A.: Tetanus toxin: Direct evidence for retrograde intra-axonal transport. Neurology 25:367, 1975.

Serial No. ZO1 NS 01792-06 MN
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Electrophysiology Applied to the Study of Neurologic Disease

Previous Serial Number: Same and incorporating Project Nos.
ZO1 NS 00926-13 MN
ZO1 NS 01049-12 MN
ZO1 NS 01050-12 MN
ZO1 NS 01416-09 MN
ZO1 NS 01809-06 MN

Principal Investigators: W. King Engel, M.D.
Richard B. Rosenbaum, M.D.
Jay B. Wells, Ph.D.
Richard L. Irwin, Ph.D.

Other Investigators: Ahmed Hassan Mohamed, Ph.D.

Cooperating Units: Department of Physiology, Ein Shams University, Cairo, Egypt

Man Years:

Total:	2.7
Professional:	2.7
Other:	0.0

Project Description:

Objectives: (1) To develop more precise methods of electromyography (EMG) recording and analysis; (2) to establish better EMG indices of early myopathic or neuropathic involvement; (3) to improve the value of the EMG in distinguishing the neural vs. myopathic basis of atypical disorders; (4) to investigate mechanisms regulating motor unit recruitment in normal subjects and those with neuromuscular disorders; (5) to correlate the electrical behavior of motor units with their histochemically defined properties; (6) to use clinical electrophysiologic parameters to evaluate course of disease and response to treatment; (7) to use electrophysiologic techniques to analyse induced animal-models of human neuromuscular diseases; (8) to study certain very limited aspects of normal muscle fiber function from the basic point of view.

Methods Employed: Recordings are made in humans using the Teca-Medelec electrophysiologic analysis system. Permanent data are stored by high-speed magnetic tape recording and photographic recording. Correlation studies involve the (a) recruitment threshold, (b) rank order of recruitment, (c) preferential stable discharge frequency, (d) relative rhythmicity, (e) amplitude, (f) duration, and (g) configuration of EMG recorded individual motor unit action potentials in human subjects with a variety of neuropathic and myopathic conditions, and with neuromuscular disease of uncertain type, as well as in subjects without definable neuromuscular disease. We continue to use a method we previously developed for recording electrophysiologically in the operating room during open biopsy and excising the region of fibers directly around the tip of the EMG electrode ("Open Biopsy EMG").

A variety of parameters of neuromuscular transmission are repeatedly evaluated in individual myasthenia gravis patients before and during treatment. Partial curarization of subjects with myasthenia gravis and other fatigue states are performed with special attention directed to the behavior of single motor units.

Electrophysiological abnormalities are sought in various systemic diseases affecting the neuromuscular system, such as hyperparathyroidism and non-metastatic distant cancer, and correlated with clinical and histochemical data (see our Amyotrophic Lateral Sclerosis Project).

Small, brief pulses of stretch were applied to one end of a frog muscle at various times during the latent period. The evoked tension response was recorded at the other end by a sensitive high frequency transducer. The onset of stiffness was the time, after stimulation, when the tension response to stretches just exceeded corresponding responses from identical stretches applied to the resting muscle. The stiffness estimate consisted of the application of perturbations, produced in the acoustic frequency range, to one end of an isolated frog muscle with a sensitive high frequency force transducer located, to record the arrival of the impulse at the other end. The transmission velocity was calculated from the estimate of transmission time as described above and from muscle fiber length, with the "measurement time" of stiffness less than 0.05 milliseconds.

Patient Material: Patients with a variety of neuromuscular disorders and without definable neuromuscular disease from the Medical Neurology Branch and the neurologic consultation service we provide throughout the Clinical Center, in whom EMG studies are being done for diagnostic purposes.

Major Findings: Several new aspects of clinical electromyography (EMG) have been published this past year. Our previously introduced new descriptive term for the pattern of short duration, small amplitude, excessively abundant potentials for a given amount of voluntary effort, viz., "SSAP", used to replace the generally employed term "myopathic" (a designation which we consider to be misleading at times) was modified in acronym (but not in substance). The "short" was changed to "brief", i.e., "BSAP", allowing one to use the two subacronyms of BAP and SAP when the situation applies. We consider BSAPs just as likely to be due to neuronal disease as to myopathic disease (v.i.). This information has been published this year.

In lectures and writings we have continued our "campaign" against designation of the BSAP EMG pattern as "myopathic" -- nevertheless, many authors in a steady flow of articles still continue not only to write "myopathic" but, worse, to think that the BSAP pattern is diagnostic exclusively of a myopathy. We continue to propose, on what we consider very sound theoretical grounds, that the BSAP EMG merely denotes either (a) small (few-fiber or "hypocomplemented") motor units of type I (or both types of) or (b) motor units uncomplemented but with smaller-than-normal-diameter fibers. The hypocomplemented units could be due either to a defect in development ("hypoplastic units") or acquired fractionation. Although any of these changes theoretically could be myopathic, any could also be neuropathic. We therefore conclude that the BSAP EMG pattern is absolutely non-diagnostic and that there is nonesuch animal as a "myopathic EMG".

In our formulation that abnormality of the lower motor neuron or its axonal branches cause a BSAP pattern, we hypothesize, on the basis of correlated electromyographic and histochemical study and in comparison to theoretical possibilities, that a neural abnormality is the basis of all or most of the muscle abnormality in the following diseases with BSAP EMGs: myasthenia gravis, central core disease, myotonic atrophy (formerly called myotonic dystrophy), myotonia congenita, paramyotonia congenita, type-I-fiber-hypotrophy-with-central-nuclei, type-I-fiber-smallness-without-central-nuclei, and the form of benign congenital hypotonia with type I fiber predominance (see our ALS, Myopathies and Myasthenia Gravis Projects).

Using a micromarking technique we previously developed to give a 50-100μM dia. spot in histochemical sections in conjunction with open biopsy electromyography, we are studying the correlation of spontaneous electrical activity at rest (fibrillations and positive sharp waves) with the presence of extrajunctional acetylcholine receptors using our newly developed alpha-bungarotoxin stain for acetylcholine receptors, in patients with amyotrophic lateral sclerosis and with dermatomyositis/polymyositis.

In a number of systemic diseases, the presence and type of neuromuscular involvement was identified by EMG correlated with histochemistry. For example, in secondary hyperparathyroidism (with NIAMD), we reported that nearly all patients studied were considered to have mild to moderately-severe lower motor neuron involvement, sometimes mimicing aspects of amyotrophic lateral sclerosis (see our ALS Project). In very early non-metastatic, distant cancer the histochemical type II muscle fiber atrophy found in most patients was correlated with EMG studies, which were generally normal (or showed only slight decreased number of recruitable units).

Our clinical electrophysiologic studies form a major basis of diagnosing our patients as described in our other projects (e.g., Myopathy, Amyotrophic Lateral Sclerosis, Myasthenia Gravis, and Periodic Paralysis Projects) and following the course of disease and results of treatment.

Some diagnostic electrophysiologic studies are provided, within the limitations of our staffing, as part of our consultation service to other clinical units of the Clinical Center.

In frog muscle it was found that the onset of stiffness to occur at about two thirds of the latent period with a Q_{10} above 2. The significance of these findings are apparent when one considers that heat liberation after stimulation, begins at about the same time or slightly before this mechanical change. It now seems certain to conclude that the observed stiffness change does result from the chemical reactions which underlie the contractile event. Stiffness preceded isometric tension production. Stiffness and twitch tension rose at about the same rate after initial stimulation, but the stiffness peaks at a higher fraction of maximum than twitch tension. During relaxation of tension, the stiffness remains at a higher fraction of maximum than twitch tension until the resting levels are reached. At increased temperature, the magnitudes of stiffness and twitch tension were reduced.

Significance to Bio-Medical Research and the Program of the Institute: Treatment of the various, and numerous, human neuromuscular diseases is based on precise diagnosis and understanding of the pathophysiology. As a group, human diseases of the lower motor neurons (including peripheral neuropathies) and muscle are just beginning to have their pathophysiology partly understood. The detailed neurophysiologic techniques herein described are leading to more precise delineation of these diseases, and in some instances causing us to completely change our concepts of their pathogenesis. Investigation of the pathogenesis is also partly dependent on knowing whether a disease is primarily one of motor neuron or muscle -- for example, it would be useless to do biochemical assays on muscle homogenates of a disease if the disorder is essentially one of the lower motor neuron.

EMG is also important for detecting subclinical presence or dissemination of neuromuscular disease, and its type, in patients with various generalized disorders, e.g., distant cancer or hyperparathyroidism.

We believe that if we can eliminate the term "myopathic EMG" or "the EMG was myopathic" from neurologic jargon, and, more importantly, from the neurologic "thinking", clinicians and investigators will have a freer approach to ponder the possible pathogenesis of various ill-understood neuromuscular diseases.

Demonstration of the prolonged (1-2 hr.) action of edrophonium (Tensilon) on neuromuscular transmission is of importance in the managing of patients with myasthenia gravis.

The study of "stiffness" of muscle provides new information on basic properties of muscle.

Proposed Course of Project: This project will be continued and elaborated upon, e.g., with improved and more diverse detailed clinical diagnostic techniques and with even more detailed correlation between EMG and histochemistry in patients with various diseases.

Keyword Descriptors: electromyography, neuropathy, myopathy, muscle physiology, myasthenia gravis, neuron-to-muscle trophic influence, amyotrophic lateral sclerosis

Honors and Awards: None

Publications:

Engel, W. K.: Brief, small, abundant motor-unit action potentials. Neurology 25:173-176, 1975.

Rosenbaum, R. B., Bender, A. N. and Engel, W. K.: Prolonged response to edrophoinium in myasthenia gravis. Trans. Am. Neurol. Assoc., 1975, in press.

Schoenberg, M., Wells, J. B. and Podolsky, R. J.: Muscle compliance and the longitudinal transmission of mechanical impulses. J. Gen. Physiol. 64:623-642, 1974.

Serial No. Z01 NS 00926-14 MN
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Electromechanical Coupling in Muscle and Drug Activity

During FY 1975 this project was incorporated with Project No. Z01 NS 01792-06 MN

Serial No. Z01 NS 01049-13 MN
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechanical Properties of Muscle in Relation to Drug Action

During FY 1975 this project was incorporated with Project No. Z01 NS 01792-06 MN

Serial No. Z01 NS 01050-13 MN
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Functional Activity and Properties of Striated Muscles

During FY 1975 this project was incorporated with Project No. Z01 NS 01792-06 MN

Serial No. Z01 NS 01416-09 MN
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A Pharmacological and Toxicological Study of Neurotoxic Venoms

During FY 1975 this project was incorporated with Project No. Z01 NS 01792-06 MN

Serial No. Z01 NS 01809-06 MN
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Impaired Transfer of Information Between Nerve and Muscle

During FY 1975 this project was incorporated with Project No. Z01 NS 01792-06 MN

HOW TO USE
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Use one page for each separation.

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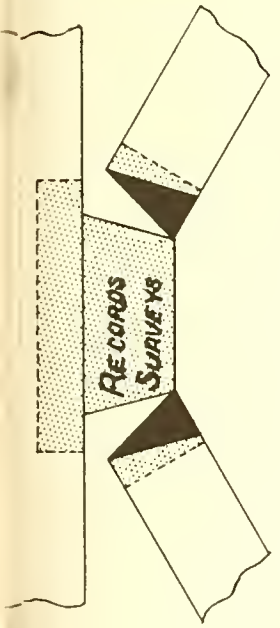

TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

LAB. OF BIOPHYSICS

SURGICAL NEUROL. BRANCH

CNS STUDIES LAB.

EEG & NEUROPHSY. BR.

CL. DEVELOP. & METAB. NEUROL. BR.

LAB. OF NEUROPATHOLOGY & NEUROANATOM. SC.

LAB. OF NEURAL CONTROL

LAB. OF NEUROPHYSIOLOGY

ANNUAL REPORT
July 1, 1974 through June 30, 1975
Surgical Neurology Branch, IR
National Institute of Neurological and Communicative Diseases and Stroke

J. M. Van Buren, M.D., Chief

Summary of Studies in Surgical Neurology

The primary orientation of the branch remains to study the form, function and disease of the human nervous system, taking advantage of the technical opportunities of neurological surgery to observe, stimulate and sample.

Long-term projects deal with studies in the following categories: I. Dyskinesias; II. Epilepsy; III Speech, Memory and Cognitive Function; IV. Trauma of the CNS; V. Tumors; and VI. Vascular Disease of the CNS.

I. Dyskinesias (Stereotaxic Program)

A. Effects of depth stimulation on Speech, Memory, and Cognitive Function are given below (III).

B. Combined use of human material following stereotaxic lesions and experimental anatomical studies in the anthropoid ape (chimpanzee) have demonstrated that the large-celled posterior thalamic nucleus, usually called n. ventralis posterior (and considered to have only sensory function), is actually separable into two regions: an anterior, n. ventrointermedius (V im) and a posterior, n. ventrocaudalis (Vc). The cerebellar afferents enter V im on the way to anterior thalamic regions (not the small-celled VL, as classically described) and the medial lemniscus enters Vc. There was no overlap of the two projections in the two nuclei. In human material, a discrete lesion in Vc produced contralateral paresthesias, while one of similar size limited to V im produced none, further supporting a non-sensory role for V im.

C. Restudy of the human supplementary motor area with implanted electrodes reconfirmed the bilateral tonic, usually extensor, motor responses described earlier, tho without the contraversive turning considered to be characteristic by Penfield. In addition, prominent sensory responses were produced both from the supplementary and cingulate regions. The cingulate sensory responses showed wide trunkal and extremity referral with only questionable contralateral preponderance. The sensations from the supplementary area were more focal, contralateral and often distal. Comparison of speech and memory deficits, using quantitative techniques, on stimulation of supplementary and fronto-striatal areas showed striking similarities, probably reflecting the anatomically demonstrated connections between the supplementary motor area and the striatum.

II. Epilepsy (Surgical Epileptic Program)

Basic Studies

A. Studies of extracellular K uptake following standard cortical stimulations at varying distances from chronic alumina cream epileptic foci in macaques showed that the rate of K uptake was inversely proportional to the counts of fibrous astrocyte population in the cortex. This is the first direct demonstration of a relationship of K kinetics to the fibroglial scar. (Joint project with EEG.)

B. Study of the role of the synaptosomal membrane glycoprotein in (Na^+-K^+) ATPase action showed that utilizing lectins, only Con A can interfere with the enzyme although some other lectins bind as well. The detailed isolation procedure of the synaptosomes allowed us to extend the histochemical procedure for (Na^+-K^+) ATPase to subcellular fractions.

Clinical Studies

A. A pilot study of chronic intermittent cerebellar stimulation in severe medication-resistant seizure states demonstrated that no patients were relieved of their seizures tho most were pleased by the procedure citing "fewer attacks" or "improved personality." Three patients had cerebellar biopsies which showed by quantitative techniques the loss of between 30% and 80% of the Purkinje cells in comparison with a normal human series averaging 3.6 Purkinje cells showing a nucleolus/mm. cortical surface/10μ section. Nevertheless, the polysynaptic reflexes in the spinal cord (tonic vibration response) were decreased 20-50% in all patients during cerebellar stimulation, giving evidence of a physiological response. The monosynaptic reflexes (H reflexes) were unaltered. The therapeutic value of this technique in epilepsy must be evaluated over an extended period. (Joint project with LNLC.)

B. A technical advance has been achieved in the design of an air-supported microelectrode (Project No. Z01 NS 01687-6 LNLC). Various prototypes have been tried in the human operating room during development but in the last year, a gratifying increase in functional reliability has been achieved. It is now possible to record from a single neurone (or small group) for many minutes (15-20) despite wide respiratory excursions, and the possibility of intracellular recording in human cortex can now be considered.

III. Speech, Memory and Cognitive Function (Stereotaxic and Surgical Epileptic Program)

A. Further studies of object naming and memory during cortical speech mapping have disclosed differences between the posterior frontal and posterior temporal representations. Stimulation of

both interrupt speech, but Broca's area to a lesser degree. In addition, stimulation of Wernicke's produced disruption of internal speech, while the effect on Broca's area did not prevent the patient from recognizing or remembering the objects and stating the name after stimulation. A unique opportunity presented to map speech in Broca's area of a blind epileptic. This patient was unable to read Braille words or name objects presented tactually until after stimulation. Stimulation of adjacent frontal sites did not block speech.

B. Our earlier work suggested that the left pulvinar and left posterior temporal cortex on stimulation responded similarly with regard to identifying, registering and retrieving verbal material. Further study, however, showed that while dysphasia and amnesia were inseparable during cortical stimulation, recent memory was occasionally spared during dysphasia induced by left pulvinar stimulation. Thus the pulvinar and other posterior thalamic nuclei may function in pre-perceptual processing while the cortex serves more in coding and storage of information.

C. Preliminary comparison of a series of patients with Korsakoff's syndrome and temporal or parietal lobe lesions shows that the former are more susceptible to the effects of distraction on a wide range of memory tests. Alteration of the learning trials (massed vs. spaced practice) also showed the Korsakoff patients were more vulnerable to the effects of proactive inhibition.

D. From preliminary study of perceptual deficits partitioned according to an imagery factor it appears that right temporal injury results in difficulty forming and using visual images as mnemonic cues to offset learning disabilities. In contrast, patients with left temporal injury (intact right temporal lobe) may be able to form a picture of an object which they are unable to name due to aphasia and to use this technique to compensate for learning difficulties.

E. Investigation of linguistic skills in patients with left temporal epileptogenic foci showed impoverished vocabulary skills reflecting a concrete or stimulus bound approach in defining words and a restricted range of word usage.

IV. Trauma of the CNS

Basic

A. The new programmable head accelerating device (HAD-III) has been installed in AFRRI. Instrumentation has been completed permitting correlation of cerèbral blood flow and O_2 levels (using O_{15}) with cerebral evoked potential (visual, somatosensory and auditory) data. This forms a totally unique facility.

B. Current observations have been as follows:

1. The ability of the CNS to process input stimuli (visual and somatosensory) at varying rates is roughly proportional to its integrity; e.g. in the post concussive state, cerebral evoked potentials can follow input stimuli only at lower frequencies -- recovery of the subject is accompanied by return of ability to follow higher frequencies.

2. Observations relating cerebral compliance $\left(\frac{\Delta V}{\Delta P}\right)$ to cerebral circulation suggest that brain death produced by space occupying lesions is caused by failure of capillary perfusion, which is relatively independent of the intracranial pressure. Further study will be made of the cerebral compliance factor as a prognostic index for brain survival in neural trauma.

Clinical

A. Diagnostic

1. Spinal arteriographic studies in acute spinal injuries resulting in para- and tetra-plegia with direct magnification demonstrated the discrepancy between the gross bony-ligamentous damage and the relative sparing of the vascular system. In one case, contrast medium was seen in the epidural space indicating "ongoing" bleeding. In another case, postmortem microangiography could be compared with the angiogram.

2 In chronic spinal trauma due to herniated thoracic disc, angiography provided important information regarding whether the surgical approach should be through the usual posterior laminectomy or through a lateral or anterolateral approach. The material on angiography in chronic cervical spondylotic myelopathy is now being evaluated.

V. Tumors of the CNS (Clinical Tumor Program)

Basic Studies

A. Murine glioma model

Pre-immunization with glioma cell membrane leads to complete failure of tumor growth in these animals. The value of chemo- and radio-therapy has been confirmed. Splenectomy prior to innoculation has some protective effect but this is not seen postinnoculation.

Clinical Studies

A. Diagnostic Methods

1. Isotope-Ventriculography and Cisterography

a. Confirmed frequent lack of isotope migration over hemispheral tumors.

b. Studied dynamics of spinal descent of isotope in patients and macaques and effects upon this of neoplasms, meningitis, and low pressure hydrocephalus.

c. Applications included assessment of CSF shunts, intrathecal drug administration, hydrocephalic status, and neoplastic invasion.

B. Clinical Studies

1. Immunotherapy of Malignant Gliomas

Studies to date have indicated that immunotherapy can only be effective if the antigenic tumor mass can be reduced to less than 1 $cm.^3$ of tissue. Also needed is the capability of localizing the immune response to the tumor site. Further studies of gel electrophoresis of human normal and malignant glioma brain tissue extracts showed wide variation in glycoprotein patterns, which showed no resemblance to the "carcinoembryonic antigen" of colon carcinomas. It has been concluded that glioma immunotherapy can not be effective in our present state of knowledge.

2. In review of angiograms of gliomas of the spinal cord, myxopapillary ependymomas of the conus and filum terminale present characteristic - ? pathognomic - angiographic findings, viz. enlarged spinal arteries, striking visualization of the anastomotic loop(s), markedly slowed circulation.

3. Angiographic Methods

a. A threshold of drug-producing postangiographic paraplegia in monkeys has been established.

b. In macaques, we have studied postradiation myelitis angiographically.

4. Radioactive Scanning Tomography

Experience with 30 cases suspected of harboring intracranial disease has indicated that blurring of activity outside the plane of interest is clearly possible, permitting a layer-by-layer analysis of the brain.

5. Computerized Axial Tomography

A number of projects are being undertaken to evaluate

clinical performance (comparison with radionuclide scans, introduction of tracers and/or markers), and the physical characteristics (various algorithms, power of resolution and sensitivity of three fundamental groups of detectors: scintillation, gas chamber and solid-state devices of CAT. With computer simulation, the effect of beam hardening has been studied. A novel pilot study using protons for reconstructive transmission tomography is being started.

VI. Vascular Disease of the CNS (No In-House Clinical Program)

Basic Studies

A. Cerebral Venous Obstructive Disease (Macaque)

Using angiographic evaluation, this animal has promise of being a good human model. Angiography is being carried out before and after ligation of one or more large endocranial venous channels. The findings of microangiograph, gross and microscopic studies are being evaluated.

B. Blood Flow in Normal and Infarcted Brain (Macaque)

A consistent infarct model has been produced by clipping the middle cerebral artery. Studies include clinical evaluation, radionuclide scanning, periodic angiography, ^{133}Xe washout, O_2 electrodes and terminal microangiography. Early results indicate that both hypo- and hyper-capnea decrease perfusion of zones of ischemia and changes in vessel morphology. In most animals brain scintigraphy becomes positive 2 weeks after infarction, related to neovascularization about the infarct, as shown by microangiography. The scintigraphy becomes negative in 4-6 weeks. This is related to decreased vascularity, peripheral gliosis and central cavity formation.

Project No. Z01 NS 00100-22 SN
1. Surgical Neurology Branch
2.
3. Bethesda, MD

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Epileptogenic Mechanisms in the Brain of Man and Other Primates

Previous Serial Number: Same

Principal Investigators: J. M. Van Buren, M.D., C. Ajmone-Marsan, M.D. and A. Daniel, Ph.D.

Other Investigators: P. Fedio, Ph.D., D. Lewis, M.D. and N. Mutsuga, M.D.

Cooperating Units: NS-EEG and NS-LNLC

Man Years

Total:	1.6
Professional:	1.2
Other:	0.4

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 examination.

2. Special radiographic and other contrast examinations.

3. Electrographic, including electrocorticographic, examination.

4. Physiological and psychological techniques as indicated.

5. Histological and chemical examinations as required.

Major Findings:

1. Basic Studies in Epilepsy

a) Fibrous astrocyte transformation and extracellular K uptake

We have supplied the preparations and histological control for a collaborative study with EEG (Z01 NS 02121-01 EEG). In brief, this relates the gradient in extracellular K uptake following a standard period and intensity of cortical stimulation to the cortical population of fibrous astrocytes quantified with the gold chloride sublimate method. The cortical fibrous astrocyte population was altered by the chronic application (few to several months) of alumina cream. In a given preparation the fibrous astrocyte population was a function of the distance of the recording site from the alumina lesion. The studies have given evidence that the rate of uptake of K extruded from the neurones by the electrical stimulation was inversely proportional to the population of fibrous astrocytes in the cortex. This is the first direct demonstration of the impairment in K uptake in an epileptic focus characterized by a fibroglial scar.

b) Biochemical basis of epileptogenic mechanism in the brain

i The 3-mercaptopropionic acid model

In order to study the etiology of the disease in man, it was essential to develop a small animal model where convulsions could be produced rapidly and reliably. Injection of rats IP with 3-mercaptopropionic acid (3MPA) proved to be the most suitable model. Radioactive 3-mercaptopropionic acid was obtained and the preliminary work of adopting the methodology for radio autography has been done. Since we did not get the services of the enzymologist, we had to gear up to do the glutamic acid decarboxylase studies ourselves.

ii Study of the synaptic membrane fraction during epilepsy

Published cellular fractionation procedures of both human and rat epileptic and normal brains were utilized to obtain the synaptosomal membrane preparations. A method was extended to radioactively (^{3}H) label plant lectins which will be utilized as surface specific probes. We have started to investigate the biochemical makeup of the CNS synaptosomes, especially in regard to the binding of radioactive lectins. A quantitative binding picture for six different lectins revealed wide variations in end-point titration amounts for the different lectins, indicating a variety of different glycoproteins on the synaptosomal surface. This research will be submitted for publication shortly.

In this regard the radioactive lectins were used by Dr. Quarles and his group (Developmental and Metabolic Neurology Branch, Project No. Z01 NS 01808-5) to look at myelin structure and especially the glycoprotein associated with it. The findings were published.

iii Role of the synaptosomal membrane glycoprotein in (Na^+-K^+) ATPase action

The investigation of the synaptosomal membrane also entails an inquiry into the role of the glycoprotein in the (Na^+-K^+) ATPase action. Utilizing lectins, and in collaboration with Dr. W. Albers' group (Neurochemistry), we were able to show that only Con A can interfere with the enzyme, although some of the other lectins bind as well. Some implications of these findings were included in a paper submitted to Biochem. Biophys. Acta. The detailed isolation procedure of the synaptosomes allowed us to try to extend the histochemical procedure for (Na^+-K^+) ATPase to subcellular fraction. The results of this project were published in Exp. Neurol., April 1975.

c) Histochemical studies in epilepsy

This year has been employed in setting up techniques. The following has been achieved:

i The histochemical technique for Na-K ATPase recently described and applied to rat spinal cord by Guth was successfully extended in our laboratory to stain (1) rat brain, (2) cat spinal cord and brain and (3) in the next few weeks, monkey spinal cord and brain.

ii Acetyl cholinesterase stains in use were found inadequate to study monkey cortex because of enzyme diffusion, and experiments to improve localization have met with only partial success. In the process, however, a study of delayed anterior horn and intermediolateral cell changes following dorsal and ventral rhizotomy was instituted and is nearing completion.

iii Experiments in norepinephrine histofluorescence were delayed by loss of essential equipment early this year. Replacements were received in November 1974; the first successful demonstration of NE terminals was achieved shortly thereafter. Monkey cortex will be studied after some additional preliminary experiments.

2. Clinical Studies

a) A pilot study of the effects of chronic intermittent cerebellar stimulation was made on five individuals in whom the devices were implanted between March-July 1974. The only complications have been the chronic accumulation of CSF about the chest receivers due to difficulty in achieving a watertight seal about the wires as they pass through the dura of the posterior fossa. Two patients have required wound revisions to correct this, and one has had two wound revisions, yet with further recurrence.

All patients had had an average of 1^{+} fit/day and have been maintained on full medication. The Laboratory of Neural Control has provided support for testing the stimulation devices and calibrating the current output in situ during the implantation operation. Dr. Hambrecht has also provided a simple and effective method of double-blind stimulation.

No patient has been relieved of seizures by chronic intermittent cerebellar stimulation. The families of two of four have been enthusiastic about the results on the basis that the patient is more alert, responsive etc. This may be the result of monitoring of antiepileptic medication. One patient carried on an epileptic drug trial program on 2-West for 8 mos. and considered to be an intractable epileptic was entirely seizure free from March-September 1974, then has had only rare attacks so has been able to resume work on the farm. In this case, electrical stimulation was never started after implantation of the device.

b) Study of the TVR and H reflexes with chronic cerebellar stimulation

The H wave (H), the response of a monosynaptic reflex activated by stimulation of group I afferent fibers proximal to the muscle spindle, was recorded in relation to the corresponding M wave (M) or muscle response preoperatively, post operatively, post stimulation and after followup periods up to 9 mos. in four of five patients receiving cerebellar stimulation. No alterations in H/M relationship or major alterations in thresholds for evocation of H response were noted with cerebellar stimulation. The tonic vibration response or TVR, a polysynaptic reflex causing a tonic increase in contraction of the muscle vibrated, was additionally measured in these patients. With followup periods identical to the H wave study, the records to date indicate a decrease in TVR during cerebellar stimulation of 20-50% in amplitude in all of the patients. In one patient, only baseline pre-stimulation data has been recorded to date.

c) Study of the cerebellum of epileptics

Quantitative studies of the Purkinje cell population in three of the patients with cerebellar stimulators show severe atrophy, with counts of 0.6, 1.0 and 2.6 Purkinje cells/mm. of cortical surface (Purkinje cells showing a nucleolus with counts adjusted for 10μ sections) with biopsies taken over the horizontal fissure in crus I and II about 1 cm. from the midline. Similar samples from four other epileptic brains in our collection showed counts of 0.7, 1.1, 1.3 and 1.6. Five normals varied from 3.3-3.8 cells/mm. surface.

If the rationale of cerebellar stimulation is to increase the inhibitory discharge of the Purkinje cells, this finding brings the basic rationale into question.

d) The air supported microelectrode developed by Project No. Z01 NS 01687-6 LNLC has been subject to mechanical problems in application to the human brain

during craniotomy for epilepsy. When it functioned reliably, the extracellular records were remarkable in permitting the continued recording from the same neurone (or small group) for many minutes despite the wide respiratory and cardiac pulsations of the cortex. Serious study of the ECoG spike-extracellular unit activity interrelationship has as yet not been practical. The progress in design, however, is very encouraging.

Proposed Course of the Project:

The promising neurochemical support for the epileptic project has been terminated so that further work along these lines cannot be pursued. More importantly, we have lost the needed support and consultation for the histochemical studies. Since no slots appear available for recruitment in this field in FY'77, the future in this area remains uncertain. In the meantime personnel training in EM techniques is proceeding which will support the histochemical work.

On the clinical side, technical work to increase the ease of application to the human operating theater of apparatus for NADH and microelectrode recording is continuing. Studies with these instruments will be pursued in FY'77 if facilities are available.

Keyword Descriptors:

Epilepsy; K kinetics; astroglial scar, 3-mercaptopropionic acid fits; synaptosomal membrane; N^{+}-K^{+} ATPase stain; histofluorescence; cerebellar stimulation, man; cerebellar atrophy; H reflexes; tonic vibration response; microelectrode, man.

Honors and Awards: Moderator of the Functional Neurosurgery Section, American Association of Neurological Surgeons, Miami 6-10 April 1975

Publications:

Daniel, A. and Guth, L.: Histochemical demonstration of (Na^{+}-K^{+}) activated ATPase activity in synaptosomes and synaptosomal membranes. Exp. Neurol. 47: 181-188, 1975.

Fedio, P. and Van Buren, J. M.: Memory deficits during electrical stimulation of the speech cortex in conscious man. Brain and Language 1: 29-42, 1974.

Horwitz, D., Clineschmidt, B. V., Van Buren, J. M. and Ommaya, A. K.: Temporal arteries from hypertensive and normotensive man. Reactivity to norepinephrine and characteristics of alpha-adrenergic receptors. Circ. Res. 34-35: Suppl. 1, 109-115, 1974.

Matthieu, J. M., Daniel, A., Quarles, Q. H. and Brady, R. O.: Interaction of conconavalin A and other lectins with CNS myelin. Brain Res. 81: 348, 1974.

Schuette, W. H., Whitehouse, W. C., Lewis, D. V., O'Connor, M. and Van Buren, J. M.: A television fluorometer for monitoring oxidative metabolism in intact tissue. J. Assoc. Adv. Med. Instrum. 8: 331-333, 1974.

Swann, A. C., Daniel, A., Albers, R. W. and Korval, G. J.: Interaction of lectins with (Na^+-K^+) adenosive tryphosphotase of eel electric organ. Biochim. Biophys. Acta. In press.

Van Buren, J. M., Ajmone-Marsan, C., Mutsuga, N. and Sadowsky, D.: Surgery of temporal lobe epilepsy. In Purpura, D. P., Penry, J. K. and Walter, R. D. (Eds.): Advances in Neurology. New York, Raven Press, 1975.

Project No. Z01 NS 00200-21 SN
1. Surgical Neurology Branch
2.
3. Bethesda, MD

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Involuntary Movements

Previous Serial Number: Same

Principal Investigators: J. M. Van Buren, M.D. and P. Fedio, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.4
Professional:	0.3
Other:	2.1

Project Description:

Objectives:

Neurological disease characterized by dyskinesias offers a twofold opportunity for research. The pathological aspects of the disease itself may be studied as well as the pathophysiology of the motor system. The second aspect is the unparalleled opportunities afforded for neurophysiological studies in man by stereotaxic surgery in the treatment of dyskinesias. Studies made directly of the disease itself have not received great emphasis in the present research since they require biochemical and pathological support.

Methods Employed:

Depth electrode stimulation employs specially built electrodes and a current-monitored stimulator. Special apparatus used in quantitative psychological testing is described in Dr. Fedio's project report, No. Z01 NS 01658-08 SN.

Major Findings:

Under circumstances of current support, the research aspects of electrode implantation have been utilized for studies of the effects of stimulation upon speech and cognitive function (see Z01 NS 01658-08 SN).

Significance to Bio-Medical Research and the Program of the Institute:

The use of a human subject who is cooperative and unsedated permits many tests of sensory and psychological function which are impossible in lower animals, even with time-consuming conditioning experiments. The use of the human subject is, therefore, not a duplication of animal experimentation but an extension of this and, of course, studies of speech function may only be carried out in man.

Proposed Course of the Project:

Dyskinesia patients will be studied as they become available in support of projects related to neuropsychological studies. At the present time the personnel support does not permit further studies utilizing the neurophysiological opportunities offered by the surgical approach.

Keyword Descriptors: Depth electrodes, man; Depth stimulation, man.

Honors and Awards: Councillor, American Society of Stereotaxic and Functional Neurosurgery.

Publications: See Project No. Z01 NS 01658-08 SN

Project No. Z01 NS 00304-20 SN
1. Surgical Neurology Branch
2.
3. Bethesda, MD

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effect of Lesions Upon the Function and Structure of the Human Central Nervous System

Previous Serial Number: Same

Principal Investigator: J. M. Van Buren, M.D.

Other Investigators: R. C. Borke, P. Fedio, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.4
Professional:	0.3
Other:	2.1

Project Description:

Objectives:

This project is directed toward the study of basic neuroanatomy and neurophysiology in man, making use of pathological material and the opportunities for study afforded by the operative treatment of neurological disease.

Methods Employed:

Anatomical studies:

1. Serial sections of human and animal brains in celloidin for myelin and Nissl series.

2. Section and staining of primate brains with Nauta technique for demonstration of degenerating pathways.

Major Findings:

1. In man and chimpanzee the large-celled region in the postero-inferior portion of the lateroventral thalamic mass, commonly designated as the n. ventralis posterior thalami, is obviously separable into two regions. The

anterior portion is called the n. ventrointermedius (Vim) and the posterior part n. ventrocaudalis (Vc)(Vogts, Hassler). In macaque, the separation is generally ignored due to its poor development, although Olszewski called the anterior part n. ventralis posterior lateralis pars oralis (1952).

Earlier studies in chimpanzee (Van Buren and Borke) demonstrated that the fibers from the superior cerebellar peduncle entered Vim on the way to distribution in the anterior half of the lateroventral thalamic mass. Fibers from the nuclei of the posterior spinal columns enter Vc with no overlap into Vim.

The present case was a 51 yr. old male with Parkinson's disease who had a left cryothalamotomy on 7/70 and a right thalamic lesion in 7/72. Following the right lesion he had persisting paresthesias of the left corner of the mouth and tongue. He died in 2/73 of bronchopneumonia.

The brain was serially sectioned in celloidin and pairs of sections at 50 section intervals were stained for cells and myelin. The lesions were clean cavities 3-4 mm. in diameter. On the left the lesion lay in Vim with slight extension anteriorly into n. ventralis oralis externus (inferior part of VL). On the right (opposite to the paresthesias) the lesion lay in the inferomedial part of Vc, anterolateral to n. centralis (Ce)(CM) and just above the tegmental field. No extension into Vim was seen. Secondary degeneration in Ce (presumably of thalamostriatal fibers) was seen on both sides.

This unusual human material supports the position that the posterior (Vc) portion of the classical n. ventralis posterior thalami alone has sensory function, while the anterior part (Vim) is not a somatic sensory relay nucleus.

2. The opportunity arose to study the sensorimotor responses to stimulation of the parasagittal (medial) frontal and cingulate area in seven patients. In three of these, speech function was studied by quantitative methods.

Sensory and motor responses appeared from the supplementary motor area. These were complex postural synergies involving the trunk and proximal extremities on the contralateral side or, occasionally, bilaterally. The strong contraversive turning of head and eyes with raising of the hand before the face, often considered to be a classical characteristic of the supplementary motor response in man, was not observed. Sensory responses, when present, were referred in a contralateral and focal fashion.

Sensory responses from the cingulate gyrus were widely referred over the body and extremities with a questionable contralateral preponderance.

Use of an object naming task in one patient showed difficulty naming with retention of use of a filler word and recall during stimulation. In another case, there was loss of both the ability to name a test object and later recall of it. In the last case, use of a test requiring both an oral and a

motor (pointing) response showed concomitant loss of both the oral and the motor responses with variable recall during stimulation.

Comparison of the speech impairment from stimulation in the supplementary motor area with the impairment from stimulation in the frontostriatal region or lateral aspect of the frontal lobe in these subjects showed striking similarities. The evidence is discussed supporting the hypothesis that interference with striatal function is the basis of speech inhibition produced by stimulation of the frontal lobe.

Proposed Course of Project:

The availability of new techniques for the study of enzyme histochemistry offers the exciting prospect of having the means to undertake topographical studies in high primates of metabolic processes and relate the relative activities to the cytoarchitectural entities of the thalamus, basal ganglia and remaining diencephalon and brain stem. Once these basic maps are made up the technique can be applied to other studies such as that of the experimental epilepsy (see Project No. Z01 NS 00100-22 SN).

The problems, of course, center upon the specialized talent as well as time and space required to produce the "stains." Since we cannot hope to obtain this talent within the Branch, it is obvious that some type of collaborative work must be undertaken. We are fortunate in having access to Dr. Bloom at St. Elizabeth's Hospital and Dr. Kaufmann in Building 36 (who has a preparation to detect tyrosine hydroxylase). Particular interest has been paid to Dr. Roberts who can selectively label glutamic acid decarboxylase with perioxidase, which would apply well to the epileptic project. The logistics of collaboration here are difficult since he resides in California. The possible use of a tritium label opens further possibilities for topographical studies. With the exception of one paper from Dr. Roberts' group, no studies of this type were reported at the meeting of the American Association of Anatomists, indicating our opportunity to advance rapidly in this field if proper liaison can be made. Personnel training in electron microscopy is continuing.

Keyword Descriptors:

Human neuroanatomy; Human neurophysiology; Thalamus; Cingulate region; Supplementary motor region; Cortical stimulation.

Honors and Awards: Discussor of papers on Functional Neurosurgery, American Association of Neurological Surgeons, Miami, 6-10 April 1975.

Publications: Fedio, P. and Van Buren, J. M.: Memory and perceptual deficits during electrical stimulation in the left and right thalamus and parietal subcortex. Brain and Language 2: 78-100, 1975.

Van Buren, J. M.: The neuroanatomical basis for functional neurosurgery. In Sano, K., Ishii, S. and Le Vay, D. (Eds.): Recent Progress in Neurological Surgery. New York, American Elsevier Publishing Co., 1974, pp. 219-233.

Van Buren, J. M.: The question of thalamic participation in speech mechanisms. Brain and Language 2: 31-44, 1975.

Van Buren, J. M. and Fedio, P.: Functional representation on the medial aspect of the frontal lobes in man. J. Neurosurg. In press.

Project No. Z01 NS 00907-14 SN
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Trauma to the Nervous System.

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, M.D., F.R.C.S., F.A.C.S.

Other Investigators: T. A. Gennarelli, M.D., N. Gunby, M.D., L. Thibault, M.S. and G. Di Chiro, M.D.

Cooperating Units: Division of Research Services, Biomedical Engineering and Instrumentation Branch, NIH; Department of Neurobiology, Armed Forces Radiobiology Research Institute; Departments of Neurosurgery and Radiology, George Washington University Medical Center.

Man Years:

Total:	3.5
Professional:	2.5
Other:	1.0

Project Description:

Objectives:

1. To understand the mechanisms of mechancial trauma to the nervous system and its responses to grades of such trauma.
2. To design rational methods for protection, prevention, diagnosis, prognosis and treatment of trauma to the brain and spinal cord.

Methods:

1. Utilizing programmable head accelerating devices, sub-human primates are subjected to controlled head accelerations, to obtain reproducible injury at three levels; subconcussive, concussive with no sequelae and concussive with sequelae.
2. Utilizing O_{15}, a study of cerebral blood flow and metabolism at varying levels of brain injury in our primate model is being developed. This study will compare the flow and metabolic changes with alterations in neurophysiologic functions as measured by somatosensory, visual and auditory evoked potentials. Correlation of these data with behavioral and pathologic data will enable a clear analysis of the primary and secondary effects of neural trauma.

3. Utilizing microneurosurgical cathetersation of pial vessels and implanted extradural balloons, the effect of controlled brain distortions on the cerebral circulation and intracranial hydrodynamics in the rhesus monkey has been determined.

4. The evoked potential study is also part of a neurophysiologic assay for primary injury severity and non-invasive diagnosis of secondary brain lesions, which is being applied both in our animal models and in head injured patients.

5. Utilizing simple neural preparations (initially frog sciatic nerve) to determine the effect of mechanical loading at various frequencies on neural functions and structure at the microscopic level (microtrauma project).

6. Utilizing phantoms and biopsy specimens, the linear attenuation coefficients of various chemical solutions and tissues is being determined via the technique of C.A.T. The use of subtraction techniques and radio-dense agents given intravenously will enable measurement of intracranial volumetric changes as well as alterations in blood-brain barrier after neural trauma.

Major Findings:

1. A great part of the past year has been devoted to re-organization and re-location of our Neural Trauma Laboratory at its new site within the A.F.R.R.I. With the new HAD-III installation we have been able to confirm in the larger brain (≈100 gm.) of the rhesus monkey our findings on the mechanism of focal and diffuse effects in head injury previously demonstrated in the squirrel monkey (brain weight ≈ 26 gm.). We have also completed instrumentation lay-out and development modifications so that the planned correlation of cerebral blood flow and 0_2 metabolism studies (using 0_{15}) with cerebral evoked potential data (visual, somatosensory and auditory) is now feasible in three grades of neural trauma viz, sub-concussive head injury, concussive injury with no neurologic sequelae, and concussive injury with significant neurologic sequelae. (Concussive injury is defined as the onset of paralytic coma in the animal). Our unique facility (the only one of its kind in the world) now enables us to initiate a definitive study of the physiopathology of head injury in the sub-human primate.

2. We have observed that the ability of the central nervous system to process input stimuli (visual and somatosensory) at varying rates is directly related to its degree of integrity. Thus in the immediate post-concussive state, the cerebral evoked potentials can follow input stimuli only at lower frequencies, higher frequencies causing significant distortions of the wave form. Recovery of the subject is accompanied by return of the ability to follow higher frequency inputs. This finding has been confirmed and is now being extended in our animal models as well as in patients with varying grades of head injury.

3. Our observations on the relation of cerebral compliance ($\frac{\Delta V}{\Delta P}$) to cerebral circulation suggest that brain death produced by space occupying lesions is caused by failure of capillary perfusion which is independant of the intracranial pressure (ICP). These findings have clarified a number of clinico-pathologic problems, e.g. why the absolute level of ICP is not a useful prognosticator for eventual outcome after brain injury, and why brain death occurs by the same mechanism at high and at low levels of ICP. Our data

also suggest that the brain's haemodynamic behaviour is analogous to that of an erectile organ with arterial pressure defining the compliance of the system. This introduces the concept of an "optimal compliance factor", a quantitative index of brain viability under varying disease states.

Significance to Bio-medical Research and the Program of the Institute:

1. Our work is continuing to provide the standard criteria nationally and internationally for experimental studies in neural trauma research.
2. Correlation of neurophysiologic (evoked potential) physiologic (cerebral blood flow and metabolism) and pathologic data in our experimental models will enable us to seperate the primary and secondary effects of neural trauma.

Proposed Course:

1. Develop the techniques of evoked potentials and C.A.T. as clinically practical serial, non-invasive methods for diagnosis and prognosis of neural trauma severity. C.A.T. will be used to measure volumetric and blood brain barrier changes in the brain, both in the animal model and in patients after head injury.
2. Develop the method of cerebral compliance measurement as a prognostic index for brain survival in neural trauma.
3. Test new therapies aimed at blocking secondary responses to trauma and accelerating neural re-integration after injury.

Keyword Descriptors: Trauma, concussion, cerebral blood flow, brain edema, pressure-volume curve, evoked potentials, computerized tomography, cerebral compliance, microtrauma, membrane, reintegration.

Honors and Awards: 1. Nominated U.S. Representative on Neurotraumatology and Glossary Committees of the World Federation of Neurosurgeons.

Publications:

Ommaya, A.K. and Gennarelli, T.A.: Cerebral Concussion Correlation of experimental and clinical observations. Brain 97: 633-654, 1974.

Ommaya, A.K. and Gennarelli, T.A.: Experimental head injury. In: Handbook of Neurology Vol. 24. (In press)

Ommaya, A.K., Murray, G., Ambrose, J., Richardson, A. and Hounsfield, G.: Computerized axial tomography: Estimation of spatial and density resolution capability. Br. J. Radiol. (In press)

Post, R.M., Allen, F.H. and Ommaya, A.K.: Cerebrospinal fluid flow and idodide ^{131}I transport in the spinal subarachnoid space. Life Science 14: 1885-1894, 1974.

Nakatani, S. and Ommaya, A.K.: Intracranial volume-pressure relationships and pial vascular pressure gradients in the rhesus monkey. In: Intracranial Pressure II. N. Lundberg and U. Ponten (Eds.) Springer-Verlag, 1975.

Advani, S.H., Ommaya, A.K. and Yang, W.J.: Head injury mechanisms: characterizations and clinical evaluation. In: Biomechanics. Applications to Physiologic Monitoring Medical Diagnosis and Rehabilitation. (Ed) D. Ghista. Publ. Saunders, 1975

Peters, N.D. and Ommaya, A.K.: Adjustable microvascular clamp for cerebrovascular surgery. J. Neurosurgery 41: 644-645, Nov. 1974.

Ommaya, A.K.: A physiologic basis for prognostic techniques in neural trauma. In: Head Injury. (Ed) R. McLaurin) Publ. Saunders, (In press)

Project No. Z01 NS 01025-13 SN
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Tumors of the Nervous System

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, M.D., F.R.C.S., F.A.C.S.

Other Investigators: W. Terry, M.D., Amiram Daniel, Ph.D., Max Cohen, M.D., Ph.D., Leslie Cahan, M.D., D. P. Houchens, M.D. and A. Ketcham, M.D.

Cooperating Units: Immunology Branch and Surgery Branch, Division of Cancer Biology and Diagnosis, National Cancer Institute.

Man Years:

Total:	3.0
Professional:	2.5
Other:	0.5

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 evaluate the:
   a) immunological identification of tumor specific antigens.
   b) chemical make-up of tumor-associated antigens, initially assumed to be in glycoproteins isolated from tumor cell membranes.
   c) antigenicity of tumor associated glycoproteins.

Methods Employed:

1. Microneurosurgical 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 (conventional or fast-neutron therapy at the NRL Cyclotron).

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 intraturmoral delayed hypersensitivity reactions.

4. Patients were randomized into a prospective controlled study to evaluate combined chemotherapy alone versus similar chemotherapy combined with immunotherapy.

5. The program of combined chemotherapy utilizes oral CCNU 130 mgm/sq. meter body surface and intratumoral 8-Azaguanine 100 mgm. by infusion; the oral drug being given for six-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 six weeks and then once a month for one year and once a month indefinitely after that.

6. The immunotherapy consisted of immunization with BCG followed by intratumoral PPD to elicit a delayed hypersensitivity reaction in the residual glioma. In addition the patient's tumor cells were given subcutaneously after pre-treatment with mitomycin and neuraminidase. In patients receiving both chemotherapy and immunotherapy the two modes of therapy were given at alternate intervals.

7. Cytotoxicity (^{51}Cr) assays are used to compare the ability of normal lymphocytes and brain tumor patients' lymphocytes to kill brain tumor cells.

8. 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 also to check the effect of splenectomy on tumor growth with and without therapy.

Major Findings:

1. The murine glioma model has clearly shown that pre-immunization of the mice with glioma cell membrane brings about complete failure of tumor growth in these animals. Glycoproteins extracted from these glioma cell membranes are also effective but to a lesser degree. The therapeutic value of chemotherapy and radiotherapy has also been confirmed in this model.

2. Splenectomy in the mouse confers some protective effect on subsequent tumor inoculation reminiscent of the pre-immunization effect in this model system but splenectomy after tumor inoculation is not effective in causing more tumor rejection either with or without varying combination of immunotherapy and chemotherapy. Both adoptive and active immunotherapy proved to be equally ineffective in this system, i.e. these modes of therapy did not extend survival beyond that achieved by chemotherapy and/or radiotherapy as previously reported.

3. The pilot study in immunotherapy for patients with glioblastoma has been terminated because of two reasons. First, the initial five patients receiving this modality succumbed to their tumor at times significantly shorter than the mean survival time achieved by combined chemotherapy plus radiotherapy. Secondly, the results of the various trials of immunotherapy in the murine model failed to suggest useful alternatives for clinical testing.

4. A review of all our clinical and experimental data enables us to make the following general recommendation for glioma management:

a) Immunotherapy for malignant gliomas cannot succeed unless the critical antigenic mass of the tumor is reduced significantly, e.g. to less than 1 cm^3 of tissue. Because of the practical problems of referral of such patients to the Clinical Center we have succeeded in doing this in only one patient to date who is alive and well, 6 years after tissue diagnosis. The need for more efficient reductive therapy (surgical, radiotherapeutic and chemotherapeutic) is thus clear and should be pursued before immunotherapy can be considered.

b) General factors enhancing the patients immunoreactivity are probably as important as specific immunoreactivity to the tumor antigens. Both are facilitated by methods which produce localization of the immune response to the tumor site. Failure to localize the immune response is probably the main reason why the immune system fails to prevent tumor growth, i.e. we are proposing the hypothesis that the immune system is competent at killing diffusely spread but low levels of "foreign" cells but incompetent to control such cells if they aggregate at any site in numbers exceeding about 10^9 cells.

5. Comparitive gel electrophoresis of human normal and malignant glioma brain tissue extracts showed large variation in glycoprotein patterns. We could not however find any immunologic identity of these antigens with the colon carcinomas "carcinoembryonic antigen."

Significance of Bio-medical Research and the Program of the Institute:

1. We have established that immunotherapy of gliomas will not add significantly to glioma management at the present stage of our knowledge.

2. Our data suggest that optimal results will follow more efficient reductive therapy,enhancement of general immunologic factors and therapy designed to improve localization of the immune response to foriegn antigens.

Proposed Course:

1. The current glioma immunotherapy protocol in patients with malignant gliomas is being phased out. Further studies on brain tumors will be focused on vascular factors in their growth and control, microsurgical techniques for more radical surgical removal and studies aimed at producing a more localized immune response at tumor margins.

2. The nature of the glycoprotein of the cell surface of the murine model is to be investigated by gel electrophoresis. Any differences among the glycoproteins of normal mice brain, brain glial tumor, subcutaneous glial tumor, and tissue culture grown glioma cells will be exploited in the murine model for an immunotherapy attack on the glioma.

Keyword Descriptors: Gliomas, chemotherapy, immunotherapy, microneurosurgery, Murine model, reductive therapy, antigens, glycoproteins, cytotoxicity, intratumoral cyst.

Honors and Awards: None

Publications:

Ommaya, A.K.: Immunotherapy of gliomas. In: Recent Advances in Brain Tumor Research. (Ed.) R. Green. Publ. Raven Press, 1975

Cohen, M.H., Chretien, P.B., Felix, E.L., Loyd B.C., Ketcham, A.S., Albright, L.A. and Ommaya, A.K.: Augmentation of lymphocyte reactivity in guinea pigs, mice, monkeys and humans sensitised to BCG, dinitrochlorobenzene or nitrogen mustard. Nature 249: 656-658, June 14, 1974.

Madigan, J., Albright, L. and Houchens, D.P.: Therapy studies in intracerebral murine glioma model. J. Neurosurg. (In press)

Ketcham, A.S., Chretien, P.B., Schour, L., Herdt, J.R, Ommaya, A.K. and Van Buren, J.M.: Surgical treatment of patients with advanced cancer of the paranasal sinuses. In: Neoplasia of Head and Neck. Year Book Medical Publishers, N.Y. 1974.

Project No. Z01 NS 01047-13 SN
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Isotope-Ventriculography and Isotope-Cisternography

Previous Serial Number: NDS(I)-63 SN/NR 1047(c)

Principal Investigators: Giovanni Di Chiro, M.D.
Mary K. Hammock, M.D.

Other Investigators: Gerald S. Johnston, M.D.
A. Eric Jones, M.D.
Michael V. Green
Thomas H. Milhorat, M.D.
Archie W. Bleyer, M.D.

Cooperating Units: Department of Nuclear Medicine, Diagnostic Radioisotope Section, Clinical Center, NIH

Department of Neurosurgery, Children's Hospital of D.C.

Pediatric Oncology Branch, NCI, NIH

Man Years:

Total:	.5
Professional:	.5
Others:	.0

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-ventriculography) and of the subarachnoid intracranial spaces (isotope-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 radioisotope 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 region 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 or 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 cerebral spinal 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 radioisotope ventriculography was carried out, were also followed up with spine scanning for the purpose of gaining experience with the spinal CSF descent.

Major Findings: During the current fiscal year we have:

1) Gained additional experience with cisternography in cases of porencephaly.

2) Added to the number of cases of supratentorial and hemispheric gliomas studied by radioisotope cisternography. The characteristic pattern of lack of ascent of the radiopharmaceutical on the tumoral side has been confirmed.

3) Injected radiopharmaceuticals within the lateral ventricles of Rhesus monkeys with the intent of studying the spinal CSF flow. A pattern of early spinal subarachnoid descent has been noted. This is followed first by equilibration, and later by decrease of spinal radioactivity as well as concomitant augmentation of cerebral convexity subarachnoid activity. When the radiopharmaceutical is introduced via the cisterna magna, the observed downward spinal pattern is even more marked, whereas an injection into the anterior basal cisterns is followed by a prevalent ascending direction of the tagged albumin toward the convexity of the brain.

4) Injected radiopharmaceuticals within the lateral ventricles of human patients (newborns, children and adults) and followed the spinal descent of the radionuclide.

5) Gained additional experience with radionuclide ventriculography in

patients with intracerebral neoplasm, meningeal infection and low pressure hydrocephalus. Important applications were assessment of CSF shunts, determining distribution of intrathecally administered drugs, evaluation functional status of hydrocephalus, and assessing the progression of neoplastic invasion of the ventricular system.

Significance of 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 follow-up 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 the CSF will be gathered by the techniques of isotope-cisternography and isotope ventriculography.

Keyword Descriptors: Cerebrospinal fluid radionuclide scanning, radioisotope cisternography, radioisotope ventriculography, circulation of cerebrospinal fluid, hydrocephalus, pathways of resorption of cerebrospinal fluid, human patients studies and animal experiments, spinal pathways of cerebrospinal fluid.

Honors and Awards: None

Publications:

Di Chiro, G.: The 'Third Circulation'. In Wagner, H.N. (Ed.): Nuclear Medicine. HP Publishing Co., Inc., New York, 1975, pp. 103-111.

Hammock, M.K., Milhorat, T.H. and Davis, D.A.: Isotope cisternography and ventriculography in diagnosis of hydrocephalus. Studies in Hydrocephalus and Spina Bifida. Supp. 32: 58-71, 1975.

Project No. Z01 NS 01195-11 SN
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Radiographic and Radioisotopic Angiography of the Spinal Cord

Previous Serial Number: NDS(I)-65 SN/NR 1195(c)

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Louis Wener, M.D.
Jean R. L. Herdt, M.D.
Gerald S. Johnston, M.D.
A. Eric Jones, M.D.
Mark M. Mishkin, M.D.
Eugene L. Timins, M.D.

Cooperating Units: Department of Radiology, Cafritz Memorial Hospital, Washington, D.C.

Diagnostic Radiology Department, Clinical Center, NIH

Department of Nuclear Medicine, Clinical Center, NIH

Department of Radiology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania

Medical Examiner's Office, City of Philadelphia, Department of Public Health, Philadelphia, Pennsylvania

Department of Neurosurgery, George Washington University Medical School, Washington, D.C.

Man Years:

Total:	.3
Professional:	.3
Others:	.0

Project Description:

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

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

The usefulness of selective arteriography in cases of spinal cord arteriovenous malformations is now well established. We are continuing to use this technique to: 1) Learn more about the pathophysiology of the spinal cord arteriovenous malformations so that a better treatment of these important and frequent lesions may be developed. 2) Evaluate how useful spinal cord angiography is in cases of spinal cord tumors. 3) Establish whether or not this technique can be of diagnostic value in the study of obstructive spinal cord vascular disease. 4) Assess the usefulness of this technique in intervertebral disc pathology. 5) Evaluate the diagnostic possibilities of this procedure in posttraumatic 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 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 or central arteries and other perforating or penetrating branches). This goal is accomplished by postmortem 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 99mTc 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 postmortem 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: During the current fiscal year we have been able to further develop the technique and to augment our experience with selective arteriography of the spinal cord. In particular:

1) Additional experience has been gathered with arteriovenous malformations and hemangioblastomas of the spinal cord.

2) We have reviewed our entire material of gliomas of the spinal cord. Myxopapillary ependymomas of the conus and filum terminale present characteristic--pathognomonic?--angiographic findings: enlarged spinal arteries, striking visualization of the anastomotic loop(s), markedly slow circulation.

3) We have increased our experience with obstructive vascular disease of the spinal cord.

4) Additional cases of arteriographic studies of the spinal cord in patients with posttraumatic para- and tetra-plegia have been carried out. Direct radiographic magnification has been particularly useful in these cases. The most impressive finding in these cases has been the discrepancy between the gross bony-ligamentous damage and the relative sparing of the vascular system. It is remarkable that in cases of total posttraumatic tetra- or para-plegia and massive skeletal-ligamentous injuries, the continuity of the vascular pathways (at least the large vascular channels) may be intact.

Three "firsts" have been attained in our angiographic studies of spinal cord injuries:

I) We have demonstrated, by high magnification, the sulco-commissural arteries in one patient.

II) Extravasation of contrast medium into the epidural space has been shown in one case, indicating "ongoing" bleeding.

III) One of the tetraplegic patients whom we had studied by angiography, died several weeks later. We succeeded in obtaining a microangiographic cord study. In this instance *in vivo* angiography and postmortem microangiography of the cord may be compared.

5) We have studied many patients with spinal cord pathology due to intervertebral disc disease. Particularly rewarding has been the information obtained in cases of herniation of the thoracic discs (six patients). Direct radiographic magnification has been especially useful in these cases. By spinal cord arteriography, we can reach a definite diagnosis of this condition together with an evaluation of the degree of impingement on the cord and its vessels by the protruding disc. This diagnosis is indispensable for a correct surgical therapy of this pathological condition: The exposure of the herniated thoracic disc should not be achieved through posterior laminectomy, but rather through a lateral retropleural or lateral transpleural approach. Our experience with angiography in cervical spondylotic myelopathy is now large (over 20

cases). We are evaluating this material.

6) We have continued our exploration of the angiographic findings in cases of suspected postradiation meylitis of the spinal cord.

7) A large number of patients have been studied with radioisotope angiography of the spinal cord. Striking demonstration of such lesions as arteriovenous malformations and hemangioblastomas are obtained by this simple, innocuous, and informative method. No diagnostic technique can match radioisotope angiography of the spinal cord in the screening and follow-up of patients with progressive myelopathy and suspected of harboring arteriovenous malformations or vascular tumors of the spinal cord.

8) The micrangiographic evaluation of the aged human cord and a comparative appraisal of the intrinsic vasculature in the various segments (cervical, high- and mid-thoracic, and thoracolumbar) of the cord is underway.

9) In several patients who, in other hospital centers, developed paraplegia immediately after abdominal aortography, flushing out of the CSF and substitution with normal saline solution has been carried out, on an emergency basis, under our suggestion and guidance. This lavage has resulted in every instance in a rapid amelioration of the paraplegia. The improvement has been concomitant with a decrease of the CSF iodine content. For comparison purposes we have been gathering CSF samples during the various stages of different angiographic procedures, particularly selective arteriography of the spinal cord. In patients without neurological complications the postangiographic raise of the iodine content in the CSF is minimal.

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: We intend to concentrate our efforts on the angiographic evaluation of posttraumatic tetra- and para-plegia. Considering that by selective techniques we may bring the injectant directly into the damaged area, it is conceivable that we could introduce in this district drugs antagonists to the pressor amines. (These amines are apparently released and contribute to the spreading of damage in the traumatized cord (Osterholm)). Thus, a diagnostic technique could be extended to be also a therapeutic method.

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 follow-up 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.

Postmortem 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.

Keyword Descriptors: Spinal cord vascularization, radiographic angiography spinal cord, radionuclide angiography spinal cord, arteriovenous malformations spinal cord, tumors spinal cord, spinal cord injury, postradiation myelitis, spinal vascularization in the aged, postangiographic myelopathy.

Honors and Awards: None

Publications:

Di Chiro, G., Wener, L.: Angiography of ependymomas of the spinal cord and filum terminale. Amer. J. Roentgenol. 122: 628-633, 1974.

Di Chiro, G., Wener, L.: Radiographic and radioisotopic angiography of AVM and hemangioblastomas of spinal cord: Proceedings of Fifth International Congress of Neurosurgery - Tokyo. Excerpta Medica 158-163, 1974.

Wener, L., Di Chiro, G., Gargour, G.W.: Angiography of cervical cord injuries. Radiology 112: 597-604, 1974.

Di Chiro, G.: Unintentional Spinal Cord Arteriography: A warning. Radiology 112: 231-233, 1974

Serial No. Z01 NS 01245-10 SN
1. Surgical Neurology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: EEG Learning Correlates Using Scalp and Intracranial Depth Electrodes

Previous Serial Number: Same

Principal Investigator: Paul Fedio, Ph.D., and William Sheriff, M.A.

Other Investigators: J. Van Buren, M.D., Ph.D.; A. Ommaya, F.R.C.S.; Monte Buchsbaum, M.D.

Cooperating Units: Section on Technical Development, NIMH; Adult Psychiatry Branch, NIMH

Man Years:

Total:	0.4
Professional:	0.3
Other:	0.1

Project Description:

Objectives: To identify cortical and subcortical mechanisms which mediate intellectual and emotional functions in man. Specifically, to understand the role of the temporal lobe and the thalamus in perception, learning, and memory (storage and retrieval of past and present information). To evaluate the significance of temporal lobe or limbic dysfunction in psychiatric disorders.

Methods Employed: Several perceptual and memory tests were used, the stimuli being patterned to yield either meaningful or nonsense information. Electroencephalographic (EEG) activity was recorded from scalp electrodes positioned over the left and right hemispheres. For certain tests, the stimuli were tachistoscopically projected to either visual field in order to facilitate rapid and selective processing by the left or right brain. Included for study were patients who had undergone unilateral temporal lobe resections for the relief of epilepsy and patients with psychiatric disorders in the absence of obvious neurologic problems. In addition, subcortical recordings were obtained via chronic electrodes in the thalamus for patients with motion disorders or in the temporal lobe for epileptic patients.

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 psychiatric patients is in progress. Neutral stimuli produced a habituation effect which was reflected in EEG activity. When the original stimuli were associated with meaning and guided the subject in a problem-solving task, correct and incorrect choices by the subject produced dissimilar EEG patterns predominantly in the left hemisphere. Evoked potentials from depth electrodes in the temporal lobe and the pulvinar nucleus of the thalamus reflected a comparable asymmetry.

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 temporal surgery and to the specific character of the material. The technique employed in this project affords a more precise method for outlining the network of cortical and subcortical structures in the human brain which support learning and memory. The research also provides direct comparisons of patients with neurologic or psychiatric disorders for possible brain dysfunctioning in schizophrenia or manic-depressive psychosis.

Proposed Course of the Project: To develop additional behavioral tasks and computer programs for EEG analysis with wider application to patients with different neurologic or psychiatric disorders.

Keyword Descriptors: Cerebral dominance, evoked potentials, scalp-depth electrodes, perception, memory, thalamus, temporal lobe epilepsy, parkinsonism, schizophrenia.

Honors and Awards: None

Publications: None

Project No. Z01 NS 01413-09 SN
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Radioactive Scanning Tomography of the Brain

Previous Serial Number: NDS(I)-67 SN/NR 1413(c)

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Floro Miraldi, M.D., Ph.D.
Eugene T. Yon, Ph.D.

Cooperating Units: Department of Biomedical Engineering, and University Hospital, Case Western Reserve University, Cleveland, Ohio

Man Years:

Total:	.0
Professional:	.0
Others:	.0

Project Description:

Objectives: Radioisotopic brain scanning is fast becoming one of the essential diagnostic methods to explore the brain. Its reliability in detection and localization of intracranial space occupying lesions is high (about 80%) but still somewhat lower than that of other neuroradiologic procedures (cerebral angiography, pneumoencephalography). To improve the accuracy of brain scanning, we may essentially follow two research avenues: 1) better tracers; 2) improvement of the equipment. The improvement of the equipment is aimed at increasing the resolution without a substantial loss of sensitivity. This double goal will undoubtedly be reached if an effective and efficient method of layer body section (tomography) associated with scanning can be developed.

Methods Employed: A radioactive brain scan (obtained with a rectilinear scanner) or a scintiphoto (obtained with a fixed device such as a gamma scintillation camera) represents a complex record of signals (photons) originating from the endocranial, cranial and facial tissues. All these signals superimpose in a pattern in which it is often difficult to discriminate between the various anatomical elements. An improved analysis of the scintigraphic record of the head would be attained by tomography. We have been theoretically analyzing the various methods tried by other investigators to solve the problem of scanning tomography. These methods fall into four

general categories: 1) focusing collimator systems; 2) systems using collimators inclined to the plane of interest; 3) scanning with collimators in the plane of interest; 4) time-of-flight positron systems.

We have performed a mathematic appraisal of the three fundamental parameters: Sensitivity, resolution, and efficiency.

We have proposed a new approach and built a prototype device: The strong focusing multi-layered Tomoscanner.

Major Findings: The prototype Tomoscanner is in Cleveland, where significant modifications have been recently carried out. In particular, the angulation of the detecting probe has been changed to 30 deg, which seems to represent a satisfactory compromise.

Preliminary experience with patients using the improved Tomoscanner has proven promising. About 30 cases suspected of harboring intracranial space occupying lesions and a few patients with liver pathology have been studied so far. The blurring of the activity outside the plane of interest is clearly accomplished. By multiple sequential cuts of the head a layer by layer analysis is possible.

Significance to Bio-Medical Research and the Program of the Institute: If the resolution of brain scanning can be significantly improved by tomography, cerebral scintiphotography would acquire further diagnostic importance for the detection of intracranial tumors, bleedings, and infarctions following strokes. Some of these lesions--especially the ones of the infiltrating type--which today escape detection by other diagnostic neuroradiologic methods, would then probably be "picked up." An increase, in absolute terms, of the detecting accuracy of the neuroradiologic methods, taken as a "whole" would then occur.

Proposed Course of Project: Project terminated.

Keyword Descriptors: Radionuclide scanning tomography, body layer radionuclide analysis, design and construction of original device, testing in phantoms, testing in human patients, improvement of diagnostic possibilities of radionuclide scans.

Honors and Awards: None

Publications: None

Serial No. Z01 NS 01424-09 SN
1. Surgical Neurology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Response Modulation by the Limbic System in Man: Neuropsychological and Physiological Changes with Amygdaloid and Cingulate Lesions

Previous Serial Number: Same

Principal Investigators: Paul Fedio, Ph.D. and Ayub K. Ommaya, F.R.C.S.

Other Investigators: J. Van Buren, M.D., Ph.D.; T. Zahn, Ph.D., and W. Sheriff, M.A.

Cooperating Units: Laboratory of Psychology, NIMH and Section on Technical Development, NIMH

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To identify structural-functional interplay of limbic brain mechanisms which regulate emotional behavior in man.

Methods Employed: Patients undergoing bilateral cingulomotomy to alleviate pain are examined by psychophysiologic procedures before and after surgery. A conditioned avoidance technique is used to obtain psychological and physiological indices of the patients' tolerance to pain. These measures include pain thresholds, response latency to avoid pain, respiration rate, galvanic skin response (GSR) and EEG.

Major Findings: No patients were added to this research project during the past year. Computer programs are currently being developed to evaluate the effects of surgery in changing the patients' tolerance and psychophysiological responsivity to pain.

Significance to Biomedical Research and the Program of the Institute: This project provides reliable behavioral and autonomic measures which may be used to evaluate the efficacy of cingulumotomy or related neurosurgical proce-

dures for pain control. The observations contribute to a better understanding of the neuropsychologic mechanisms involved in the emotional appreciation of pain and stress.

Proposed Course of the Project: To continue the study and to extend the protocol to include patients with lesions in other areas of the limbic or emotional brain.

Keyword Descriptors: Limbic system, cingulum-amygdala, pain relief, brain lesions, brain stimulation, conditioned avoidance, skin resistance (GSR), respiration rate, electroencephalograph (EEG).

Honors and Awards: None

Publications: None

Project No. Z01 NS 01654-08 SN
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Experimental Spinal Cord Angiography

Previous Serial Number: NDS(I)-68 SN/NR 1654(c)

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Mary K. Hammock, M.D.
Jean R. L. Herdt, M.D.
Jack Fein, M.D.
Giulio J. D'Angio, M.D.
Kenneth Earle, M.D.
Eugene L. Timins, M.D.

Cooperating Units: Diagnostic Radiology Department, Clinical Center, NIH

Armed Forces Radiobiology Research Institute, Bethesda, Maryland

Department of Radiation Therapy, Memorial Sloan-Kettering Cancer Center, New York, New York

Armed Forces Institute of Pathology, Washington, D.C.

Department of Neurosurgery, George Washington University Medical School, Washington, D. C.

Man Years:

Total:	.2
Professional:	.2
Others:	.0

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 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 dealt 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 mondeys. 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: During the past fiscal year three irradiated monkeys have developed postradiation myelitis. This condition occurred six to eight months after the end of irradiation. We are evaluating the angiographic, microangiographic and histological data obtained. We have lost a large number of animals due to nonneurologic (most frequently hemorrhagic enteritis) postradiation damage.

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 the 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.

Keyword Descriptors: Spinal cord vessels, radiation damage to spinal cord, paraplegia after radiation, experiments in monkeys, angiography of spinal cord, microangiography of spinal cord, pathology of spinal cord radiation damage

Honors and Awards: None

Publications: None

Serial No. Z01 NS 01658-08 SN
1. Surgical Neurology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Hemispheric Development and Specialization of Intellectual Functions

Previous Serial Number: Same

Principal Investigators: Paul Fedio, Ph.D. and John Van Buren, M.D., Ph.D.

Other Investigators: D. Bear, M.D.; M. Mishkin, Ph.D.; N. Butters, Ph.D.

Cooperating Units: Laboratory of Psychology, NIMH; Veterans Administration Hospital, Boston, Massachusetts

Man Years:

Total:	0.8
Professional:	1.6
Other:	0.3

Project Description:

Objectives:

1. Outline brain mechanisms which support speech and language functions, and code information to be held for immediate use (short-term) or for later, delayed recall (long-term).

2. Investigate whether the human brain registers visual and auditory information in different cortical areas, and whether the verbal or nonverbal character of the information is vital to this neural process.

3. Examine hypothesis that heightened sensory-limbic associations are the bases for psychosocial problems in temporal lobe epilepsy. Also, study relationship between personality traits and the epileptic process - laterality of focus, duration, age at onset, frequency of seizures, etc.

4. Compare the effects of brain lesions during infancy vs. adulthood on the development and recovery of intellectual abilities.

Methods Employed:

1. The laterality and general outline of cortical zones instrumental in perception and immediate memory of verbal and nonverbal material were mapped by stimulation of the cortex during neurosurgical treatment of epileptic patients. The behavioral tests utilized verbal or nonverbal materials: a series of photographs of common objects and random visual patterns. In each case, the subject was instructed to name the object or to discriminate several patterns: a short-term memory command was also included for each task.

2. The organization of cortical and subcortical systems which enable man to speak and to use language or to remember events over varying periods of time was studied during electrical stimulation with therapeutic electrodes in thalamic nuclei. Behavioral indices during stimulation were monitored on the object-naming task and a random-form discrimination test described earlier.

3. Perceptual and intellectual deficits were studied for neurosurgical patients with focal damage limited to the parietal or temporal lobes. The test protocol included several visual and auditory memory tasks which measure the effects of distraction or interference on learning. In a separate project, imagery, or the "ability to see something in mind's eye," is being studied in patients with injury to the left or right temporal lobe.

Major Findings:

1. 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 indispensible for identifying verbal material. Stimulation of the left, posterior frontal cortex (Broca's area) also interfered with object naming, albeit to a lesser degree than with stimulation of Wernicke's area. Moreover, stimulation of Wernicke's area produced disruption of internal language, the patient being unable to find the name of simple objects. In contrast, stimulation of Broca's area did not prevent the patient from recognizing or remembering objects but from stating the name until after stimulation.

Recently, speech mapping over Broca's area was performed with a blind, epileptic patient undergoing a frontal craniotomy. The patient was unable to read Braille words or to name objects presented tactually until after stimulation. Stimulation of adjacent frontal sites did not block or arrest speech.

These data suggest that Wernicke's zone integrates and codes information from different modalities, which is then transmitted to Broca's area for speech elaboration and production. Stimulation of the anterior cortical areas of the left temporal lobe and a wide area of the right brain failed to disrupt naming skills.

Memory presses the anterior and posterior temporal regions into different support services. The primary or immediate memory system may be intimately linked with the anterior temporal lobe and medial structures (hippocampal complex), while the posterior temporo-parietal cortex may support secondary or long-term memory. Our findings correlate anterograde memory errors with stimulation of the anterior temporal lobe, and retrograde errors with posterior temporal stimulation. With anterior cortical stimulation, information could be retrieved from an immediate memory store, but newly perceived information could not be deposited into the same storage system for subsequent recall. This may be a defect in the consolidation rather than retrieval mechanism in that stimulation prevents the memory trace from being established for immediate recall.

In contrast, with cortical stimulation of the posterior temporal region, recently stored information could not be retrieved even though other information could be simultaneously transferred into the same storage system. This indicates that, in man, memory for recent and remote information may be served by a common retrieval mechanism. Therefore, patients who suffer stroke or other forms of insult which invade the primary language zones are apt to display aphasia and a significant memory disorder.

2. Mechanisms for perception and memory were probed by electrical stimulation via therapeutic electrodes in the lateral thalamus. Stimulation within the left pulvinar nucleus induced transient dysphasia and a retrograde loss in recent memory for verbal memoranda. In contrast, comparable stimulation of the right pulvinar failed to disrupt verbal behavior and instead, interfered with perception and recognition of complex visual patterns. The findings suggest that an asymmetry in the functional organization of linguistic and nonverbal processes appears to exist at the level of the lateral thalamus.

At first glance, the left and right pulvinar nuclei behave like the ipsilateral cortex with regard to identifying, registering and retrieving from immediate memory, verbal and nonverbal material.

However, the behavioral deficits accompanying cortical and subcortical stimulation appear to be different. Specifically, the data suggest that dysphasia and amnesia were inseparable during cortical, but not subcortical, thalamic stimulation. That is, if the patients were unable to name objects during cortical stimulation, they also experienced severe memory impairment. With thalamic stimulation, recent memory was occasionally spared while at the same time, the patient experienced dysphasia. This suggests that the registers or stores vital for coding information are dependent upon cortical mechanisms.

The contribution of the pulvinar, and other posterior thalamic nuclei may be limited to pre-perceptual processing, that is, attenuating and shuttling sensory information to the cortical stations for interpretation and analysis.

These results complement our earlier work with Ommaya involving stimulation of the cingulum, using the same tests and procedures. A parallel dissociation was established wherein verbal memory deficits resulted during left cingulum stimulation, nonverbal recognition impairment was associated with right cingulum stimulation. The error profiles were dissimilar indicating that with pulvinar stimulation, the patients may have experienced a retrograde or retrieval memory loss, stimulation of the cingulum produced an anterograde or storage memory disorder.

A separate study involved stimulation in the more anterior thalamic nuclei and adjacent strio-capsular structures. The behavioral observation, in brief, indicate that these structures may support speech by providing attentional or orientation cues for incoming stimuli (see report by Dr. John Van Buren, Serial No. Z01 NS 00200-21-SN).

3. In collaboration with Dr. Butters, Aphasia Unit, Boston VA Hospital, patients with parietal lesions or Korsakoff's Syndrome (VA Hospital), or with temporal lobe lesions (NINCDS) are being studied on a wide range of memory tests. Preliminary data show that Korsakoff patients (with presumed subcortical or limbic damage) are more susceptible to the effects of distraction during learning than patients with cortical injury regardless of the type of material to be memorized. Experimental manipulation of learning trials (massed vs. spaced practice) also showed the Korsakoff patients to be more vulnerable to the effects of proactive inhibition.

In a study with Dr. Mishkin, Laboratory of Psychology, NIMH, the perceptual deficits that accompany temporal lobe injury were partitioned according to an imagery factor. On the basis of preliminary results, it appears that patients with right temporal damage experience difficulty in forming and in using visual images as mnemonic cues to offset learning disabilities. In contrast, patients with left temporal injury, may be able to form in their "mind's eye" (intact right temporal lobe) a picture of an object which they may be unable to name due to aphasia, and to use this technique to compensate for learning difficulties.

In a separate investigation of linguistic skills, patients with left temporal epileptogenic foci showed impoverished vocabulary skills. This impairment reflects a concrete or stimulus-bound approach in defining words and restricts the semantic range of word usage.

4. A pilot study of psychosocial traits of patients with temporal lobe epilepsy was completed. Preliminary results show different personality profiles for the left and right temporal patients, and the project will be expanded to include control groups and to increase the number of epileptic patients.

Significance to Biomedical Research and the Program of the Institute: The investigators advance understanding of the development and organization of structural-functional relationships in the human central nervous system. This research program advances basic knowledge to better understand 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 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.

Keyword Descriptors: Asymmetry of cerebral functions, brain damage, brain stimulation, cortex, thalamus, limbic system, speech-language, memory, epileptic personality, motion disorders.

Honors and Awards: None

Publications:

Fedio, P. and Van Buren, J. M.: Memory deficits in man with electrical stimulation in the left and right thalamus. Proceedings of the American Association of Neurological Surgeons, Bal Harbor, Florida, 1975.

Rosenthal, L. S. and Fedio, P.: Recognition thresholds in the central and lateral visual fields following temporal lobectomy. Cortex, (in press).

Project No. Z01 NS 01791-07 SN
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Angiography of Cerebral Venous Obstructive Disease

Previous Serial Number: NDS(I)-69 SN/NR 1791(c)

Principal Investigators: Giovanni Di Chiro, M.D.
Mary K. Hammock, M.D.

Other Investigators: Kenneth Earle, M.D.

Cooperating Units: Experimental Surgery and Clinical Medicine Section
Veterinary Resources Branch, Division of Research Services, NIH

Armed Forces Institute of Pathology, Washington, D.C.

Man Years:

Total:	.0
Professional:	.0
Others:	.0

Project Description:

Objectives: "Stroke" is one of the pathological conditions which recently has been a target attacked from many different sides. For instance, in the last ten years, a great amount of research has been dedicated to the angiographic evaluation of the cerebrovascular obstructive disease. The major emphasis, however, has been put on the arteriographic findings. A perusal of the literature clearly shows that only scattered observations have been made regarding the phlebographic changes. The neuropathological studies (Hahn, Loeb, Zülch), on the other hand, have pointed out the fact that the obstructive venous pathology, although not as frequent as its arterial counterpart, represents an important entity.

We intend to explore the angiographic changes occurring in the cerebral vessels after experimental obstruction of the various brain venous channels (veins and dural sinuses) in a primate experimental model. We are particularly interested in establishing, on the basis of these experiments, which venous occlusions can be well tolerated and which are, instead, incompatible with good function. We will try to appraise, again angiographically, what types of compensatory mechanisms take place after the venous ligations. Do new venous draining pathways open, and if so, which and how efficient are

these reserve discharge routes? The angiographic work will be carried out in close connection with an analysis of the clinical status of the operated animals.

The knowledge acquired in our experimental models will be extended to human material.

Methods Employed: Catheter arteriographic studies in the monkey (Macaca mulatta) are performed before and after ligation of one or several of the large endocranial venous channels.

Together with the post surgical angiographic evaluation, an analysis of the clinical condition of the monkeys is carried out. After various periods of time following the venous ligations, the animals are finally sacrificed in order to carry out microangiographic and gross and microscopic neuropathologic studies.

We are planning microangiographic studies of brains (supplied by the AFIP) of patients who have died of possible cerebral thrombophlebitis.

Major Findings: We have acquired a significant degree of experience in the evaluation of the monkey cerebral angiography. We have been impressed by the remarkable similarity of the major, and even medium size vessels (arteries, veins, dural sinuses) in the Rhesus monkey and man. We have good evidence, therefore, that the primate model will be applicable to the goals of our research project.

We have carried out in a group of Rhesus monkeys, ligation of the superior longitudinal sinus in its anterior, middle or posterior third. Cerebral angiographic studies have been performed before and at various time intervals after the ligation. We are now in the process of evaluating our angiographic, surgical, gross and light microscopic anatomo-pathologic findings in the operated monkeys.

Significance to Bio-Medical Research and the Program of the Institute: This project can increase our understanding of the pathophysiology of the cerebrovascular occlusive disease. Possibly, we will be able to recognize the separate specific conditions (mainly due to venous drainage impairment) in the large group of pathologic entities which are classified under the heading of "stroke."

Proposed Course of Project: We intend to continue our experimental investigation in monkeys.

Later on we intend to apply the acquired experimental knowledge to the human clinical experience.

Keyword Descriptors: Stroke, venous stroke, cerebral veins thrombosis, obstruction of cerebral veins, angiography of cerebral veins thrombosis, pathology of cerebral veins thrombosis, experiments in monkeys.

Honors and Awards: None

Publications: None

Project. No. Z01 NS 01866-05 SN
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Cerebral Blood Flow in Normal and Infarcted Brain by Radiographic and Radioisotopic Methods

Previous Serial Number: NDS(I)-71 SN/NR 1866(c)

Principal Investigators: Giovanni Di Chiro, M.D.
Mary K. Hammock, M.D.
Apichati Pongpatirojana, M.D.

Other Investigators: Norman D. Peters, M.D.
Michael Green
Harry Agress, Jr., M.D.
Gerald S. Johnston, M.D.
A. Eric Jones, M.D.
Stephen Bacharach,Ph.D.
Kenneth Earle, M.D.

Cooperating Units: Laboratory of Applied Studies, Division of Computer Research and Technology, NIH

Department of Nuclear Medicine, Clinical Center, NIH

Armed Forces Institute of Pathology, Washington, D.C.

Man Years:

Total:	2.3
Professional:	2.3
Others:	0.0

Project Description:

Objectives: Although an enormous number of studies have been carried out on the cerebral blood flow (CBF), the actual behavior of the small cerebral vessels (arteries, capillaries, and veins) in physiologic and pathologic conditions is not yet clear. In fact, in recent years, new concepts--luxury perfusion, intracerebral steal, blue arteries and red veins, loss of autoregulation--have been introduced. These concepts have not brought about increased clarification, but rather additional confusion. We are convinced that morphological analysis of the vessels still remains a potential source of significant information in the area of normal and pathological CBF. We intend, therefore, to pursue the matter of extracting information regarding

the circulatory conditions of the brain from radiographic observations, substantiated in selected instances by radioisotopic methods and possibly other diagnostic and research techniques.

Methods Employed: Radiographic and radioisotopic observations of the cerebral circulation represent the main tools to be used in this study.

An experimental stroke model in Rhesus monkeys has been developed. The middle cerebral artery is occluded at its origin through the superior orbital fissure after the removal of the eyeball. This technique (Garcia) offers various advantages. Particularly important is the fact that the produced infarction is consistent in its anatomical location (medial-inferior district of the ipsilateral basal ganglia) and its extent. Also, because no craniectomy is needed, subsequent studies with radioisotope scanning are easily interpretable.

After production of the anemic infarction our multi-pronged evaluation includes:

A) Periodic neurologic testing.

B) Periodic conventional static radionuclide brain scanning.

C) Periodic magnification internal carotid angiography (an indwelling catheter is positioned in the internal carotid artery of some of the stroked monkeys).

D) Periodic radioisotopic angiographic studies (Tc pertechnetate) and periodic ^{133}Xe washout studies (through indwelling internal carotid catheter).

All the radionuclide flow data are gathered with an Anger camera and stored on tape. Subsequently, the data on tape are analyzed by a computer-assisted program. Multiple cursors are drawn in the central part and at the periphery of the infarcted area. The purpose of this approach is to evaluate the centripital and centrifugal blood flow in the infarcted area at various stages of development of the infarction. The data should be of assistance in the evaluation of the circulation parameters in the initiating, evolving, complete, and resolving stroke. The centripital and centrifugal flow is evaluated second by second after the intracarotid injection of the radiopharmaceutical.

E) Acute polarographic deep electrodes are used in some of these animals for recording oxygen availability.

F) At various time intervals after the creation of the stroke, the animals are sacrificed by a "sudden death" technique and immediately afterwards perfused with a special micròpaque solution for postmortem microangiographic studies.

G) The "sudden death" is accomplished under various attempts at modifying

the pathophysiology of the CSF: hyperventilation, hypercarbia, anoxia, hyper- and hypo-tension, use of vasodilators.

H) In a few monkeys perfusion with the radioiodinated albumin macro-aggregates for scanning appraisal of the infarcted hemisphere has also been carried out.

I) In selected monkeys the changes of the superficial vasculature are followed up by means of fluorescein angiography of the exposed cortical surface.

J) A day-by-day histological analysis of the anemic infarction is under way. Stroked monkeys are sacrificed for this purpose, one at each sequential day, after the production of the stroke.

K) We plan to study the infarcted monkeys by computerized axial tomography (CAT) as soon as a device for this technique (EMI-Scanner) will be available to us.

L) We are getting ready to start "revascularization" of our infarcted monkeys with an original method of by-pass procedure to the middle cerebral artery distal to the occlusion. The "revascularized" monkeys will be subjected to all the above multi-pronged testing.

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).

Major Findings: We are very pleased with the experimental stroke model which we have developed. The possibilities of investigation with this model are practically unlimited. We have started evaluating the many data available to us from the first group of infarcted monkeys.

1) The stroke model used gives a consistent area of infarction in deep cerebral layers with a minimal amount of brain exposure.

2) Microangiograms prepared in the manner described can fix at a point in time the dynamic changes taking place in the microvasculature.

3) The method allows us to duplicate in deeper structures some of the observations of others on the reaction of surface vessels.

4) We can demonstrate changes in vessel morphology following $PaCO_2$ changes both in normal and infarcted monkeys.

5) Both hypo and hypercapnia decrease the perfusion of zones of ischemia during the acute phase. Hypocapnia accelerates the rate of ischemia to a greater extent than hypercapnia.

6) In completed stroke at the five day interval, hyperventilation hypocapnia may increase perfusion of nonischemic portions of the brain as compared to normocapnic and hypercapnic animals.

7) By polarographic depth electrodes determination a reversal of the normal O2a response in the ischemic brain to either low or high $PaCO_2$ levels has been observed. This would suggest that attempts at manipulation of cerebral blood flow to ischemic or infarcted brain using hypo or hypercapnia would not be consistently successful due to the variable response obtained.

8) A correlative study between sequential radionuclide brain scanning and time-lapse microangiograms has been completed. In the majority of animals brain scintigraphy becomes positive by two weeks to regress toward negative by four to six weeks after the ligation of the middle cerebral artery. The increased radioisotope uptake in the affected area is clearly related to neovascularization around the area of infarct as shown by the microangiograms. Decreased vascularity, peripheral gliosis and central cavity formation are the main factors determining the diminution of the radionuclide penetration in the involved area at later stages.

Significance to Bio-Medical Research and the Program of the Institute: We are hopeful that the computer analysis of our radioisotope angiography data in and around the infarcted area will be informative. In particular the determination of the centripital and centrifugal blood flow in the area of the lesion should enable us to draw some conclusion on the mechanisms of resolution of the infarction and perhaps a better understanding of the important phenomenon of the luxury perfusion.

We are confident that with our stroke model we will be able to contribute both in the basic area of CSF pathophysiology of the infarcted brain, as well as in the practical management of the patients with stroke. Perhaps we will shed some light on such fundamental questions as: a) if and when we should hyperventilate patients with stroke; b) when, if ever, induced hypercarbia may be useful in these patients; c) what is the optimal level at which the blood pressure should be maintained during the various stages of the stroke; d) if, when, and which vasodilators should be used.

We have hopes that our "revascularization" approach may prove to be efficacious, practical, and applicable to human patients.

The arterial spasm production after subarachnoidal injection of blood into the chiasmatic cistern is an excellent model simulating the important problem of human arterial spasm following subarachnoidal hemorrhage. This event generally follows rupture of arterial and arteriovenous aneurysms of of the brain.

Proposed Course of Project: This is a long-range research project. The evaluation of the cerebral blood flow both in experimental models and in clinical material is still in its infancy, but we are convinced that it will

develop into a full discipline.

Keyword Descriptors: Stroke, cerebral infarction, cerebral circulation, cerebral blood flow, cerebral angiography, dynamic radioisotopes studies, fluorescein angiography, micro-angiography stroke, histology stroke, computerized axial tomography, experimental monkeys, brain revascularization.

Honors and Awards: None

Publications: Di Chiro, G., Timins, E.L., Jones, A.E., Johnston, G.S., Hammock, M.K., Swann, S.J.: Radionuclide scanning and micro-angiography of evolving and completed brain infarction in monkeys. Neurology 24: 418-423, 1974.

Project No. Z01 NS 02072-02 SN
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Blood-Brain Barrier and Radiographic Contrast Media

Previous Serial Number: NDS(I) SN/NR 2072(c)

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Norman D. Peters, M.D.
Mary K. Hammock, M.D.
Eugene L. Timins, M.D.
Milton W. Brightman, Ph.D.
Kenneth Earle, M.D.
Jean R. L. Herdt, M.D.
John Fox, M.D.
Torsten Almeń, M.D.

Cooperating Units: Department of Neurosurgery, George Washington University, Medical School, Washington, D. C.

Section on Neurocitology, LNNS, NINCDS, NIH

Armed Forces Institute of Pathology, Washington, D. C.

Diagnostic Radiology Department, Clinical Center, NIH

Department of Diagnostic Radiology, Malmö General Hospital, Malmö, Sweden

Man Years:

Total	.3
Professional:	.3
Others:	.0

Project Description:

Objectives: Neurological complications after intravascular (angiography) and intrathecal or intraventricular (myelography, ventriculography) introduction of hydrosoluble radiographic contrast media (various types of iodinated compounds including the recently developed polimeric, ion-balanced, and non-ionic) still represent a real and serious risk in the neuroradiological procedures. In turn, this inherent danger constitutes a deterrent which prevents the performance of frequently needed procedures.

There is general agreement that the critical phenomenon in the chain of events leading to neurotoxicity by radiographic contrast media is the passage of the media from blood, respectively from CSF, into the neural tissue. This passage is probably determined, among other factors, by the osmotic shrinkage of the cerebro-vascular endothelial cells and opening of the so-called tight junctions.

We have initiated a long range research project to evaluate the disruption of the Blood Brain Barrier (BBB) and Blood-CSF Barrier (BCSFB) by radiographic contrast media either introduced into the blood stream or within the CSF cavities.

Methods Employed: Our attention will be focused on the monkeys spinal cord. This model has many advantages. We have had a long experience with selective contrast media introduction within the cord vessels (arteriography) and in the CSF around the cord (myelography). Arteriograms and myelograms will be performed in monkeys using the various types of contrast media available. The procedures will be carried out to the threshold of cord damage and beyond--to the point of first production of actual cord damage. Evoked spinal cord potentials will be used to determine the point of early cord damage. Appropriate perfusions will enable us to study by electron microscopy (EM) the damage of the tight junctions.

Major Findings: We have established the threshold of production of post-angiographic paraplegia in monkeys using one type of radiographic contrast medium.

Significance to Bio-Medical Research and the Program of the Institute: Increase our understanding of the neurological complications connected with use of radiographic contrast media. Establish the point of reversibility--respectively irreversibility--of these complications.

Proposed Course of Project: To follow our research according to the above described guidelines.

Keyword Descriptors: Postangiographic paraplegia, angiographic damage to spinal cord, mechanism of damage to spinal cord from angiography, electron microscopy studies, experimental monkeys.

Honors and Awards: None

Publications: Di Chiro, G.: Unintentional spinal cord arteriography: A warning. Radiology 112: 231-233, 1974.

Project No. Z01 NS 02073-02 SN
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Computerized Axial Tomography

Previous Serial Number: NDS(I)-74 SN/NR 2073(c)

Principal Investigators: Giovanni Di Chiro, M.D.
Rodney A. Brooks, Ph.D.

Other Investigators: Robert Ledley, D.D.S., M.A.
Dieter Schellinger, M.D.
Stewart Axelbaum, M.D.
Alan M. Cormack, Ph.D.
Andreas M. Koehler
Richard N. St. Onge, Ph.D.

Cooperating Units: Department of Radiology, Georgetown University Medical School, Washington, D.C.

Physics Department, Tufts University, Medford, Mass.

Cyclotron Lab., Harvard University, Cambridge, Mass.

Physics Department, University of New Hampshire, Durham, New Hampshire

Man Years:

Total:	.10
Professional:	.10
Others:	.00

Project Description:

Objectives: Computerized Axial Tomography (CAT) is a revolutionary diagnostic method which is having a stunning impact on the practice of neuroradiology and which soon will affect the entire field of roentgenology and nuclear medicine. The technique, which was introduced in early 1972 in England, has already been described in detail in a large number of reports in the European and American literature. At least six types of devices for transmission reconstructive tomography (EMI-Scanner - about 80 units all over the world -; ACTA-Scanner prototype; DELTA-Scanner prototype; Siretom prototype; Neuroscanner prototype; EMI-Body Scanner prototype) are already operational and being used in patient evaluation. In the area of emission reconstructive

tomography, which actually preceeded the transmission approach, one center (University of Pennsylvania) is in the forefront. A large number of other devices both for transmission and emission reconstructive tomography are being tested in phantoms and animal work, are being built, or are in the planning stage. These new units are either modifications of the first built device (EMI-Scanner) or they incorporate fundamental technical innovations.

We have been involved from the beginning with one of the original units for CAT, the ACTA-Scanner.

In a few weeks one EMI-Scanner to be used for clinical work will be installed in the NIH Clinical Center (Department of Radiology). We will immediately start with patient evaluation from our hospital as well as from other centers in the Washington area, particularly government military hospitals (Army, Navy, Air Force). Late in the summer a second EMI-Scanner unit, this one devoted primarily to experimental work, will be installed in the Department of Nuclear Medicine. Our experimental EMI-Scanner will be used for a number of projects including evaluation of the physical parameters of the machine, determination of absorption coefficients of specimens and tissues (normal and abnormal), as well as followup of absorptometric changes in experimental infarctions and intra and extracerebral bleedings in primates. The second EMI-Scanner will also be used in a limited fashion for clinical studies such as 1)Comparison with radionuclide brain scans and 2) CAT of the head after systemic, intracarotid or intrathecal introduction of various types of contrast media and other tracers and/or markers.

We have completed an analysis of the theory and practice of image reconstruction, with particular emphasis on the various programs (algorithms) used or useful for CAT. With the help of computer simulation we have finished an assessment of the important physical parameter, beam hardening. This factor is essential in the determination of the quality of the image and is responsible for the creation of some of the most disturbing artifacts. Other physical parameters are being examined as well.

We are building a bench model for evaluation of one component of the CAT equipment, the detector. We will attempt to assess the reliability, power of resolution and sensitivity of three fundamental groups of detectors: scintillation types, gas chamber models, and solid state devices.

Finally, we are in the planning stage, and have actually started a pilot study, to evaluate the possibility of a totally novel approach, i.e., the use of protons for reconstructive transmission tomography.

Methods Employed: Our direct working experience with the ACTA-Scanner located at the Georgetown University Medical Center is continuing.

Our clinical experience with the first NIH EMI-Scanner will begin very soon. Our experimental experience with the second NIH EMI-Scanner will begin next fall.

Our theoretical evaluation of the various parameters of transmission and emission reconstructive tomography is underway.

Building of a bench model for evaluation of various types of detectors is in the planning stage. A pilot study has been initiated for studying the feasibility of a protons (or other heavy particles) device for reconstructive transmission tomography.

Major Findings: We have demonstrated that CAT with the ACTA-Scanner is a useful procedure to evaluate syringomyelia as well as other spinal cord diseases -- tumors and malformations. In conjunction with conventional myelography, CAT gives us significant information in the diagnosis and for treatment of spinal cord cavitations including syringomyelia, hydromyelia and tumoral and other cysts. CAT is innocuous, causes no discomfort and is fast and simple. Thus, it is ideal for screening and followup of patients affected by obvious or obscure myelopathies.

We have carried out a preliminary comparison of the ACTA-Scanner versus the EMI-Scanner for head studies. It has been found that the power of resolution is better for the EMI than the ACTA. On the other hand, lack of need of a water bag for the ACTA-Scanner makes this machine preferable in the evaluation of patients with acute head trauma, large heads, claustrophobia, as well as in the study of infants and small children. The water bag, we have found, is a serious drawback for the EMI-Scanner.

Beam hardening seems to be an important parameter which affects the final quality of reconstructive transmission tomography. This has been found in a computer simulation study. Beam hardening is particularly disturbing when heavy bony parts have to be penetrated. The use of a water interface (EMI-Scanner) does help to alleviate the image deterioration due to beam hardening.

A review of various algorithms, useful for reconstructive transmission and emission tomography, has been prepared. The advantages and disadvantages of the various programs (back-projection, analytical reconstruction - Radon's, Fourier's and convolution -, iterative or algebraic reconstruction) have been assessed.

Significance to Bio-Medical Research and the Program of the Institute: Computerized analysis-display of anatomic cross-sections obtained by transmission or emission reconstructive tomography is already revolutionizing neuroradiology. Diagnoses which were previously reached only with the use of many, complex, and not always safe radiographic and radioisotopic procedures, are now made with extreme reliability, simplicity, speed and lack of risk for the patient. A typical example is the CAT diagnosis of intracerebral bleeding which is totally straightforward. Further developments of CAT will certainly lead to profound modifications of our diagnostic abilities and to more efficient health related services. Also, the economics of health delivery will be affected, generally in a favorable fashion, by the elimination of other costly and hospitalization-requiring diagnostic procedures. At this stage, the greatest benefit of CAT

is in the area of evaluation of morphological changes, but dynamic studies should soon make possible the study of functional derangements and metabolic alterations.

Proposed Course of Project: In the Section of Neuroradiology CAT will be the main area of research for years to come. We will proceed with a multi-pronged approach: theory (mathematics, physics, computer systems); testing of components (particularly various types of detectors: scintillation, gas ionization, semiconductors); planning and building new types of CAT devices. We will continue our clinical work with the ACTA-Scanner in the head and in the spinal cord; we will start our direct clinical experience with the EMI-Scanner; we will also proceed with experimental work (phantoms, specimens and selected patients) using this type of device.

Keyword Descriptors: Computerized axial tomography, computer assisted tomography, reconstructive transmission tomography, computed tomography in syringomyelia, computed tomography in spinal cord diseases, comparison of computed tomography devices, beam hardening in computed tomography, algorithms in reconstructive tomography.

Honors and Awards: None

Publications:

Ledley, R.S., Di Chiro, G., Luessenhop, A.J., Twigg, H.L.: Computerized transaxial X-ray tomography of the entire human body. Science 186: 207-212, 1974.

Di Chiro, G.: Of CAT and other beasts. Amer. J. Roentgenol. 122: 659-661, 1974.

Di Chiro, G., Axelbaum, S.P., Schellinger, D., Twigg, H.L., Ledley, R.S.: Computerized axial tomography is syringomyelia. New Engl. J. Med. 292: 13-16, 1975.

Schellinger, D., Di Chiro, G., Axelbaum, S.P., Twigg, H.L., Ledley, R.S.: Early clinical experience with the ACTA-Scanner. Radiology 114: 257-261, 1975.

Di Chiro, G.: A neuroradiologist's analysis of two devices for reconstructive transmission tomography. Proceedings of Workshop on Reconstruction Tomography in Radiology and Nuclear Medicine (In Press).

HOW TO USE
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LAB. OF NEUROPHYSIOLOGY

ANNUAL REPORT

July 1, 1974 through June 30, 1975

Central Nervous System Studies Laboratory

National Institute of Neurological and Communicative

Disorders and Stroke

The Central Nervous System Studies Laboratory is an outgrowth of the Study of Child Growth and Development and Disease Patterns in Primitive Cultures, under the direction of D. Carleton Gajdusek, M.D. It was the 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 this disease is caused by a most unconventional and atypical virus. In addition, the successful transmission of kuru provided the necessary techniques for the subsequent demonstration of a viral etiology for many forms of transmissible virus dementias, particularly the Creutzfeldt-Jakob type of sporadic and familial presenile dementias, and the transmissibility of certain forms of familial Alzheimer's disease.

These discoveries made possible the recognition that Creutzfeldt-Jakob disease may be transmitted for corneal graft, as has occurred accidentally in this country. There is now just cause for concern for possible contamination in all tissue transplantation. It has also been possible to identify the virus of transmissible virus dementia as the cause of death in a neurosurgeon and to raise the possibility of occupational hazard. In the past year the demonstration of the transmissibility of scrapie from American sheep to many species of monkeys, with a disease in the experimental monkey indistinguishable from transmissible virus dementia, has phrased the urgent question of the possible relationship between scrapie of sheep and the spongiform encephalopathies of man.

Initially, the Study of Child Growth and Development and Disease Patterns in Primitive Cultures included direct field and laboratory studies on a wide variety of conventional virus diseases, and study of the ecology and seroepidemiology of arboviruses in remote populations, the evaluation of efficacy, immune-response, and long-term sequellae following use of live attenuated measles virus vaccine in previously unexposed, immunologically virgin populations, and the identification of the antigenic strain of influenza that was responsible for the 1918-1919 world pandemic, by analysis of the antibody pattern in isolated islanders who have remained virgin to flu since the 1918-1919 pandemic.

Concomitantly, the Study of Child Growth and Development and Disease Patterns in Primitive Cultures was engrossed in the investigation of deaf-mutism, mental subnormality and other congenital CNS 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 on isolated Melanesian groups. Ethnic drug abuse (particularly of kava), strange patterns of psychosexual development, 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, amyotrophic lateral sclerosis and Parkinsonism, were also being investigated. Genetic studies in the investigations 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.

With greater emphasis being placed on studies to determine the nature of the viruses causing subacute spongiform encephalopathies (i.e. kuru, Creutzfeldt-Jakob disease and scrapie and transmissible mink encephalopathy), and with intensified efforts to demonstrate infection as the etiology of multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease and a wide variety of other central nervous system diseases, it became obvious to the director of the program that the laboratory had developed into two mutually dependent and closely interrelated projects which diverged at their technical extremes into very different disciplines. However, it is significant to note that the development and maturation of both projects resulted from cross-fertilization of each other since their origin, and that the second has grown from the first. These two projects which constitute the Central Nervous System Studies Laboratory are: Neurobiology of Population Isolates:Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures; and, Chronic Central Nervous System Disease Studies:Study of Slow, Latent and Temperate Virus Infections.

Although the two projects, each composed of several sub-sections, differ markedly in the enquiries 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. They 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.

Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures

A basic understanding of human biology, including in depth data on neurobiology, immunological "original sin", and behavioral patterns and diverse styles and modes of learning have thus far been best obtained in the study of rapidly vaishing primitive societies in the remote areas of the world. Our research is directed towards problems of the neurological development and learning patterns in children from diverse cultural experiments in the human condition, which are found in these isolated primitive populations. Laboratory studies on human biology, genetics, and associated molecular biology, on immunology, virology and biochemistry, and on the biological adaptation to diverse and extreme environmental conditions, have all been directed at solving problems which have been carefully chosen from small isolated bands still living in the primitive situation in which these problems may be more appropriately studied than in the larger civilized societies. Nutritional studies, studies on reproduction and fertility, in neuroendocrine influences on age of sexual maturation and aging, and on studies of the selective advantage and establishment of population equilibrium with genetic polymorphisms are all under way. The unudual and odd alternative methods of employing the central nervous system in its higher cerebral function of patterns of language, cognitive style, computation (number sense and calculation without a number system), and psychosexual culturally-modified behavior and methods of visual, auditory and tactile learning are providing data on alternative forms of possible neurological functioning for man. We would remain unaware of such alternative forms of neurological functioning and be unable to produce or investigate them in the clinic or laboratory, once the natural cultural experiments in primitive human population isolates had been finally amalgamated into the modern civilized cultural veneer which is now imposed on almost all members of the community of man.

Growth and development studies in primitive cultures have yielded evidence for delayed puberty and slow growth rates in certain peoples, particularly for the short-statured populations in the highlands of New Guinea. Preliminary evidence suggests high levels of pituitary growth hormone in such populations from the pituitary glands, obtained at autopsy, and in serum specimens. The growth rate seems to be proportional and the age of puberty, inversely proportional to the mean adult stature.

Congenital mental defect and a wide range of neurological problems associated with the most severe foci of endemic goiter and cretinism in the world, in the highlands of Western New Guinea, as a result of severe iodine deficiency, are under further investigation. These are contrasted with enclaves of familial goiter and cretinism associated with thyroid dysfunction in the coastal populations on small islands. The frequent but irregular association of familial deaf-mutism with goiter and cretinism is under study.

The discovery of new genetic factors, including haptoglobins, hemoglobins and red cell enzyme pleomorphisms, with the elucidation of their biochemical structure and their later use as markers in human population genetic studies, has been a by-product of these investigations. The discovery of immunologically virgin populations without exposure to respiratory-and enteroviruses which are ubiquitous in the civilized world, has permitted fundamental investigations of the immune response in man, investigations possible no where else, on the persistence of the immune response after natural measles infection

and live attenuated measles virus immunization in the absence of circulating virus, yielding data important to the diagnosis and understanding of delayed slow measles encephalitis (subacute sclerosing panencephalitis, or SSPE), and to establishing the serological identity of the agent of the 1918 influenza pandemic to aid in the genetic-historical elucidation of the serial mutation of influenza virus. By virtue of limited travel during their entire lifetime and exposure to their natural ecology, members of primitive groups serve as unequaled sentinel populations for revealing the focal microbial agents that infect man in his environment. Thus, for infections ranging from Chagas disease and toxoplasmosis to arbovirus infections, primitive groups offer unusually fruitful samples for investigation. On toxoplasmosis, filariasis, yaws, and malaria, as well as on arbovirus encephalitides, we have studies in progress.

With the current interest in papovaviruses (SV40, J.C., BK) latent in man as the cause of progressive multifocal leucoencephalopathy, the presence and the pattern of the acquisition of antibodies to these in isolated primitive groups, with no contact either with primates or vaccines, help settle the problem of the origin of these infections in man. In most of the isolated population groups examined, representing over one thousand serum specimens from Micronesia, Melanesia, South American Indians, Australian aborigines and Alaskan Eskimos, isolated Icelandic villages, and diverse groups from the Middle East, we have confirmed a high level of exposure to BK virus in the absence of association with frank disease, with prevalence ranging from 60% to 90%. Furthermore, SV40 antibodies occur almost exclusively in individuals with high levels of antibody to BK virus, suggesting far more cross-reactivity than has been previously supposed. Similar antibody acquisition patterns for cytomegalovirus, Epstein-Barr virus, and herpesviruses types 1 and 2, and other simian (including the foamv viruses) viruses are also being studied in these isolates to add to our knowledge of their biological role. We have, for instance, noted the very early acquisition of cytomegalovirus and Epstein-Barr virus antibodies in these isolated groups, in some cases with positive titers reaching 100% by mid-childhood. Populations virgin to BK, and others to J.C. virus are found.

Our efforts to document the development and neurological patterning in the disappearing primitive cultures has resulted in one of the largest archives of such documentation in the world. The collection and preservation of cinema data from New Guinean, Oceanian, African, Australia aborigine and American Indian groups as they live as hunter-gatherers or primitive hoe and digging stick agriculturists, provides the only such documentation of the life and behavior of man as he lived and evolved for most of his evolutionary history of about three million years. Our many and diverse investigations have evolved about the theme of elucidating the medical problems in the cultures of primitive man, or man living in small isolated communities, by carefully selecting such problems of immunological, virological, biochemical, genetic, reproductive biology, endocrinological, and nutritional interest--all pertinent to the long-term studies of growth, behavior, emotional response to stress, and human biology in these groups.

Potent tools of mathematical population genetics have been used with our massive genetic pleomorphism data on primitive groups, for the analyses of the multilocus genetic effects in determining expression of neurological diseases, such as kuru, and a similar approach is under way for the transmissible virus dementias. Genetic distance techniques, including genetic tree construction and principal components analyses, are used.

During our last (1974 and 1975) long-term field studies in Western New Guinea (Indonesia), we delineated the extent and completed the epidemiological documentation of a focus of extremely high incidence of amyotrophic lateral sclerosis (ALS) among the primitive and very isolated Auyu and Jaqai peoples of the inland southern coastal plain. Here we found in this intense focus of ALS a higher prevalence than that among the Chamorro people on Guam. We found that this situation has persisted over the last twelve years, since the principal investigator originally described it (New Eng. J. Med., 1963). He has also found an associated high incidence of Parkinsonism, Parkinsonism-dementia and myoclonic dementia, all of which have been documented clinically and with cinema. In a population of under four thousand people, over two dozen cases of these syndromes were found, and no other cases among the over-twenty thousand surrounding peoples who were as intensively surveyed.

On 3 recent long expeditions to Papua New Guinea, we have concentrated on the further delineation of kogaisantamba (an essentially tremor syndrome) and of puriripiram (the Western Highlands kuru-like syndrome) which have mistakenly been called "kuru" at times. These we are now describing in detail.

Laboratory studies are continuing, sorting and testing our vast medical, genetic and human biological data collected during the Alpha Helix expedition three years ago, but we anticipate that it will be some time before all these studies are completed. The knowledge we are gaining from such studies will, no doubt, as has been demonstrated thus far, have wider impact on medical science.

Chronic Central Nervous System Disease Studies: Study of Slow, Latent and Temperate Virus Infections

Slow virus infections of the central nervous system have been a postulated possibility in man since we first launched an inquiry into the possible slow virus etiology of a wide variety of chronic, subacute and degenerative central nervous system diseases thirteen years ago. This study was an outgrowth of our attempts to solve the riddle of an epidemic of a fatal subacute degenerative neurological disease, kuru, in New Guinea natives which had attained enormous proportions in a small isolated primitive population. This exotic model was chosen for such intensive study for the obvious implications its solution would have to other central nervous system degenerative diseases which it resembled in basic pathological lesions and even to degenerative processes active in all aging brains.

The choice was wise. The intense localized phenomenon of kuru offered the possibility of a breakthrough. Kuru became the first chronic human disease proved to be a slow virus infection. This set the field for intensive investigation of many more important degenerative diseases that we then realized might have related pathogenesis. Soon thereafter, the presenile dementias of the Creutzfeldt-Jakob (C-J) type were proved to be the equivalent of kuru, but with worldwide non-exotic distribution. Both the sporadic and familial forms of this presenile dementia were shown to be transmissible.

Recognition of slow virus infection pathology in the human brain led next to the demonstration that subacute sclerosing panencephalitis of children and adolescents, and progressive multifocal leucoencephalopathy were also slow virus diseases. Slowly data has accumulated both from the virus laboratory

and epidemiological studies which suggest that multiple sclerosis and Parkinson's disease should be attacked by the same approach, since slow infection with a masked and probably defective virus now appears a likely cause of these diseases.

The study of kuru was carried on simultaneously with a parallel attack on multiple sclerosis and Parkinson's disease. In addition, other degenererative dementias, such as Alzheimer's disease, Pick's disease, and Huntington's chorea, have been similarly studied and such work continues. Amyotrophic lateral sclerosis, Parkinsonism-dementia, chronic encephalitis, focal epilepsy and degenerative reactions to schizophrenia, are among the other diseases under investigation. As we have attempted to define the range of illness caused by the C-J virus, a wide range of clinical syndromes involving dementia in middle and late life have been shown to be such slow virus infections. These include even cases that have been diagnosed clinically as Alzheimer's disease, senile dementia, or stroke (CVA) at some time in their clinical course. Thus, the urgent practical problem is to delineate the whole spectrum of subacute and chronic neurological illness that is caused by this established slow virus infection.

Furthermore, no approach to therapy or prevention to these disorders can even be considered until the nature of the related C-J and kuru viruses are further determined. We have discovered that these newly isolated human viruses form a group with the two viruses causing somewhat similar diseases in farm animals: scrapie in sheep and goats, and mink encephalopathy. These agents are very strange and unconventional viruses lacking classical virus antigenicity and failing to evoke either an immune response or inflammatory reaction in the victim. Attempts to elucidate the basic structure and biology of these agents are under way using scrapie virus in mice as the only practical means we have for approaching the problem. The scrapie studies are paralleled with similar investigations on the viruses causing the human diseases, as funds and facilities permit.

All four of these viruses--those causing the two human and the two animal diseases--have been successfully cultivated in in vitro tissue cultures of brain cells from infected human patients or animals.

The ultimate goal of this work is 1) to define the range of chronic human diseases caused by these atypical slow virus agents, and 2) to elucidate their biology and structure of these unconventional viruses with an aim to both prevention and treatment. The methods that have been successful in producing the surprising and alarming discovery that the chronic degenerative diseases, even of the familial type, may be caused by slow virus infections, obviously needs further application to the major targets of multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, chronic progressive epilepsy, chronic encephalitis and other presenile and senile degenerations of the brain, even schizophrenia. This work is well advanced in our laboratories.

The problem of the long survival or persistence of chronic, latent, masked, recurrent and long incubation infections, and of the carrier state, were all well known phenomena already studied at the dawn of virology. As we pointed out in 1964, "these are neither new ideas nor new problems, nor do they necessarily involve new viruses." The accuracy of this perspective in virology was proven following the successful transmission of kuru with the isolation and identification of defective measles virus as the etiological agent of subacute

sclerosing panencephalitis (SSPE) in children and young adults, the isolation and identification of J.C. and SV40 papovaviruses from the brains of patients with progressive multifocal leucoencephalopathy (PML), the demonstration of intracranial synthesis of specific antibody to measles in the cerebrospinal fluid of some patients with multiple sclerosis, the detection of virus inclusion bodies in the brain of patients dying of epilepsy partialis continua, or chronic focal epilepsy, with progressive brain pathology and the demonstration of the tick-borne encephalitis virus (RSSE) as the agent causing this type of disease in the Soviet Union and, finally, the demonstration of the rubella etiology of some cases of SSPE-like, or MS-like, disease.

Techniques developed during the early studies of kuru led us to the remarkable demonstration for the first time that presenile dementias of the Creutzfeldt-Jakob type, which are not ethnically nor geographically restricted, are caused by a similar virus. The demonstration of a 30-fold higher incidence of C-J disease in Israel in Jews of origin from Libya above that in those of European origin, may offer important epidemiological clues toward the elucidation of the source of infection. The further pursuit of these similar diseases has made possible the definition of the subacute spongiform virus encephalopathies as a group of related diseases of man (kuru and C-J disease) and animals (scrapie of sheep and transmissible mink encephalopathy). During the past two years, further study of patients with progressive dementia has provided the surprising demonstration that even the heredofamilial form of C-J disease is transmissible and of virus etiology. Although most cases are sporadic there are families in which the disease has occurred in siblings, parents and close maternal and/or paternal relatives over several generations. From five such families the disease has now been experimentally transmitted to primates, demonstrating for the first time in man that disease with apparently genetic determination may be caused by a slow virus. Most important has been the demonstration that the virus of C-J disease is present not only in the brain, but in the liver, spleen, kidney and lymph nodes of a patient that died with the familial disease. The onset of clinical signs and the acute progression of the disease in a patient seventeen months following transplantation of a cornea from a donor who died with Creutzfeldt-Jakob disease, and the occurrence of the disease in a neurosurgeon, are of concern. This has added impetus to our already extensive studies of Huntington's chorea, Alzheimer's and Pick's diseases, Parkinsonism-dementia, and even senile dementia. These investigations have so broadened the picture of C-J disease that we now must refer to transmissible virus dementias. This is necessary, since the diseases in patients with subacute or chronic dementia whose clinical and pathological diagnoses have been Alzheimer's disease and papulosis atrophicans maligna of Degos, have been transmitted to primates as subacute spongiform encephalopathies. Thus, the trail from kuru to C-J disease now embraces studies of presenile and senile dementias of all sorts, including "dementia praecox", the organic brain disease associated with late uncontrolled schizophrenia.

The demonstration that non-inflammatory chronic degenerative, even heredofamilial, central nervous system disorders could be of virus etiology, greatly stimulates the search for possible virus etiology in the demyelinating and sclerosing CNS diseases and chronic or actual encephalitides. The seroepidemiologic approach to multiple sclerosis with antibody determination in the spinal fluid and serum has led to our suspicion that at least some of the patients are suffering from delayed and slow defective virus infections, prob-

ably with measles virus, as Adams and others have earlier suggested. Intensive studies with whole virus and virus subunit antigens of the serial spinal fluid and sera, and gammaglobulin extracted from autopsy brain specimens for antibody activity is under way along with nucleic acid homology studies using MS and ALS brain tissue and nucleic acid probes made from candidate viruses such as measles, rubella, vaccinia, influenza, parainfluenza and poliomyelitis viruses.

In an attempt to unmask viruses in these diseases, brain cells have been grown in vitro by both primary explant and trypsinized cell layer techniques from specimens obtained at biopsy or early autopsy. Cocultivation and cell fusion and BUDR and IUDR induction are major techniques employed. The presence of a virus in these cultures is sought by electron microscopic scanning, staining for inclusion bodies, search for CPE, hemadsorption, fluorescent antibody, interferon production, virus interference and animal inoculation. The same techniques are applied to surgically sterile tissue obtained from experimental animals affected with kuru, Creutzfeldt-Jakob disease, and other slow virus infections. As a byproduct to the work, many masked and latent viruses from chimpanzee and monkey hosts have been isolated. These include herpesviruses (including cytomegaloviruses and Epstein-Barr virus), new ape and simian adenoviruses, a wide series of new foamy viruses, and several strains of type C oncornaviruses from the brains of gibbon apes. The foamy viruses are of particular interest, in that they are oncornaviruses characterized by the production of a reverse transcriptase, yet they have not caused cell transformation in vitro or tumors in monkeys. It has been shown that they are present in the brain and other organs of most chimpanzees and monkeys. We have recently developed a complement fixation test for studying the seroepidemiology of foamy viruses. The gibbon brain oncornaviruses are of interest, in that they belong to the group of woolly monkey and gibbon type C oncornaviruses which cause lymphosarcoma in gibbons, share their G-S antigens and p-30 protein antigens with the virus particles isolated by Gallo, presumably from human leukemia.

The long maintenance of brain cells, presumably astroglial cells, dedifferentiated on passage to resemble fibroblasts, regularly result in cell lines which propagate indefinitely and show many properties that suggest transformation. More blatant transformation with full loss of contact inhibition has occurred in three episodes with human brain cells: in the first instance, from a C-J disease patient's brain tissue, with regular presence of a Mason-Pfizer monkey virus-like MP-MV oncornavirus in the transformed human brain cells. The other two transformations have occurred in two brain cell lines from C-J patients which transformed with full loss of contact inhibition, but without the presence of recognizable virions in the transformed cells. In addition, a transformed cell line from a class II astrocytoma has been established and attempts to demonstrate virions are in progress. These transformations have given further support to the speculation that the slow-virus cell interaction in the degenerative central nervous system diseases may be closely related to the slow virus-cell interaction in cell transformation and carcinogenesis. The use of the term "temperate" in naming our laboratory in 1962 seems to have been prophetic.

A group of atypical viruses unlike any others known to microbiologists appear to cause the subacute spongiform virus encephalopathies. The other CNS

degenerations caused by slow viruses are caused by rumbling non-productive, even defective infection, with the more conventional measles, papovaviruses (SV40 and J.C.), rubella virus, cytomegalovirus, herpes simplex virus, adenovirus type 32, and RSSE. The atypical viruses (kuru, C-J disease, scrapie and TME) have unusual resistance to ultraviolet radiation and ionizing radiation, to ultrasonication, to heat, proteases and nucleases, and to formaldehyde. They are not associated with a recognizable virion on electron microscopic study of infected cells in vivo or in vitro, or concentrated virus preparations (zonal banding). This has led to the speculation that they represent infectious agents lacking a nucleic acid, perhaps even a self-replicating membrane fragment. A major effort in this laboratory has been and is now being directed toward the molecular biological elucidation of the nature and structure of this group of atypical viruses.

The scrapie virus has been partially purified using fluorocarbon precipitation of proteins and density gradient banding using the zonal rotor. Other semi-purified preparations have been made using ultra-filtration and repeated complete sedimentation, and washing of the scrapie virus using ultrasonication for resuspension of the virus-containing pellets. Sucrose-saline density gradient banding of scrapie virus in mouse brains produced wide peaks of scrapie infectivity at densities of 1.18 to 1.28. On electron microscopic examination fractions of high infectivity (10^7-10^8 LD_{50}/ml) revealed only smooth vesicular membranes with mitochondrial and ribosomal debris and no structures resembling recognizable virions. Lysosomal hydrolases (n-acetyl-β-D-glucosaminidase; β-galactosidase; acid phosphatase) and a mitochondrial marker enzyme (INT-succinate reductase) showed most of their activity in fractions of lower density than the region of high scrapie infectivity. We have confirmed the previously noted resistance of scrapie virus to UV inactivation at 254nm and an UV inactivation action spectrum with 6-fold increased sensitivity at 237nm over that at 254nm or 280nm. This may not be taken as proof that no genetic information exists in the scrapie virus as DNA or RNA molecules since work with the smallest viruses, called viroids, indicates a similar resistance to UV inactivation in crude infected plant-sap preparations. There is also a great effect of small RNA size on UV sensitivity, as has been shown by the high resistance of the purified viroid RNA to UV inactivation, and a similar high UV resistance with the purified very small RNA (about 80,000 daltons) of tobacco ring spot satellite virus. Partial purification of fluocarbon only slightly increases UV sensitivity at 254nm. Fluorocarbon purified scrapie was not inactivated by RNAase I, RNAase III or DNAase I. Autoclaving (121°C/20lbs psi/45mins.) completely inactivated scrapie virus in crude suspensions of mouse brain.

Freeze fracture studies of membranes in the scrapie-affected mouse cerebellar cells have been initiated in the hope of better defining the membrane sub-unit structures we assume are the infectious agent of scrapie. Preliminary studies already reveal no unique structures obviously related to virus particles inside the membranes, even those forming the walls of vacuoles. However, the innermost limiting membranes of vacuole walls in neurons and astrocytes show large areas devoid of the usual 8-13nm intramembranous particles. The membranes around vacuoles in astrocytes show abnormally high numbers of "assemblies", which are specific structures for astrocytes, and unusual clusters of large particles with large bare spaces between these clusters. This

rearrangement of intramembranous particles and denuding of areas of intramembranous surfaces is a structural abnormality which may be an aspect of astrocytic hypertrophy known to occur in scrapie. Preliminary studies already reveal the same type of changes in Creutzfeldt-Jakob dises-affected cells. Too little is known about the appearance of various pathological states in freeze fractured neural membranes to determine whether any of these changes are specific to scrapie and C-J disease. They are changes absent in the normal mouse and may represent changes in the organization of proteins associated with the development of virus infectivity in membrane subunits.

After our discovery that the cat is susceptible to Creutzfeldt-Jakob disease, we have now found that the mink is similarly susceptible to kuru and these two non-primate hosts for the human virus infections may expand enormously the possibility of the study of these viruses. We are trying to confirm recent reports of the transmission of C-J disease to mice and to guinea pigs, which have been resistant to these viruses in all our earlier work.

The transmission of scrapie virus from American scrapied sheep brain, as well as from goats and mice infected experimentally, to several species of New and Old World monkeys, with disease clinically and pathologically indistinguishable from that produced by C-J disease in the experimental primates, has reawakened our earlier suspicions that scrapie may be closely related to the occurrence of the subacute sclerosing virus encephalopathies in man. In the absence, as yet, of proven antigenciity or identified nucleic acid in the agents, neither serological specificity nor nucleic acid homology can be used to answer the compelling question of the relationship between the viruses of kuru, Creutzfeldt-Jakob disease, scrapie, and transmissible mink encephalopathy.

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

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

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, pre-senile 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 vivo and in vitro host range of the subacute spongiform virus encephalopathies.

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

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

Sub-Project VIII: Tissue and cell culture in vitro studies of virus induced slow infections of man and animals.

Sub-Project IX: The syncytium forming viruses (simian and human foamy viruses).

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

Sub-Project XI: Isolation and characterization of cytomegalovirus from the urine of chimpanzees.

Sub-Project XII: Characterization and identification of new herpes viruses from explant cultures of tissues from sub-human primates.

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

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

Sub-Project XV: Development of serological and immunological test system for use in the study of slow infections of the CNS.

Sub-Project XVI: 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 XVII: Animal management and intercurrent diseases in sub-human primates on long-term studies of slow infections.

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

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

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

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Other Investigators: Herbert L. Amyx, D.V.M.; David M. Asher, M.D.; Richard Benfante, M.A.; Paul W. Brown, M.D.; Ralph Garruto, Ph.D.; George J. Nemo, Ph.D.; Roger D. Traub, M.D.; Theodore Tsai, M.D.; Lon R. White, M.D.; Richard Yanagihara, M.D.

Visiting Scientists: Michael Alpers, M.D.; Nedar Grčević, M.D.; Chev Kidson, M.D.; Neville Stanley, M.D.; Vincent Zigas, M.D.

Technical Assistants: Alfred Bacote, Lucille Gilbert, Monica Lewis, Nancy Luber, Judith Meyer, Stephen Ono, Joseph Pazdersky, Hubert Saville, Ezra Schafer, Michael Sulima, Randolph Taylor, Leon Vance, Eugene Webster, Harry Winpigler

Cooperative Students: Charles Brittain, Greagori Brown, Judith Chavis, Gary Cooper, Jay Gorham, MaryBeth Hartman, Anthony Montanaro, Ann Rudnick, Robert Shapiro, Robert Wismer

Cooperating Units: Michael Alpers, M.D., University of Western Australia, Perth, Australia; Eric French, Ph.D., Division of Animal Health, CSIRO, Victoria, Australia; Robert L. Kirk, M.D., Department of Genetics, Australian National University, Canberra, Australia; Eric Shaw, Ph.D., Red Cross Blood Transfusion Service, Brisbane, Australia; F. Seitelberger, M.D., Neurologisches Institut der Universitat Wien, Vienna, Austria; Armand Lowenthal, M.D., Laboratory of Neuropathology, l'Institut Bunge, Antwerp, Belgium; R. Luzzatto, Instituto de Neurocirurgia, Hospital de Neurocirurgia, Santa Casa Porto Alegrers, Brazil, South America; J.J. Gilbert, Victoria Hospital, London, Ontario, Canada; W.P. McInnis, Victoria Hospital, London, Ontario, Canada; Theodore Rasmussen, M.D., University of Montreal, Canada; N.B. Rewcastle, Toronto General Hospital, Banting Institute, Toronto, Canada; D.M. Robertson, Queens University Hospital, Department of Neuropathology, Kingston, Ontario, Canada; A.R. Watts, Medical Dental Building, Regina, Saskatchewan, Canada; Helena Libikova, M.D., Institute of Virology, Slovak Academy of Sciences, Czechoslovakia; Peter M. Daniel, M.D., Department of Neuropathology, Maudsley Hospital, London, England; Mrs. Elisabeth Beck, Institute of Psychiatry, Department of Neuropathology, London, England; George Dick, M.D., Bland-Sutton Institute of Pathology, London, England; E.J. Field, M.D., Medical Research Council, Royal Victoria Infirmary, Newcastle-upon-Tyne, England; P.J.H. Fletcher, Department of Pathology, N. Staffordshire Royal Infirmary, Stoke-on-Trent, England; D. Haig, D.V.M., D. Hunter, Ph.D. I.H. Pattison, Animal Research Section, Compton, England; M. Sim, Queen Elizabeth Hospital, Edgbaston, England; N. Oker-Blom, Saikku, Department of Virology, University of Helsinki, Finland; Francoise Cathala, M.D., Department of Neurology, Hopital de la Salpetriere, Paris, France; Contamin, M.D., Hopital St. Antoine, Paris, France; Lhermitte, M.D., Hopital de la Salpetriere, Paris, France; F. Pertuiset, Hopital de la Salpetriere, Paris, France; Warot, M.D., Department of Neurology, Universitat de Lille Nord, France; Klaus Mannweiler, M.D., Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie, Hamburg, Germany; Raymond Latarjet, Foundation Curie-Institut du Radium, Paris, France; A. Struppler (A. Zumsky, USA), Neurology Institute of Technology, University of Munich, Munich, Germany; Volker ter Meulen, M.D., Institut fur Virologie der Universitat, Wurzberg,

Germany, P.A. Palsson, D.V.M., Institute for Experimental Pathology, Keldur, Iceland; M.S. Bhat, Department of Neurology, J.J. Group of Hospital, Byculla, Bombay, India; C. Fieschi, Clinica Delle Malattie, Nervosa E. Mentali, Universita di Siena, Siena, Italy; R.W. Hornabrook, M.D., Institute of Human Biology, Papua New Guinea; Vincent Zigas, M.D., Public Health Department, Rabaul, New Britain, Papua New Guinea; Ewa Osetowska, M.D., Institute of Neuropathology, Polish Academy of Science, Warsaw, Poland; V. Mojica, M.D., V.A. Hospital, San Juan, Puerto Rico; Louis Herzberg, Department of Neurology, Royal Infirmary, Dundee, Scotland; E.H. Jellinek, Neurological Unit, Northern General Hospital, Edinburgh, Scotland; F. Assaad, Medical Officer Virus Disease, World Health Organization, Geneva, Switzerland; A.M. Gardashyan, M.D., Department of Immunology and Oncology, Gamelaya Institute, Moscow, U.S.S.R.; V.I. Il'yenko, M.D., All-union Research Institute of Influenza, Leningrad, U.S.S.R.; J. Vesenjak-Hirjan, M.D., Department of Virology, Medical Faculty, University of Zagreb, Yugoslavia; Jacob A. Brody, M.D., Radiation Effects Research Foundation, Hiroshima City, Japan; J.R. Baringer, M.D., V.A. Hospital, San Francisco, California; C. Espana, Ph.D., National Center for Primate Biology, University of California, Davis, California; F. Dixon, M.D., M. Oldstone, M.D., David Kohn, Ph.D., Scripps Clinic and Research Foundation, La Jolla, California; Peter W. Lampert, M.D., Department of Pathology, University of California, La Jolla, California; F. Newton, M.D., Letterman Army Medical Center, San Francisco, California; L.J. Rubinstein, M.D., Stanford Medical School, Stanford, California; R. Silton, M.D., Department of Anatomical Pathology, City of Hope Hospital, Duarte, California; W. Tourtellotte, M.D., V.A. Hospital, Los Angeles, California; Roy L. Walford, M.D., Department of Pathology, University of California, Los Angeles, California; S. Wiesenfeld, M.D., University of California, Davis, Sacramento Medical Center, Davis, California; R. Gilbert, M.D., R. Bobowick, M.D., Waterbury Hospital, Waterbury, Connecticut; L. Prockop, M.D., V.A. Hospital, Tampa, Florida; D. Camenga, M.D., R. Franco, M.D., Emory University, Atlanta, Georgia; Dr. Friedman, Department of Neurology, Medical College of Georgia, Augusta, Georgia; Freidrich Deinhardt, M.D., Loren Wolf, M.D., Department of Microbiology, Presbyterian-St. Luke's Hospital, Chicago, Illinois; Philip Y. Paterson, M.D., Northwestern University Medical School, Chicago, Illinois; M.G. Reyes, M.D., Department of Pathology, Mt. Sinai Hospital, Chicago, Illinois; Wolfgang Zeman, M.D., A.N. Siakotos, Ph.D., Department of Neuropathology, Indiana University Medical Center, Indianapolis, Indiana; D. Ziegler, M.D.,

Kansas University Medical Center, Department of Neurology, Kansas City, Kansas; William Greer, D.V.M., Gulf South Research Institute, New Iberia, Louisiana; W.J. Mogabgab, M.D., Department of Medicine, Infectious Disease Section, Tulane University, New Orleans, Louisiana; E. David, M.D., Bangor, Maine; 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., Leslie Weiner, M.D., Johns Hopkins University Hospital, Baltimore, Maryland; Theodore Diener, Ph.D., Department of Plant Biology, U.S. Department of Agriculture, Beltsville, Maryland; Paul Albrecht, M.D., Lewellys F. Barker, M.D., Hope E. Hopps, J. Anthony Morris, Ph.D., Bureau of Biologics, FDA, Bethesda, Maryland; Samuel Baron, M.D., Hilton Levy, M.D., NIAID, NIH, Bethesda, Maryland; Paul MacLean, M.D., NIMH, NIH, Bethesda, Maryland; King Engel, M.D., David A. Fuccillo, M.D., Luis Horta-Barbosa, M.D., John Sever, M.D., NINCDS, NIH, Bethesda, Maryland; A. Dekaban, NIH Clinical Center, Pediatric Neurology, Bethesda, Maryland; Drs. Kroot and Leshner, Department of Neurology, Bethesda Naval Hospital, Bethesda, Maryland; L.S. Adelman, New England Medical Center, Boston, Massachusetts; M. Fleming, Department of Neurology, St. Elizabeth's Hospital, Brighton, Massachusetts; Dr. Frei and William Schoene, M.D., Peter Bent Brigham Hospital, Boston, Massachusetts; M. Oxman, M.D., Children's Medical Center, Boston, Massachusetts; David Poskanzer, M.D., Massachusetts General Hospital, Boston, Massachusetts; K. Scott, M.D., New England Medical Center, Boston, Massachusetts; M. Shelnasky, M.D., Boston Children's Hospital, Boston, Massachusetts; M. Jones, M.D., MSV Department of Pathology, St. Lawrence Hospital, Lansing, Michigan; I. Selah, M.D., William Beaumont Hospital, Department of Neurology, Royal Oak, Michigan; Y. Franck, M.D., Department of Neurology, V.A. Hospital, Minneapolis, Minnesota; R. Torack, M.D., Department of Neurology, Washington University School of Medicine, St. Louis, Missouri; W. Hadlow, D.V.M., Rocky Mountain Laboratory, NIAID, NIH, Hamilton, Montana; J.A. Newman, M.D., St. James Community Hospital, Butte, Montana; N. Haidri, M.D., Martland Hospital, Newark, New Jersey; G. Budzilovich, M.D., N.Y. University School of Medicine, New York; A.J. Lapovsky, M.D., P.N. Sawyer, M.D., Downstate Medical Center Hospital, Brooklyn, New York; Robert Ledeen, M.D., Department of Neurology, Albert Einstein College of Medicine, Bronx, New York; Donald Harter, M.D., Richard L. Masland, M.D., Department of Neurology, Columbia University, College of Physicians and Surgeons, New York, New York; R. Katzman, M.D.,

Yeshiva University; Gerald Ward, D.V.M., Otisville, New York; R. Carp, Ph.D., Halldor Thormar, Ph.D., Institute for Basic Research in Mental Retardation, Staten Island, New York; N. Peress, M.D., Department of Neurology, State University, Stony Brook, New York; P. Phillips, M.D., Hospital for Special Surgery, New York, New York; John Griffith, M.D., David Lang, M.D., Department of Pediatrics, Duke University Medical Center, Durham, North Carolina; J. Hammerstedd, M.D., Department of Neurology, University of Oregon School of Medicine, Portland, Oregon; Dr. Black, Department of Neurology, Hershey Medical Center, Hershey, Pennsylvania; Gertrude Henle, M.D., Werner Henle, M.D., P. Koldovsky, M.D., Children's Hospital, Philadelphia, Pennsylvania; D. Gilden, M.D., University of Pennsylvania Hospital, Philadelphia, Pennsylvania; Hilary Koprowski, M.D., Wistar Institute, Philadelphia, Pennsylvania; Drs. Mancall, Cutler, J. Parr, Hahnemann Medical Center, Philadelphia, Pennsylvania; Y. Mussio, M.D., Conemaugh Hospital, Johnstown, Pennsylvania; J. Powers, M.D., Department of Neurology, V.A. Hospital, Charleston, South Carolina; E.J. Kozlowski, M.D., V.A. Hospital, Dallas, Texas; V. Rivera, M.D., Department of Neurology, Houston Medical Center, Houston, Texas; J. Davenport, M.D., Department of Neurology, V.A. Hospital, Seattle, Washington; F. Chu, M.D., Dr. Heferin, Department of Ophthalmology, Georgetown University Hospital, Washington, D.C., Dr. Guinn, V.A. Hospital, Washington, D.C.; J. Hourrigan, D.V.M., Animal Research Section, U.S. Department of Agriculture, Washington, D.C.; Drs. Jenkins and Lawrinson, Washington Hospital Center, Washington, D.C.; A.B. White, M.D., Walter Reed Medical Center, Washington, D.C.; K. Earle, M.D., Sam Chou, M.D., Department of Neuropathology, West Virginia University Medical Center, Morgantown, West Virginia; Stu Aaronson, M.D., Robert Martin, M.D., Wallace Reichert, M.D., George Todaro, M.D., NIH, Bethesda, Maryland; Donald Gustafson, Ph.D., Purdue University, Department of Virology, West Lafayette, Indiana; Asao Hirano, M.D., Division of Neuropathology, Montefiore Hospital and Medical Center, Bronx, New York; Philip Schwartz, M.D., Warren State Hospital, State Institute for Geriatric Research, Warren, Pennsylvania; Stuart Aaronson, George Todaro, NCI, Wallace Reichert and Robert Martin, NIH, Bethesda, Maryland.

Administrative: Marion Poms, Linda Poole, N. La-Donna Tavel

Man Years:

Total:	24
Professional:	14
Other:	10

Project Description: Chronic Central Nervous System Diseases Studies (described fully on pages 4 through 10)

Keyword Descriptors:
Viruses and chronic disease
Slow virus infections
Defective virus infections
Unconventional viruses
Kuru
Creutzfeldt-Jakob disease
Transmissible virus dementia
Scrapie
Transmissible mink encephalopathy
Viroids
Multiple sclerosis
Amyotrophic lateral sclerosis
Epilepsy, chronic
Alzheimer's disease
Heredofamilial degenerative diseases
Immunological deficiency syndromes
Primate models of disease
Chimpanzees in medicine
Oncornaviruses
Papovaviruses
Herpesviruses
Cytomegaloviruses
Membranes associated viruses
Arboviruses
Tissue culture
Transplantation, infection from

Honors and Awards: None

Publications: Listed on pages 18 through 23

The projects (I through XX) listed herein, as itemized in the Project Reports of previous years, have continued throughout this year and have been expanded, as are reflected in the extensive list of publications and the summary in pages 1 through 10. Conractural phases of this work are being conducted at:

Gulf South Research Institute, New Iberia, Louisiana	$451,000.
Public Health Research Institute of New York, Otisville	116,000.
Center for Primate Biology, University of California, Davis, California	100,000.
Department of Molecular Biology, University of Connecticut	66,330.

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Serial No. Z01 NS 01282-11 CNSS
1. Laboratory of Central Nervous System Studies
2.
3. Bethesda, Maryland
PHS - NIH

Project Report
July 1, 1974 through June 30, 1975

Project Title: Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures

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, 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.

Previous Serial Number: NDS (I)-65 CNSS 1282

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

Other Investigators: David M. Asher, M.D.
Ralph M. Garruto, Ph.D.
Roger D. Traub, M.D.
Richard Yanagihara, M.D.

Visiting Investigators: Michael Alpers, M.D., Francoise Cathala, M.D., Chev Kidson, M.D., Robert L. Kirk, Ph.D., Marek Jablonko, Robert MacLennan, M.D., Jack Ono, M.D., Roger Rodrique, M.D., Stephen Wiesenfeld, M.D., Vincent Zigas, M.D.

Student Investigators: Richard Benfante, M.A., Yavine Borima, Peter Fetchko, M.A., Josede Figirliyong, M.A., Laura Kreiss, Ivan Mbagintao, Jesus Maluwetig, Nancy Luber, Judith Meyer, Jesus Mororui, Stephen Ono, Donald Rubinstein, Jesus Tamel

Cooperating Investigators: Timothy Asch, Harvard University, Cambridge; Dr. P.T. Baker, Department of Anthropology, Pennsylvania State University; University of Perth; Stephen Brown, M.D., School of Public Health, University of California, Berkeley; Dr. N. Chagnon, Department of Anthropology, Pennsylvania State University; Dr. Edwin Cook, Department of Anthropology, University of California, Davis; Dr. Cyril C. Curtain, Division of Animal Health, CSIRO, Melbourne; Richard Feinberg, Department of Anthropology, University of Chicago; Dr. T. Velasquez, San Marcos University, Lima, Peru; Dr. Gordon Gibson, Department of Anthropology, Smithsonian Institution, Washington, D.C.; Dr. M. Godelier, Laboratory of Social Anthropology, L'Ecole Pratique Des Hautes Etudes, Paris; Dr. Jean Guiart, Musee des Sciences Naturelles, Paris; Dr. C. Hoff, Department of Anthropology, University of Oregon, Eugene; Mr. and Mrs. Marek Jablonko, Perugia, Italy; Perry Kennedy, Department of Anthropology, University of Buffalo, New York; Department of Anthropology, Thomas Kiefer, Brown University, Providence; Allan Lomax, Applied Social Research, Columbia University, New York; Dr. R.L. Kirk, Dr. N.M. Blake and Dr. K. Omoto, Department of Human Biology, Australian National University, Canberra; Dr. D. Kitchin, Opthalmology Institute, Columbia-Presbyterian Medical Center, New York; Dr. H. Lehman, Department of Biochemistry University of Cambridge, England; Dr. R. MacLennan International Agency for Cancer Research, Lyon; Margaret Mead, Museum of Natural History, New York; Dr. Denise O'Brien, Department of Anthropology, Temple University, Philadelphia; Dr. Sol Riesenberg, Division of Ethnology, Smithsonian Institution; Dr. Theodore Schwartz, Department of Anthropology, University of

California, Los Angeles; Dr. R. Rodrique, Wilmington, Delaware; Dr. R.T. Simmons, Melbourne, Australia (deceased); Dr. M.P. Alpers, Dr. Neville Stanley and Dr. J. Sheridan, University of Western Australia, Perth; Dr. Malcolm Simons, W.H.O. Immunology Centre, Singapore; Dr. Kok Ann Lim, Department of Bacteriology, University of Singapore; Dr. S.A. Wurm, Australian National University, Research School of Pacific Studies, Canberra; Richard Lloyd, Summer Institute of Linguistics, Papua New Guinea; Dr. H.A. Brown, London Mission Society, Port Moresby, Papua New Guinea; Dr. R. Hornabrook, Institute of Medical Research, Goroka, Papua New Guinea; Dr. Vincent Zigas and Dr. Jack Ono, Public Health Department, Papua New Guinea; Dr. R. Tesch, Dr. Gordon Wallace, and Dr. Leon Rosen, Pacific Research Section, NIAID, Honolulu; Dr. Jan ten Brink, Vught, The Netherlands; Dr. Charles Wisseman, Department of Microbiology, University of Maryland, Baltimore; Dr. Arthur Steinberg, Department of Biology, Case Western Reserve University, Cleveland; Dr. B. Padget, Department of Microbiology, University of Wisconsin, Madison; Dr. Raymond Roos and Dr. K. Shah, Johns Hopkins University, Baltimore; Dr. David Lang and Dan Domizio, Duke University Medical School, Durham; Dr. Richard Ferber, Children's Medical Center, Boston; Dr. S. Wiesenfeld, Sacramento Medical Center, University of California, Davis; Dr. Kenneth Brown and Mr. W. Leyshon, NIDR; Drs. F. Neva and L.M. Miller, NIAID; C. Plato,NICHD; Dr. Paul MacLean, NIMH; Dr. J. Sulianti-Sarosa and Dr. Ben Kawengian, Ministry of Health, Jakarta, Indonesia; Dr. Surjadi Gunawan (Jayapara), Dr. Lukas Kristanda (Jayapura), Dr. Leode R. Tumada (Abepura), Dr. Budi Subianto (Enaratobi), Dr. Widodo (Kepi) and Dr. K. Dresser (Senggo), Public Health Department, West New Guinea (Irian Jaya), Indonesia; Bishop A. Sowada, Fr. Ben Van Oers, Fr. F. Trenkenshuh, D. Gallus, Catholic Mission, Irian Jaya, Indonesia; Dr. Kurt Sorenson, NAMRU 2 Auxillary Laboratory, Jakarta, Indonesia; Dr. E.M. Kosasih and Dr. Bernard Salafsky, University of North Sumatra, Medan, Sumatra; Dr. P. de Carfort, K. Woodward, Dr. D. Bowdin, Dr. R. Greenough, Medical Services, New Hebrides; Dr. B. Eyers, Dr. J. MacGregor, and Dr. R. Lee, Medical Service, British Solomon Islands Protectorate; Dr. A. Nahmias, Department of

Pediatrics, Emory University, Atlanta; Dr. Harry Hoogstraal, NAMRU 3 Laboratory, Cairo, Egypt; Dr. Chev Kidson, Department of Biochemistry, University of Queensland, Brisbane, Australia; Dr. Palmer Beasley, NAMRU 2 Laboratory, Taipei, Taiwan.

Administrative: Marion Poms, Linda Poole, La-Donna Tavel

Man Years:

Total:	12
Professional:	8
Others:	4

Project Description: Neurobiology of Population Isolates (described fully on pages 3 and 4)

Keyword Descriptors:

Child development
Child behavior
Developmental human biology
Human biology
Human evolution
Genetics of human populations
Population genetics
Language aquisition
Puberty
Aging
Psychosexual development
Perception, acquisition
Nurological information processing
Sensory information processing
Coding of neurological information processing
Coding of sensory information
Ciphers and notation
Learning
Primitive cultures
Melanesia
New Guinea
Micronesia
Polynesia
Australian aborigines
Indians, American
Amerindians

Honors and Awards:

Dyer Lecturer, National Institutes of Health
The Heath Clark Lecturer, University of London
Alexander D. Langmuir Lecturer, Communicable Disease Center, Atlanta
Chairman: Symposium on Slow Virus Infections, Tokyo
Lecturer: Application of Concepts of Modern Biological Research to Investigation of Slow Virus Infections of the CNS, Institut für Virologie der

Universitat, Würzburg, Germany
Biennial Lecturer: Microbiological Societies of Yugoslavia and Society of Yugoslav Infectologists, Radenci, Yugoslavia
Organizer: Workshop on Slow Infections by Unconventional Viruses, Fourth International Congress of Virology, Madrid
William Withering Lecturer, University of Birmingham, England
Elected Member, National Academy of Science
Distinguished Service Award, DHEW
Benjamin Knox Rachford Memorial Lecturer, University of Cincinnati

Publications: Listed on pages 18 through 23.

The projects (I through XI) listed herein and their related studies, as itemized in the Project Reports of previous years, have continued throughout this year and are reflected in the extensive list of publications. Laboratory studies on human biology, genetics and associated molecular biology, immunology, virology, and biochemistry, are all directed at solving problems chosen from small isolated bands still living in the primitive situation in which these problems may be more appropriately studied than in larger civilized societies. Studies in Micronesia, the New Hebrides, and the British Solomon Islands resulting from our participation in the expedition of the Research Vessel Alpha Helix of the Scripps Institution of Oceanography, have gradually been coming to fruition and some were published in the past year (see bibliography). A full prospectus of the studies on these islands was published in the 1973 Annual Report. The specimens and data collected on the expedition continued to be vigorously studied during this past year, and the results of many new studies are in preparation. The 1974 and 1975 expeditions to Micronesia, Malaysia, Indonesia, especially West New Guinea, and Papua New Guinea, have resulted in much new kuru epidemiological data, and to the delineation of the new focus of amyotrophic lateral sclerosis, parkinsonism, and parkinsonism-dementia, and also a peculiar form of myoclonic epilepsy with dementia, which are all being reported shortly. Our long-term studies of kogaisantamba, an essential tremor syndrome in the highlands of Papua New Guinea, and of puriripiram, a Western kuru-like syndrome with shock-like hemiballistic jerks, are now also being summarized for publication, along with surveys of other neurological disorders and neuropsychiatric disorders in various regions of Melanesia, especially New Guinea.

LAB. OF BIOPHYSICS

EEG & NEUROPHSY. BR.

CL.

DEVELOP. & METAB. NEUROL. BR.

LAB. OF NEUROPATHOLOGY & NEUROANATOM. SC.

LAB. OF NEURAL CONTROL

LAB. OF NEUROPHYSIOLOGY

HOW TO USE
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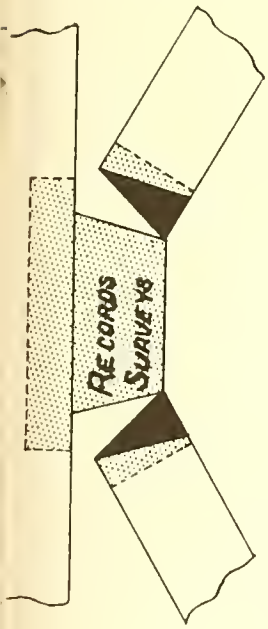

TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT

July 1, 1974 through June 30, 1975
Branch of Electroencephalography and Clinical Neurophysiology
National Institute of Neurological and Communicative Disorders and Stroke

Cosimo Ajmone Marsan, M.D., Chief

Summary of Program Activity

1. Clinical-Diagnostic Service

This service has still represented a considerable portion of the overall activity of the Branch, although the man/years devoted to it has decreased to a total of 3.6 (professional 0.5; technical-secretarial 3.1) from the 5 man/years of the preceding fiscal period. The service is provided to all patients in the Clinical Center (both in- and out-patients) referred for an electroencephalographic examination either as part of their routine clinical work-up or for specific research projects originating outside of this Branch and/or Institute.

From the time the last report was prepared, (April 13, 1974) to that of the present report (April 10, 1975), a total of 1348 EEG tracings have been obtained and interpreted following the corresponding requests from doctors in the various Institutes, and specifically:

INSTITUTE	NO.	%
NINCDS (OP 301)	870	64.5
NCI	175	13.0
NIMH	134	10.0
NHLI	66	4.9
NIAMD	60	4.5
NIAID	28	2.1
NEI	2	0.1
Misc.	13	0.9
TOTAL	1348	100.0

About two thirds of the referrals have been from NINCDS, mostly from the Branch of Neurological Surgery. A considerable number of the 478 examinations requested by the other Institutes were carried out in the Ward, at the patient's bed or in Intensive Care. In addition to the EEG examinations, which include those performed in patients with chronically implanted depth electrodes, 17 records were obtained directly from the exposed cortex, in the course of surgical procedures for the treatment of partial epileptic seizures, in cooperation with the Branch of Neurological Surgery, NINCDS.

This service activity also provides the opportunity for training in clinical electroencephalography. During this fiscal period one clinical associate has completed his training and another has successfully passed the Board of Qualification in clinical EEG.

2. Research Activity

Of the 10 projects listed in the previous Annual Report, 2 were completed and 2 had been terminated. The others are included in the present Report together with 7 new projects. Of these 13 projects, 8 have been completed, and 5 are still active (for 3 of these, the completion is expected in the near future).

In the experimental field, 2 projects deal with the early phases of development of epileptiform activity at the single neuron level. The experimental design includes a reversible model of highly discrete focal cortical epilepsy and the utilization of neurons with well defined and readily assessable physiological properties. Specifically the activity of a single cell in the visual cortex of cat is monitored continuously during recurrent stimulation with light patterns of optimal configuration and orientation within the cell's receptive field. This is carried out before, during and after periods of iontophoretic application of penicillin through a nearby micropipette. The process is fully reversible and multiple observations can be made with the same or different cells with repeated penicillin applications. This study has demonstrated the development of several distinct changes of neuronal activity as the passage of iontophoretic current progresses. The earliest and most persistent alteration is an increase in the number and frequency of spikes which represented the neuronal response to effective stimuli in the normal situation. This enhanced physiological response (EPR) could be elicited from a cell independently of the discharge activity of an induced epileptogenic focus, but only with stimuli appropriate for the cells' receptive field. As the iontophoresis continues, a new response develops which is consistent with an extracellularly recorded PDS (paroxysmal depolarization shift). This PDS could be triggered by preferential stimuli but also by stimuli which were ineffective

in normal conditions and, with either stimuli, it was characterized by a longer, and more variable latency, a longer recovery period and a different sensitivity to changes of stimulus intensity. A period of response inhibition could also be demonstrated in tonically responding neurons with each interictal potential. On the basis of these and other observations it can be concluded that the earliest direct effect of the epileptogenic agent on the cell or its immediate synaptic connections is represented by the EPR, whereas the PDSs seem to depend on the altered interaction with a population of such affected cells; a dynamic model of the EPR-PDS relationship can thus be proposed. The results of the first project have just appeared in published form and those of a second, related project with a modified experimental design are summarized in the individual project report and are currently written up for publication.

Four experimental projects deal with various aspects of brain metabolism in different situations. In one study, the NADH fluorescence changes induced in the cerebral cortex of cat during direct cortical stimulation are correlated with oxygen consumption, blood flow and Hgb saturation in an attempt to strengthen and clarify the relationship between fluorometric changes and oxidative processes. This project is still active but should be completed in the near future. Two other projects deal with electrographic-metabolic correlations in the cat cerebral cortex during Metrazol induced seizures and during stimulation of the brain stem reticular formation. In these experiments NADH fluorometry is carried out using fluorescein fluorescence rather than reflected UV light as the reference signal. The former appears to be a more reliable reference for NADH because of the co-existing large increases in cortical blood flow which affect UV changes. The two signals (through NADH and fluorescein filters) are measured differentially by means of video densitometry which permits a topographical survey of different cortical regions. In one of these projects the blood oxygen saturation and the blood flow in the sagittal sinus are also monitored. These two projects are still active: some of the findings obtained are mentioned in the individual reports and the projects are expected to be completed during the next fiscal period. A fourth, partially completed project deals with the application of television fluorometry in the study of oxidative responses to electrical stimuli in human cortex. This is carried out in the course of surgical interventions for the treatment of seizure disorders with the scope of differentiating normal from abnormal cortical tissue.

In 4 other projects, K^+ -sensitive microelectrodes are used in the cerebral cortex and/or hippocampus of cats to monitor the extracellular concentration of potassium, with or without NADH fluorescence changes, in various experimental situations. In a completed and recently published study, the application of brief trains of electrical stimuli to the dorsal surface of the hippocampus was found to produce an up to 6 mM increase in

extracellular K^+, as well as a simultaneous decrease in NADH fluorescence, the intensities of both changes being highly correlated. The time course of the post-stimulus decline of K^+ was an exponential decay function with half-time values of 1.3 - 6.9 sec. The speed of this K^+ reuptake was independent of the magnitude of the extracellular K^+ changes but it tended to increase following the later trains in closely repeated stimulation or when the stimulus was followed by an afterdischarge. In another similar study, carried out at the neocortical level, K^+ and NADH changes are compared in a cortical area following the establishment of an epileptogenic process (penicillin topical application) and in a distant (homologous contralateral) region. This study, which is completed, has shown the lack of appreciable changes in either extracellular K^+ or oxidative rate (i.e. NADH decrease) in coincidence with the "projected" interictal paroxysmal discharges, in contrast to the obvious changes observed during similar phenomena occurring at the site of the epileptogenic focus.

The question of K^+ reuptake has been further investigated in two other projects which are close to completion. In one, the effects of temperature upon potassium reuptake have been studied in the cat hippocampus, following direct electrical stimulation. The Q_{10} value found (2.1) would appear to be consistent with an active metabolic process. In the other, the K^+ clearance after its extracellular increase produced by direct electrical stimulation, is being studied in the motor cortex of monkeys in which a chronic epileptogenic glial scar had been produced by intracortical injection of alumina cream. The purpose of this investigation is to correlate possible changes in K^+ reuptake with the degree of fibrous astrocyte proliferation within the epileptogenic lesion. On the basis of partial analysis of the results it would appear that K^+ is cleared more slowly in densely fibrotic areas than in areas of normal cortex.

The 3 last projects are of a clinical nature. These have all been completed and the results are currently in press or have appeared in publications. One deals with a series of patients with partial seizures resulting from an epileptogenic lesion located in the orbital surface of the frontal lobe. The clinical patterns and electrographic features of this type of focal epilepsy are still poorly understood because fully documented cases of such a disorder have been rather rare in the literature. The use of chronically implanted electrodes in frontal and temporal structures of 4 patients with a seizure disorder of presumed temporal origin has permitted to demonstrate the focal onset of their ictal episodes in the orbitofrontal cortex. The characteristic clinical EEG features in these patients have been analyzed and should prove of some practical usefulness in the differential diagnosis, localization of epileptogenic focus and, possibly, surgical management

of patients affected by similar disorders. Another project deals with epileptogenic processes localized in the occipital lobe. Specifically it consists of an analysis of the seizure patterns and other clinical features in a series of 55 epileptic patients with electrographic evidence of exclusive or predominant occipital involvement and it is the continuation of a preceding similar study of seizures of front-centro-parietal origin. This investigation has demonstrated few statistically significant differences in ictal patterns between subjects with evidence of purely focal occipital involvement and those with paroxysmal activity of wider distribution (occipito-temporal, occipito-parietal, etc.) On the other hand, cases with bilateral synchronous occipital epileptiform activity seem to reflect a different type of epileptic disorder. Clinical pleomorphism is more frequent than is generally expected from the ictal activation of the occipital region. The incidence of visual auras is high but other sensations (epigastric, psychic, somatic phenomena etc.) are rather common. Ictal motor patterns tend to be of a generalized nature or are absent, but partial motor attacks and typical psychomotor seizures are not infrequently observed. This study has also established the relative localizing and lateralizing value of these and other various phenomena in relation to their incidence of occurrence in seizures of different origin.

The third clinical project is an attempt to a systemic investigation of the effects of the withdrawal of medication on the EEG of epileptic patients. This is a rather common procedure in clinical EEG, especially in epileptics who are potential candidates for surgical treatment, whenever their on-medication tracings are persistently negative or when it is important to witness and record a clinical attack. From a purely electrographic standpoint, the rationale for this procedure is that the anticonvulsant medication depresses the epileptogenic process and attenuates or abolishes its EEG manifestations, thus preventing its identification and localization. The results of this study (see individual Report) indicate that the procedure is indeed a useful one for practical diagnostic purposes in certain cases. The results also show, however, that the "activation" effects of medication withdrawal are not always simple; their interpretation may often be difficult and they may actually be misleading. In fact, 4 different types of changes could be identified in the off-medication records of 55 patients, including a characteristic "non-specific" pattern, which appears to be deprived of any diagnostic value.

3. Other Activity

The Chief of the Branch has been invited to contribute to the Clinical Neurosciences Section of the NINCDS 25th Anniversary Volume for which he has prepared the Chapter on Clinical Electroencephalography. He and one of his associates have participated

with a formal presentation in two Symposia at the 1974 Seattle Meeting of the American EEG Society and the St. Louis Meeting of the Society for Neuroscience. The Chief of the Branch is also officially and actively involved in editorial duties of several specialized Journals (Electroenceph. clin. Neurophysiol; Epilepsia; Arch. Ital. Sci. Biol.) and is co-editor of the Handbook of EEG and Clinical Neurophysiology which consists of about 30 volumes. He has official responsibility in the administrative and scientific activities of several societies and as Past President and current member of the Executive Committee of the International Federation of Societies for Electroencephalography and Clinical Neurophysiology, he is actively involved in the organization and scientific program of the IX International Congress of said Federation to be held in Amsterdam (The Netherlands) in 1977.

Project No. Z01 NS 01939 04 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Fluorometric monitoring of intracellular redox changes in human cerebral cortex

Previous Serial Number: 1939b

Principal Investigator: D.V. Lewis, M.D.

Other Investigators: W.H. Schuette, W.C. Whitehouse, M. O'Connor, M.D. and J.M. Van Buren,M.D.

Cooperating Units: Surgical Neurology Branch, IR, NINCDS. Biomedical Engineering Branch of Div. of Research Services, Television Engineering Section of Clinical Center

Man Years:

Total:	0.25
Professional:	0.1
Other:	0.15

Project Description:

Objectives: Use of the television fluorometry system previously developed to study differences in oxidative response to electrical stimuli in normal and abnormal human cortex.

Methods Employed: Using the previously described television system, fluorescence changes are recorded on video tape over a wide area of brain which has been exposed in the course of neurosurgical procedures. Energy metabolism is evaluated through analysis of transients both spontaneously occurring and induced by electrical stimulation.

Major Findings: In addition to the previous findings this rate of oxidation induced in human cortex by electrical stimulation has been found to be proportionate to the stimulus intensity. Metabolic changes are localized within 1-2 cm of the stimulated area. No increases in oxidation rate have been observed during spontaneous interictal activity in the human cortex.

Proposed Course of the Project: Project to be completed in this FY. One paper already published; a second publication dealing the patient data being prepared.

Keyword Descriptors: NADH - Redox - cerebral cortex - man - television fluorometry - metabolism - epilepsy

Honors and Awards: None

Publications: Schuette, W.H., Whitehouse, W.C., Lewis, D.V., O'Connor, M. and Van Buren, J.M.: A television fluorometer for monitoring oxidative metabolism in intact tissue. Med. Instrument. 1974, 8:331-333.

Project No. Z01 NS 02012 03 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of abnormal neuronal responses during the evolution of an epileptic focus in feline visual cortex

Previous Serial Number: 2012

Principal Investigators: J.S. Ebersole, M.D. and R.A. Levine,M.D.

Other Investigator: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.15
Other:	0.05

Project Description:

Objectives: To characterize the development of the abnormal paroxysmal depolarization shift (PDS) in visual cortex neurons during the induction of an epileptic focus in the surrounding brain.

Methods Employed and Major Findings: After defining the receptive fields of single units in cortical area 17 of anesthetized cats, recurrent on-off stimulation with bars of light of optimal configuration was presented during the induction of an epileptic focus by iontophoresis of penicillin from a second micropipette.

Progressively, three distinct alterations of neuronal activity developed. The most long-lasting and usually the earliest abnormality was an increase in the number and frequency of spikes comprising a neuron's response to stimuli that were effective prior to iontophoresis. This enhanced physiologic response (EPR) could be elicited from a cell independently of the discharge activity of an induced focus, but only with stimuli

appropriate for the cell's receptive field.

With additional iontophoresis an entirely new response developed, which was consistent with an extracellular paroxysmal depolarization shift (PDS). This high-frequency burst of spikes appeared only in association with an ECoG interictal potential. It could be triggered,however, by stimuli which were previously effective or ineffective, as well as occur spontaneously. Characteristics which further distinguished the PDS from EPR included a longer and more variable latency, a longer recovery period, and a different sensitivity to changes of stimulus intensity.

A period of response inhibition also accompanied each interictal potential and persisted with a variable duration afterward. It was most noticeable as an interruption in the activity of tonically responding neurons and was often present before the cell began to generate PDSs.

It was concluded that the EPR represents a direct effect of penicillin on the cell or its immediate synaptic connections, while the PDS appears dependent on the altered interaction within a population of such affected cells. The inhibitory phenomenon, in addition, seems a result of projected influences from cells more fully involved with the developing focus.

Proposed Course of the Project: This project has been completed.

Keyword Descriptors: Visual cortex - cat - experimental epilepsy - neuronal activity - penicillin

Honors and Awards: None

Publications: Ebersole, J.S. and Levine, R.A.: Abnormal neuronal focus in cat visual cortex. J. Neurophysiol. 1975, 38: 250-265.

Project No. Z01 NS 02014 03 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Clinical seizure patterns in patients with occipital epileptogenic foci

Previous Serial Number: 2014(c)

Principal Investigators: B.I. Ludwig, M.D. and C. Ajmone Marsan, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.45
Professional:	0.4
Other:	0.05

Project Description:

Objectives: To analyze the various sensory, motor autonomic and other symptoms which characterize the ictal episodes in patients with electrographic evidence of epileptiform activity in the occipital and para-occipital regions.

Methods Employed and Major Findings: Seizure patterns and other clinical features are analyzed in 55 epileptic patients with electrographic evidence of exclusive or predominant occipital involvement. Few statistically significant differences in clinical or ictal patterns were found between subjects whose EEGs revealed purely focal occipital involvement and those with temporal and temporo-parietal spread or minor additional independent foci. On the other hand, cases with bilateral synchronous occipital spike activity appeared to reflect a different type of epileptic disorder. Clinical pleomorphism was more apparent than is commonly conceived; thus, although the incidence of visual auras was relatively high (47%), epigastric, psychic, somatic and other sensory phenomena were not infrequently encountered.

Ictal motor patterns were most commonly (53%) non focal (grand mal) or absent (momentary lapses of consciousness) but partial or focal motor attacks and psychomotor seizures were amply represented. Ictal features with their localizing and lateralizing reliability were also analyzed and discussed in relation to those found in a companion study of seizures of fronto-centro-parietal origin.

Proposed Course of the Project: This project is completed.

Keyword Descriptors: Electroencephalography - epilepsy - occipital cortex - seizure patterns.

Honors and Awards: None

Publications: Ludwig, B.I. and Ajmone Marsan, C.: Clinical ictal patterns in epileptic patients with EEG occipital foci. Neurology 1975 (In press)

Project No. Z01 NS 02094 02 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Use of a television fluorometer to study simultaneous oxidative and K^+ changes in primary and dependent mirror epileptogenic foci

Previous Serial Number: 2094

Principal Investigator: D.V. Lewis, M.D.

Other Investigators: W. H. Schuette and W. C. Whitehouse

Cooperating Units: Biomedical Engineering Br anch of Div. of Research Services, Television Engineering Section of Clinical Center

Man Years:

Total:	0.25
Professional:	0.1
Other:	0.15

Project Description:

Objectives: To semiquantitatively study the changes in oxidation rate and K+ release accompanying interictal activity in both a primary cortical epileptogenic focus (penicillin induced) and the contralateral cortical focus of projected (mirror) activity. Possibly to study as well, oxidative changes with generalized epileptiform discharges.

Methods Employed: The previously described television fluorometer records fluorescent images of the dorsal surfaces of the two cerebral hemispheres. A penicillin focus of epileptiform activity is established in a hemisphere and bilateral ECoG leads monitor this activity from the primary focus as well as any contralateral homologous (projected) activity. Simultaneous oxidative (NADH fluorescence) changes are studied in the primary and projected foci. As often as possible K^+ sensitive microelectrodes are used simultaneously to record extracellular K^+

changes as well. The data are stored on video tape and analyzed at a later time.

Major Findings: Consistent transient increases in both oxidation rate and extracellular K^+ accompanying interictal spike activity in the primary focus. Projected epileptiform sharp waves are seen in the contralateral homologous area of about half the voltage of the primary spikes, however, no increase in either oxidation rate or extracellular K^+ has yet been observed with the projected sharp waves, even using signal averaging techniques.

Proposed Course of the Project: Publication in course of preparation. Project is completed.

Keyword Descriptors: NADH - Potassium - metabolism - Cerebral cortex - Cat-experimental epilepsy - mirror foci - television fluorometry.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02095 02 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Simultaneous monitoring of changes in extracellular K^+ concentration and oxidative metabolism in intact cat hippocampus and cerebral cortex

Previous Serial Number: 2095

Principal Investigator: D.V. Lewis, M.D.

Other Investigator: W.H. Schuette

Cooperating Units: Biomedical Engineering Branch of Div. of Research Services, Television Engineering Section of Clinical Center

Man Years:

Total:	0.2
Professional:	0.15
Other:	0.05

Project Description:

Objectives: To study the relationship between active Na^+ - K^+ transport and oxidative metabolism in intact neural tissues during electrical stimulation, evoked responses and epileptiform activity.

Methods Employed and Major Findings:

1. Short (2 s) trains of stimuli were applied to the dorsal hippocampal surface of cats, producing an increase in $[K^+]_o$ and a decrease in NADH fluorescence (the latter being indicative of an increase in tissue oxygen utilization).

2. The $[K^+]_o$ rose rapidly during stimulation ($\Delta [K^+]_o$ values from 1 to 6 mM) with larger stimulus currents producing larger changes. The time course of the poststimulus decline of $[K^+]_o$ was an exponential decay function, with $T_{\frac{1}{2}}$ values varying

from 1.3 to 6.9 s, and independent of the magnitude of the $\Delta[K^+]_o$. Consistent undershoots of $[K+]_o$ occurred following stimuli causing >1 mM change in $[K^+]_o$.

3. The maximum depression of fluorescence and the time integral of the fluorescence changes following each stimulus train were both highly correlated with the total increase of $[K^+]_o$ occurring during the stimulus train.

4. Application of several stimulus trains in close succession resulted in more rapid potassium reuptake following the later trains and an unusually large undershoot after the last train. Concomitantly, there was a progressive decrease in the fluorescence level.

5. When afterdischarges were induced by prolonged (>2 s) stimulation, larger and more sustained increases in $[K^+]_o$ and decreases of fluorescence were observed, and there was some indication that afterdischarges were followed by accelerated reuptake of extracellular potassium.

Proposed Course of the Project: This project is completed.

Keyword Descriptors: Potassium - NADH - cat-hippocampus-electrical stimulation - oxidative metabolism - afterdischarge

Honors and Awards: None

Publication: Lewis, D.V. and Schuette, W.H.: NADH fluorescence and $[K^+]_o$ changes during hippocampal electrical stimulation. J. Neurophysiol. 1975, 38:405-417.

Project No. Z01 NS 02096 02 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effects of electrical stimulation of the ponto-mesencephalic reticular formation and of Metrazol seizures on the electrical and metabolic activity of cortical areas in the acute cat.

Previous Serial Number: 2096

Principal Investigator: B. Vern, M.D., Ph.D.

Other Investigators: W. C. Whitehouse, W.H. Schuette

Cooperating Units: Biomedical Engineering Branch of Div. of Research Services, Television Engineering Section of Clinical Center

Man Years:

Total:	0.65
Professional:	0.5
Other:	0.15

Project Description:

Objectives: As reported last year, NADH fluorometry was carried out over the exposed cat cortex during the reticulo-cortical activation. Reflected ultraviolet light (UV), which was used as a reference for NADH, was found to be unreliable because of motion-induced specular UV changes during large increases in cortical blood flow. Sodium fluorescein fluoresence was substituted as a reference signal. NADH oxidation was then studied over various cortical areas during brain stem stimulation and Metrazol-induced seizures.

Methods Employed: Adult cats were anesthetized with sodium thiopental, immobilized with Flaxedil and artifically ventilated. The exposed cortex was illuminated with UV. After i.v. injection of fluorescein the cortex was viewed by a T.V. camera equipped with an image intensifier, and a filter beam splitter. Two

video signals were obtained: one through NADH filters (440-480 nM), one through a fluorescein interference filter (529 nM). These signals were measured differentially by means of video densitometry in several animals in order to obtain a measure of average relative changes in cortical blood flow. Cortical activation was induced either by high frequency brain stem reticular stimulation or by Metrazol-induced seizures.

Major Findings:

1. Sodium fluorescein fluorescence proved to be a reliable new reference for NADH during large increases in cortical blood flow. There was a 1:1 match between the NADH and fluorescein channels during hemodilution.

2. NADH oxidation was not uniform over the cortical surface during either reticulo-cortical activation or during Metrazol seizures. The distribution of this variability was inconsistent from animal to animal.

3. The initial time course of changes in sagittal sinus blood flow matched the usually homogeneously distributed fluorescein transients. Fluorescein changes tended to outlast the sagittal flow curves however.

Proposed Course of the Project: Attempts will be made to more clearly define the relationship between surface fluorescein transients and changes in sagittal sinus blood flow.

Keyword Descriptors: NADH - television fluorometry - fluorescein - cerebral cortex - cat - reticular formation - Metrazol - experimental epilepsy - metabolism - arousal response - cortical blood flow

Honors and Awards: None

Publications: None

Project No. Z01 NS 02121 01 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: $[K^+]_o$ clearance in epileptogenic glial scars of Alumina cream foci in Macaca Mulatta

Previous Serial Number: None

Principal Investigator: D.V. Lewis, M.D. and N. Mutsuga, M.D.

Other Investigator: W.H. Schuette

Cooperating Unit: Biomedical Engineering Branch of Div. of Research Services, Television Engineering Section of Clinical Center

Man Years:

Total:	0.5
Professional:	0.35
Other:	0.15

Project Description:

Objectives: To correlate any observed changes in $[K^+]_o$ reuptake with the degree of fibrous astrocytic proliferation in or near the epileptogenic lesions induced by $Al(OH)_3$ injection in the monkey motor cortex.

Methods Employed: $[K^+]_o$ measurement is done as in Project Number 02122. $[K^+]_o$ changes are induced by direct cortical stimulation. Brains are perfused, fixed and fibrous astrocytes and neurons are quantitated utilizing standard neuropathological techniques. The observed $[K^+]_o$ kinetics are then correlated with the amount of fibrous astrocytes and/or neurons observed in the region of measured $[K^+]_o$ kinetics.

Major Findings: There appears to be a correlation between astrocytic fibrosis and potassium clearance, as $[K^+]_o$ is cleared more slowly in densely fibrotic areas. No correlations with neuron counts are yet apparent.

Proposed Course of the Project: Confirm the above findings and publish results. Project is expected to be completed within the coming fiscal period.

Keyword Descriptors: Potassium - gliosis - experimental epilepsy - cerebral cortex - monkey

Honors and Awards: None

Publications: None

Project No. Z01 NS 02122 01 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effect of varied temperature on $[K^+]_o$ uptake in the hippocampus

Previous Serial Number: None

Principal Investigator: D.V. Lewis, M.D.

Other Investigator: W. H. Schuette

Cooperating Unit: Biomedical Engineering Branch of Div. of Research Services, Television Engineering Section of the Clinical Center

Man Years:

Total:	0.35
Professional:	0.2
Other:	0.15

Project Description:

Objectives: To determine the Q_{10} of potassium reuptake in the cat hippocampus following direct electrical stimulation. The speed of $[K^+]_o$ clearance during neuronal activity is important to the function and excitability of the neuronal population. Likewise this $[K^+]_o$ clearance may be impaired in epileptogenic tissue, hence knowing the mechanism of $[K^+]_o$ clearance could be helpful in understanding normal and abnormal neural function.

Methods Employed: Double micropipettes; one referential and one K^+ resistive (Corning ion exchanger-liquid) are employed to measure on line changes in extracellular K^+ activity during and following short trains of direct electrical stimulation. The hippocampus is cooled by superfusing the surface with artificial C.S.F. of varying temperatures and hyppocampal surface temperature is monitored with a thermocouple.

Major Findings: Q_{10} of potassium reuptake is 2.1, consistent with a metabolic process.

Proposed Course: Project is completed and a paper is being prepared for publication.

Keyword Descriptors: Potassium - hippocampus - temperature-cat

Honors and Awards: None

Publications: None

Project No. Z01 NS 02123 01 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Fluorometric and oxygen consumption changes in the cat cortex

Previous Serial No.: None

Principal Investigator: D.V. Lewis, M.D.

Other Investigator: W.H. Schuette

Cooperating Unit: Biomedical Engineering Branch of Div. of Research Services, Television Engineering Section of Clinical Center

Man Years:

Total:	0.35
Professional:	0.2
Other:	0.15

Project Description:

Objectives: To correlate oxygen consumption, blood flow and Hgb saturation with NADH fluorescence changes in the cat cortex during direct cortical stimulation. If the NADH fluorescence changes can be correlated with separate on line measures of O_2 consumption this will strengthen and clarify the relationship between fluorometric changes and O_2 consumption rates. In addition, with $[K^+]_o$ (see below) and NADH monitoring along with flow, some information on the local regulation of cerebral blood flow may be obtained.

Methods Employed: Intracellular redox changes as indicated by changes in the ratio of oxidized to reduced nicotine adenine dineucleotide are monitored fluorometrically in the superficial 1 mm of the cat cerebral cortex. Blood flow from the monitored area is measured by a drip meter from the cannulated sagittal sinus. Hgb saturation is measured continuously in the cannula by means of a miniature oximeter.

Major Findings: With direct cortical stimulation of both hemispheres simultaneously an increase in blood flow is seen with a transient decrease in venous saturation followed by an increase of saturation. The oxygen consumption calculated from these two parameters (arterial Hgb saturation is constant) agrees well in relative magnitude and time course with the simultaneously monitored NADH changes.

Proposed Course of the Project: Continue as above and if possible monitor $[K^+]_o$ simultaneously.

Keyword Descriptors: NADH - oxygen - cerebral cortex - cat - cerebral blood flow - redox

Honors and Awards: None

Publications: None

Project No. Z01 NS 02124 01 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Measurements of cortical oxygen consumption, in relation to NADH oxidation during reticulo-cortical activation and Metrazol seizures

Previous Serial Number: None

Principal Investigator: B. Vern, M.D., Ph.D.

Other Investigators: W. H. Schuette, and W.C. Whitehouse

Cooperating Units: Biomedical Engineering Branch of Div. of Research Services, Television Engineering Section of Clinical Center

Man Years:

Total:	0.75
Professional:	0.5
Other:	0.25

Project Description:

Objectives: To study the relationship between cortical oxygen uptake and NADH oxidation during cortical activation.

Methods Employed: Cats are anesthetized, paralyzed and artificially ventilated. The cortex is prepared and video densitometry is set up as described in Project Number 02096. A polyethylene cannula is inserted into the superior sagittal sinus and passed through an oximeter. Sagittal blood flow and blood oxygen saturation is continuously monitored, enabling the calculation of oxygen uptake from the sagittal sinus blood. Graded high frequency stimuli are delivered to the brain stem reticular formation. Electrographic seizures are induced with i.v. Metrazol.

Major Findings: Reticulo-cortical activation induces a transient hypertensive response and an increase in cerebral oxygen uptake. This oxygen uptake curve frequently shows a bimodal pattern. Surface cortical NADH oxidation, on the other hand, is generally monotonic. Fluorescein transients are either monotonic or bimodal. Metrazol seizures are accompanied by monotonic NADH, fluorescein, and oxygen uptake transients.

Proposed Course of the Project: The drainage areas of the superior sagittal sinus must be somehow delineated. It is possible that a portion of the oxygen uptake curves following brain stem stimulation might be artifactually produced, e.g. A-V shunting induced by the hypertensive response. In order to resolve this question, blood pressure changes will be eliminated by ganglionic blockade.

Oxygen uptake curves will be studied as a function of pre-stimulation or pre-seizure sagittal oxygen saturation levels, blood pressure, and baseline NADH levels.

Keyword Descriptors: NADH - cerebral blood flow - oxygen - cerebral cortex - cat - reticular formation - Metrazol - experimental epilepsy

Honors and Awards: None

Publications: None

Project No. Z01 NS 02125 01 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Initial abnormalities of neuronal responsiveness in a developing epileptogenic focus

Previous Serial Number: None

Principal Investigator: J.S. Ebersole, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	0.95
Other:	0.25

Project Description:

Objectives: To characterize the earliest neuronal response abnormalities induced by penicillin in a developing epileptic focus. More specifically, to further investigate at the origin of the focus the previously identified initial cellular abnormality, the enhanced physiologic response (EPR), and its relationship to more fully developed epileptic activity, the paroxysmal depolarization shift (PDS).

Methods: Cats are anethetized (Pentobarbital), paralyzed (gallamine) and artificially respired. Temperature, blood pressure and expiratory CO_2 are monitored and maintained in physiologic range. Eyes are refracted and vision corrected with lenses. Double barreled micro-pipettes are positioned in the visual cortex (area 17). The distal electrode records extracellular electrical activity. From the proximal electrode tip (approx. 60 um distance) penicillin G is iontophoresed in the immediate vicinity of the recorded cell. A silver ball electrode records the ECoG. Receptive fields of the visual neurons are

characterized before penicillin iontophoresis. Recurrent preferred and non-preferred projected stimuli are presented before, during, and after introducing penicillin around the recorded cell.

Major Findings: Penicillin has a direct effect upon neuronal responsiveness, which appears to be a pre-requisite for the development of epileptic activity. The initial abnormality induced by the iontophoresis of penicillin in nano-amp quantities (20-60 na) around single cells was an enhancement of the cell's physiologic response to preferred stimuli (EPR). A progressive increase in number and frequency of spikes followed step-wise advances in the rate of iontophoresis. These alterations were reversible within minutes after ending iontophoresis, repeatable multiple times, and occurred without evidence of interictal potentials. Both simple and complex visual neurons were affected, but preferentially the initial group of spikes in the more tonic simple cell response developed into an EPR. At higher rates of penicillin iontophoresis (60-100 nA) several additional abnormalities became apparent - 1) longer lasting responses to previously non-preferred stimuli and 2) multiple, additional responses following the EPR to a single stimulus. Characteristically, at high rates of iontophoresis (100 + nA) an extra response immediately following the EPR became similarly enhanced and was associated with a larger local slow potential and a longer period of subsequent response inhibition. It could also be evoked by previously non-preferred stimuli without the presence of a preceding EPR. These properties are similar to those of the classic interictal cellular phenomenon, the PDS.

The EPR appears to represent a primary alteration in the properties of an individual neuron or its synaptic connections due to a direct effect of penicillin. It is not dependent upon the interaction of a cell population or is necessarily associated with interictal epileptic activity. Its properties are distinct from those of a PDS. The multiple, additional responses following an EPR at higher rates of iontophoresis suggest an increased efficacy of secondary input to the recorded cell, probably of collateral and/or recurrent nature. The prominent secondary response which develops following the EPR at high rates of iontophoresis is probably the "larval" form of a PDS. It possesses several properties of the classic PDS. It would thus appear that penicillin induces EPRs in individual cells, which provide for each other through similarly

enhanced recurrent and collateral pathways the secondary input needed to generate a PDS.

These new observations help clarify the cellular mechanisms underlying the penicillin model of epilepsy. They suggest that the PDS is a population dependent phenomenon although the initial prerequisite is an abnormality of individual cells.

Proposed Course of the Project: This project is completed. Results are in the process of being prepared for publication.

Keyword Descriptors: Visual cortex - cat - experimental epilepsy - penicillin - neuronal activity

Honors and Awards: None

Publications: Ebersole, J.S.: Direct epileptogenic effects of penicillin upon single visual cortex neurons. Fed. Proc. 34:446, 1975. (Presented at the FASEB Meeting in Atlantic City on April 16, 1975).

Project No. Z01 NS 02126 01 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cerebral seizures of probable orbito-frontal origin

Previous Serial Number: None

Principal Investigators: B.I. Ludwig, M.D., and C. Ajmone Marsan, M.D.

Other Investigators: J.M. Van Buren, M.D.

Cooperating Units: Branch of Neurological Surgery, NINCDS

Man Years:

Total:	0.55
Professional:	0.4
Other:	0.15

Project Description:

Four patients with seizures of presumed temporal lobe origin are presented, in whom a definite focal ictal onset in the orbito-frontal cortex was revealed during recording, either by chronically implanted electrodes or ECoG. In three cases automatisms occurred concomitantly with orbito-frontal activation without spread of paroxysmal activity into temporal structures monitored. With eight additional cases of possible orbito-frontal epilepsy found on reviewing the literature, two subgroups emerge: a) patients with primary psychomotor type fits, and b) patients with loss of consciousness, head and eye deviation and generalized convulsions. Scalp EEGs in patients on whom we have available data, manifested bilaterally synchronous, paroxysmal discharges which were bifrontal, frontopolar or maximal in one anterior quadrant, with or without evidence of additional temporal lobe involvement. On the basis of anatomical and physiological studies, as well as our own electrographic data, it is felt that a posterior

orbito-frontal and temporo-limbic, or mesial orbito-frontal and parasagittal-limbic relationship exists, within which, autonomous epileptogenic zones may develop, with the ability to discharge directly and independently to subcortical centers, while eliciting similar clinical patterns.

Proposed Course of the Project: This project is completed and a paper has been published.

Keyword Descriptors: epilepsy - electroencephalography - orbito-frontal cortex - depth electrography

Honors and Awards: None

Publications: Ludwig, B.I., Ajmone Marsan, C and Van Buren, J.M.: Cerebral seizures of probable orbito-frontal origin. Epilepsia, 1975, 16: 141-158.

Project No. Z01 NS 02127 01 EEG
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: EEG changes after withdrawal of medication in epileptic patients.

Previous Serial Number: None

Principal Investigator: B.I. Ludwig, M.D. and C. Ajmone Marsan, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.55
Professional:	0.4
Other:	0.15

Project Description:

Fifty five patients with intractable partial seizures whose on-medication EEGs demonstrated either predominantly focal epileptiform lesions or absence of paroxysmal activity, were studied, and the effect of withdrawing all anticonvulsive drugs on their EEGs was observed. Four types of responses were encountered: (1) no effect (20%), (2) specific (focal) activation (25%), (3) complex activation (29%), with wide spread of the initial on-medication focus or appearance of additional independent epileptogenic foci and (4) "non specific" activation (63%), consisting of bursts of either bilaterally synchronous and frontally dominant spike and waves, triphasic waves, or sharp slow complexes, or smaller amplitude rapid and diffuse spike and wave complexes. This latter effect is thought to be secondary to metabolic derangements resulting from the withdrawal of neurotropic agents and not directly related to the specific epileptogenic process. No association was found between type of effect and any of the following parameters:

topography of on-medication focus, duration off therapy, type of anticonvulsant used, suspected underlying etiopathology, or median age when medication was withdrawn. Furthermore, no evidence could be found that the development of a "complex" or "non-specific" EEG effect carried with it a bad prognosis for surgical cure following focal cortical excision.

Performing off-medication tracings seems to be of greatest value in patients with partial seizures and EEGs revealing either a relative paucity or definite absence of epileptiform discharges. The occurrence of a "non-specific" response in a questionable epileptic during the off-medication period, on the other hand, should be interpreted with caution.

Proposed Course of the Project: This project is completed and a paper is currently in press.

Keyword Descriptors: epilepsy - electroencephalography
anticonvulsant medication

Honors and Awards: None

Publications: Ludwig, B.I. and Ajmone Marsan, C.: EEG changes after withdrawal of medication in epileptic patients. Electroenceph. clin. Neurophysiol. 1975, 39: In press.

LAB. OF BIOPHYSICS

LAB. OF DEVELOP. & METAB. NEUROL. BR.

LAB. OF NEUROPATHOLOGY & NEUROANATOM., SC.

LAB. OF NEURAL CONTROL

LAB. OF NEUROPHYSIOLOGY

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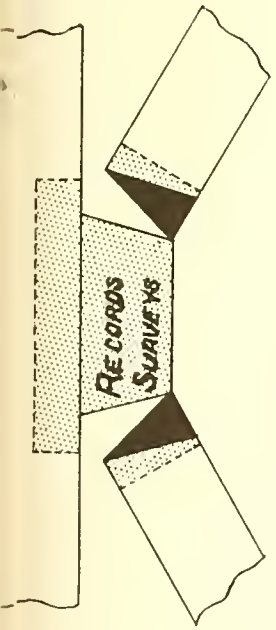


TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1974 through June 30, 1975
Developmental and Metabolic Neurology Branch
National Institute of Neurological and Communicative Disorders and Stroke
Roscoe O. Brady, Chief

The Branch has continued its high level of originality and productivity in several areas pertaining to disorders of the nervous system. Many of the discoveries have immediate application for the delivery of health care to the community including enzyme replacement therapy for lipid storage diseases, the development of reagents for the diagnosis and genetic counseling for inherited diseases, and for the treatment of epilepsy. Other experiments offer promise for obtaining insight into the control of inherited disorders of anabolism, neoplastic diseases, multiple sclerosis, mucopolysaccharidoses, and kinky hair disease, an inherited copper-deficiency syndrome. We expect that further investigations of the effect of enzyme replacement on lipids deposited in the circulatory system in patients with Fabry's disease will provide a useful model for the amelioration of conditions which predispose humans to strokes. These investigations cover a wide but related base for the control and therapy of a number of human disorders; therefore, this report is divided into the following categories.

I. Enzyme Replacement Therapy for Inherited Diseases.

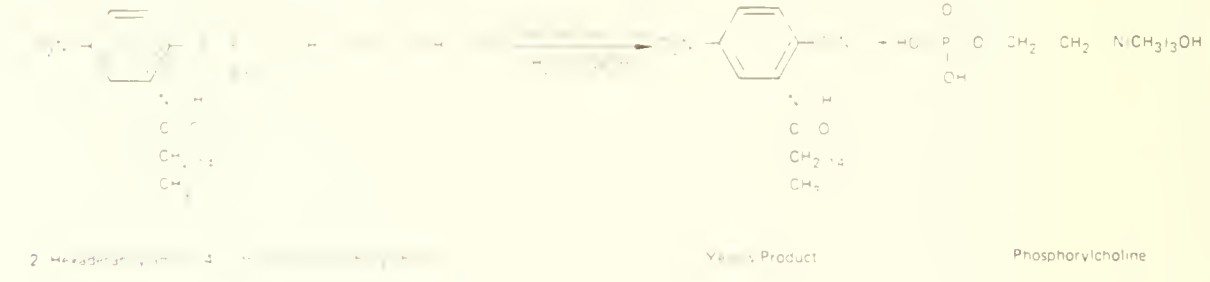
We have found that the effects of administration of glucocerebrosidase to patients with Gaucher's disease persist over a surprisingly long period of time. The first patient who received this enzyme showed a decrease in the quantity of glucocerebroside in his erythrocytes from a three-fold elevation to the normal level within 72 hours after the enzyme was injected. The amount of glucocerebroside in the red blood cells remained within normal range for a period of several months. One year after administration of the enzyme, the erythrocyte glucocerebroside was still 50% below the preinfusion level. A similar long-term effect on glucocerebroside in erythrocytes was seen in the second patient who received exogenous glucocerebrosidase. Our calculations indicate that the injected enzyme caused the clearance of a quantity of glucocerebroside from the liver of the first patient which had accumulated over a four year period. In the second patient whose disease was somewhat less severe and who received slightly more than twice the amount of glucocerebrosidase than the first patient, we found that the enzyme caused the clearance of more than 13 years of accumulated glucocerebroside. These observations support our belief that enzyme replacement therapy for Gaucher's disease is likely to be highly beneficial for patients with this disorder.

We have recently obtained data which substantiate and extend our finding that the administration of active exogenous enzyme to patients who lack a particular enzyme causes an activation of the patient's mutated catalytically ineffective enzyme. This activation was originally observed when we infused ceramidetrihexosidase into a patient with Fabry's disease. We have now confirmed this important observation in a patient with Tay-Sachs disease. When hexosaminidase A was injected into the patient, we found 2.4 times more enzyme activity in the recipient's liver an hour after the enzyme was administered than had actually been infused. The most logical explanation of

these unexpected salutary effects is that the exogenous enzymes bring about an alteration of the configuration of the mutated enzymes which restores catalytic activity to the defective enzymes. This important discovery provides much additional encouragement for enzyme replacement therapy for inherited diseases of metabolism.

II. Synthesis of Diagnostic Reagents

We have made a major breakthrough in the diagnosis of the sphingolipidoses. Previously Niemann-Pick disease and Krabbe's disease could not be diagnosed except by the use of radioactively labeled sphingomyelin and galactocerebroside respectively. These substrates are not widely available, and most clinical chemistry laboratories are either unwilling or are not equipped to perform the necessary radioactive analyses for the diagnosis of these patients, the detection of heterozygotes, or monitoring pregnancies at risk for these disorders. We have now synthesized a chromogenic analog of sphingomyelin called 2-hexadecanoylamino-4 nitrophenyl-phosphorylcholine which is an absolutely reliable diagnostic reagent for the detection of Niemann-Pick disease and the identification of heterozygous carriers of this disorder.



We are confident that a similar chromogenic analog of galactocerebroside in which the phosphorylcholine moiety is replaced with a molecule of galactose will be useful for similar determinations in Krabbe's disease. The synthesis of the latter compound is underway at this time in our laboratory. Chromogenic assays with these substrates are so facile that any clinical chemistry laboratory equipped with a colorimeter or spectrophotometer can perform these tests and we expect that these diagnostic reagents will experience wide usage when they become generally available.

III. Malignant Transformation and Heritable Disorders of Anabolism

Our work on alterations of glycolipid and glycoprotein metabolism in tumorigenic cells has continued in a productive fashion. In collaboration with the Laboratory of Molecular Biology, IR, NINCDS, we have found the phenotypic and enzymatic changes in HeLa cells which form neurite-like processes and become sessile upon the addition of short chain fatty acids to the tissue culture medium depends upon a complex interaction involving calcium

ions which most likely regulates the intracellular assembly of microtubules. We are exploring the relationship between this phenomenon and the induction of ganglioside synthesis which occurs in both HeLa and neuroblastoma cells when they are grown in the presence of short chain fatty acids.

Other investigations have been carried out on glycopeptide changes in cells transformed with the Kirsten murine sarcoma virus and Wooley monkey sarcoma virus, a tumorigenic primate RNA virus. There are conspicuous differences in the effects of these viruses on glycoprotein metabolism and this type of experimental metabolic dissection should provide additional insight into the role of glycoproteins and glycolipids in tumorigenic transformation of cells. In addition we are continuing our investigations of anabolic lesions of glycolipid and glycoprotein metabolism on the development of the nervous system and the effects of such alterations on CNS function.

IV. Myelination

Work on myelin-specific glycoproteins in the central and peripheral nervous system discovered by this laboratory has accelerated and a number of important findings were made in FY 75 concerning these substances. Procedures were developed for the isolation of these glycoproteins in small quantity and the major carbohydrate residues were identified. The precise quantitation of these components is not yet available because of the extremely limited supply of the pure proteins. The myelin-specific glycoprotein isolated from the central nervous system has been shown to be antigenic. We must now determine whether the anti-glycoprotein antibody is myelinotoxic and whether it will cause encephalomyelitis in suitable test animals.

Other work along these lines has revealed that the CNS myelin glycoprotein is a surface component and is therefore highly susceptible to virus attachment and/or alteration of its structure by viral enzymes. The larger immature form of the glycoprotein persists to an older age in copper-deprived animals and in the hypothyroid state. Both conditions are associated with hypomyelination. Administration of hexachlorophene *in vivo* causes fragmentation of the myelin sheath and myelin glycoprotein appears to be one of the most labile and earliest altered myelin component. These findings are strongly indicative of a critical role of this glycoprotein in the developing central nervous system. Its proper metabolism seems to be particularly related to the extent to which myelination occurs and the eventual formation of mature myelin sheathes.

V. Epilepsy

A method has been developed for obtaining a complete profile of the levels of anti-epileptic drugs and their metabolites in serum, cerebrospinal fluid, and the urinary excretion of these compounds. This investigation was carried out in order to place the treatment of epileptics on an objective basis rather than purely clinical estimation. Various individuals metabolize anticonvulsants differently and at varying rates. It is possible to see at a glance from the metabolic profile whether a drug was administered

in correct amount, in excess, or in too small a dose. It was found that children metabolize anticonvulsant drugs 2 to 3 times faster than adults. In addition, diphenylhydantion does not influence the metabolism of phenobarbital, and phenobarbital probably does not influence the metabolism of other anticonvulsants in chronically administered combined medications. This finding is in contrast to contrary statements in the literature derived from other investigations. In a number of patients with epilepsy, high doses of anticonvulsants cause a significant slowing of mental performance. In children this impairment can be a greater handicap than the advantage gained from a slight decrease in the frequency of seizures. We therefore advocate a comprise consisting of an optimal dose of anticonvulsants for each individual patient which will adequately control his seizures yet will not excessively impair his mental performance. This regimen can be established by assessing mental performance while the patient receives consecutively varying doses of a particular type of medication.

VI. Mucopolysaccharidoses

We have demonstrated that only a portion of the patients classified as having the Scheie syndrome have a deficiency of α-L-iduronidase activity which is also seen in Hurler patients. Therefore, the Scheie syndrome is a heterogenous condition. We have reported for the first time pathological findings in patients with the Scheie syndrome who are deficient in α-L-iduronidase. All of the major organs and tissues except the brain had lesions which were similar to those reported for patients with the Hurler syndrome. The neurons of the patient with Scheie syndrome were normal in contrast to patients with the Hurler syndrome whose neurons are in a state of degeneration and are distended with lipid-staining material. This finding provides an anatomical basis for the normal intellect in patients with the Scheie syndrome in spite of the marked reduction of α-L-iduronidase activity.

VII. Kinky Hair Disease

We have devised a treatment for patients with kinky hair disease, an inborn error of copper metabolism. Using labelled copper ^{67}Cu, we demonstrated that the absorption of copper from the gut is in the range of 11 to 13% of normal in patients with this disorder. However, once absorbed, the biological half-life of copper is three to four times longer than normal. We presume this effect is an atnempt to conserve and recycle copper by these patients. We have developed a protocol for treating kinky hair disease which consists of weekly subcutaneous slow-drip infusion of dilute solutions of copper sulfate. This procedure avoids toxic damage to tissues. Previous methods involving intravenous infusion of large volumes of fluids to small infants proved impractical for ambulatory maintenance therapy.

VIII. Stroke

We have continued our examinations of blood lipid components in stroke patients to try to identify predisposing factors in the circulation which contribute to the occurrence of cerebrovascular accidents. We have devised an experimental model for stroke therapy based on enzyme replacement

in patients with Fabry's disease who are prone to strokes. These investigations should provide an indication whether the arteriosclerosis which is widespread in these individuals is reversible by a specific lipid-catabolizing enzyme.

IX. Award

The Branch was honored in FY 75 by the election of the Chief of the Branch to membership in the National Academy of Sciences of the United States.

Project No. Z01 NS 00706-16 DMN

1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Genetic, Clinical, Biochemical and Pathological Studies of Progressive Cerebral Degenerations, Mental Retardation and Birth Defects; with Emphasis on Familial Occurrence.

Previous Serial Number: NDS(I)-60 DMNB/CIT 706(c)

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Norio Sakuragawa, M.D., George Constantopoulos, Ph.D. and Jan K. Steusing

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.1
Other:	0.4

Project Description:

Objectives: The majority of chronic neurological disorders affect patients from their early years of life and constitute one of the greatest medical and social problems of our times. According to conservative estimates for this country, over four million people are permanently handicapped by mental retardation (frequently of familial type), birth defects, and metabolic (genetic) disorders affecting the nervous system. Our main objectives are: (a) to identify those conditions with neurological involvement which have increased familial incidence; (b) to separate these patients into probably hereditary and acquired categories; (c) to study the pathogenesis and etiology of certain of these conditions; (d) to devise special diagnostic tests and (e) to institute therapeutic modifications in several groups of these diseases and to explore preventive measures.

Methods Employed:

A. Diagnostic
   1. Detailed general medical, neurological, psychological and developmental studies.
   2. Genetic (pedigree type) and cytogenetic studies.
   3. Special biochemical assays of blood, CSF, urine and saliva for partitioned carbohydrates, proteins and lipids.

4. Employment of more invasive procedures when indicated, such as pneumoencephalography and arteriography, and in special instances, brain biopsy.
5. Employment of special enzymatic assays using patients' white blood cells, plasma, fibroblast tissue cultures and, occasionally, biopsy of liver, gut or brain.

B. Therapeutic
1. Use of approved and investigative pharmacological agents employing modifications of the drugs, dosages and others.
2. Use of corticosteroids for suppressing inflammatory reaction or enhancement of enzymatic activity.
3. Use of plasma, whole blood or white blood cells (obtained by plasmapheresis), for intravenous infusions as means of supplying deficient enzymes.
4. Infusion of the deficient trace elements.
5. Identification, isolation, purification and intravenous infusion of the respective deficient enzymes in special categories of genetic disorders.

Patient Material: 17 inpatients; 25 outpatient visits.

Major Findings: Recently we have studied two new patients with mucopolysaccharidosis type V (MPS-V). The older of these siblings, a male 35 years of age, died and his brain and other organs became available for chemical and structural examinations. This is the first such study of the patient, clinically and by laboratory tests diagnosed as MPS. The importance of this investigation rests on the fact that MPS-V and MPS-I have deficiency of the same enzyme α-L-iduronidase, yet their clinical phenotypes are vastly different. Comparison of histological, histochemical, chemical and electron microscopic findings in tissues of MPS-V and MPS-I provided us with the understanding of the underlying differences between the two diseases. Thus: (a) the principal new finding in the brain of the patient with MPS-V is the presence of lesions in the perivascular 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 neuronal abnormality and damage are severe. (b) Electron microscopic studies of the brain in MPS-I demonstrated numerous complex membranous inclusions in the neurons, whereas the neurons in MPS-V contain only a small number of lipofusion-like inclusions and typical lipofusion granules. (c) There was a 3-fold increase of glycosaminoglycans (GAG) in the brain of MPS-I but only a slight increase in MPS-V; GAG in the liver and spleen of all patients was markedly 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.

Significance to Bio-Medical Research and the Program of the Institute: There are over one hundred crippling hereditary disorders and they affect usually multiple organs; however, either directly or indirectly the nervous system is involved in nearly all of them. The results presented here advance

our knowledge in the genetic mechanisms of some of these disorders. Therapeutic modifications of the course of the disease and changes in metabolism are likely to provide us with better understanding of these disorders and their eventual management.

Proposed Course of the Project: Our efforts will continue in determinations of pathogenesis and etiology of individual genetic disorders. The considerable success in preliminary therapeutic trials in mucopolysaccharidosis, Menkes disease and Fabry's disease would be extended using improved approaches as we learn more; similar attempts will be extended to other hereditary diseases.

Keyword Descriptors:

Human genetics; enzymatic defects; cerebral degenerative disease; mental retardation; pathology of the nervous system.

Honors and Awards: Clinical Associate Professor of Neurology, George Washington University Medical School (no remuneration)

Publications:

1. Dekaban, A.S.: Brain Dysfunction in Congenital Malformations of the Nervous System. In Gaull, G. (Ed.): Biology of Brain Dysfunction, Vol. 7. New York, Plenum Press, 1975.

2. Dekaban, A.S., Constantopoulos, G., Herman, M. and Steusing, J.: Mucopolysaccharidosis type V (Scheie). First postmortem study by multidisciplinary techniques with emphasis on brain. Arch. Path. (in press).

Project No. ZO1 NS 00815-15 DMN
1. Developmental & Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Metabolism of Complex Lipids of Nervous Tissue: Studies of Gaucher's Disease, Niemann-Pick Disease, Fabry's Disease, Krabbe's Disease, Metachromatic Leukodystrophy, and Tay-Sachs Disease.

Previous Serial Number: NDS(1)-61 DMN/EG 815.

Principal Investigator: P. G. Pentchev, Ph.D.

Other Investigators: R. O. Brady, M.D., A. E. Gal, Ph.D., J. W. Kusiak, Ph.D., J. M. Quirk, G. E. Mook and S. R. Hibbert.

Cooperating Units: Weizmann Institute of Science, Rehovot, Israel, Children's Hospital, University of Pennsylvania, Philadelphia, Pennsylvania.

Man Years:

Total:	6.8
Professional:	3.3
Other:	3.5

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 galactopsychosine have been similarly prepared. Ceramide-trihexoside and ceramide tetra hexoside (globoside) uniformly 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 several of these enzymes for therapeutic replacement trials is a major continuing portion of this project.

Major Findings: (1) We have previouslyreported that enzyme replacement in Gaucher's disease promises to be an effective therapeutic measure for this disorder. We have now found that the clearance of accumulated lipid extends over a period of several years. This observation is extremely encouraging for the treatment of this disease. (2) We have utilized the facilities at hand with maximum efficiency to prepare several-fold greater quantities of highly purified human glucocerebrosid than had been previously available. With this larger amount of enzyme, we are now in the position to further develop and extend our enzyme replacement studies. A third patient will be infused in May. A reliable method has been developed to insure that enzymes prepared for infusion are free of pyrogens. (4) A procedure for isolating Gaucher cells from bone marrow biopsy specimens has been developed in collaboration with investigators at Children's Hospital in Philadelphia. We shall use this added parameter for assessment of further effectiveness of enzyme replacement in forthcoming studies with Gaucher patients. (5) The "sea-blue histocyte" syndrome identified last year as a deficiency of sphingomyelinase has been shown on a molecular level to be caused by a greater cold lability of this enzyme in patients with this syndrome. (6) Infusion Of high doses of sphingolipid hydrolases into Rhesus monkeys has given us a valuable model for studying the uptake, tissue distribution, and duration of effectiveness of these enzymes. (7) The purification of ceramidetrihexosidase has been improved considerably by the introduction of two new chromatographic steps, concanavalin A-sepharose and hydroxyapatite columns. Both the degree of purification and yield of enzyme have been significantly increased. A third patient with Fabry's disease has been Infused with ceramidetrihexosidase. This patient received five times more enzyme than the two previous Fabry patients who were given the enzyme. Kidney biopsies were obtained prior to and after infusion. The results of this therapeutic trial are being assessed at the present time. (8) We continue to serve as a center for the diagnosis of patients and detection of carriers for all of the lipid storage diseases. Requests and samples come from all over the world for these assays. Much current work is devoted to the monitoring of pregnancies at risk for these heritable metabolic disorders. During the past year we monitored 62 cases and found 22 affected individuals and 20 carriers.

Significance: Gaucher patients whose deficient levels of glucocerebrosidase had been supplemented with infusions of exogenous human enzyme show a prolonged and sustained clearance of accumulated lipid which can still be observed 14 months after administration of the enzyme. These findings are immensely encouraging for the prospect of effective enzyme replacement therapy for hereditary lipid storage diseases.

Proposed Course: We will continue to carry out and monitor the long-term effects of enzyme infusions in patients with Gaucher's disease and Fabry's disease. The effect of infusing substantially larger doses of enzyme will be investigated. Preliminary studies of enzyme replacement with purified sphingomyelinase will be initiated in patients with Niemann-Pick disease.

Keyword Descriptors:

Gaucher's disease; Niemann-Pick disease; Fabry's disease; Tay-Sachs disease; enzyme replacement therapy; diagnosis of lipid storage diseases; detection of heterozygotes; prenatal monitoring; genetic counseling.

Honors and Awards:

Dr. R. O. Brady, election to the National Academy of Sciences, April 1975.

Publications:

1. Brady, R. O., The chemistry and control of hereditary lipid diseases. Chem. Phys. Lipids 13: 271-282, 1974.

2. Brady, R. O., The lipid storage diseases: new concepts and control. Ann. Int. Med. 82: 257-261, 1975.

3. Brady, R. O., Enzyme defects in the lipidoses and their prenatal detection. In: Curtius, Ch. and Roth, M. (Eds.): Clinical Biochemistry: Principles and Methods, New York, Walter de Gruyter, 1974, pp. 1277-1291.

4. Brady, R. O., Pentchev, P. G., Gal, A. E., Hibbert, S. R., and Dekaban, A. S., Replacement therapy for inherited enzyme deficiency: use of purified glucocerebrosidase in Gaucher's disease. New Engl. J. Med., 291: 989-993, 1974.

5. Brady, R. O., Gal, A. E., and Pentchev, P. G. Evolution of enzyme replacement therapy for lipid storage diseases. Life Sci., 15: 1235-1248, 1974.

6. Brady, R. O., Pentchev, P. G., and Gal, A. E. Investigations in replacement therapy in lipid storage diseases. Fed. Proc. 34: 1310-1315, 1975.

7. Pentchev, P. G., Brady, R. O., Gal, A. E., and Hibbert, S. R. Replacement therapy for inherited enzyme deficiency: sustained clearance of accumulated glucocerebroside in Gaucher's disease following infusion of purified glucocerebrosidase. J. Mol. Med., in press.

Project No. ZO1 NS 00816-15 DMN
1. Developmental & Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Structural and Metabolic Studies of Gangliosides in Normal Humans and Patients with Tay-Sachs Disease.

Previous Serial Number: NDS(1)-61 DMN/EG 816

Principal Investigator: J. F. Tallman, Ph.D.

Other Investigators: R. O. Brady, M.D., A. E. Gal, Ph.D.; J M. Quirk, G. E. Mook.

Cooperating Units: Department of Human Genetics, Tel-Aviv Medical School, Sheba Medical Center, Israel.

Man Years:

Total:	2.8
Professional:	1.3
Other:	1.5

Project Description:

Objectives: To investigate the normal relationship of human hexosaminidases A and B, their involvement in the metabolism of gangliosides and the derangement of these enzymes in patients with Tay-Sachs disease.

Methods: Highly purified β-hexosaminidase A and B have been prepared from human placental tissue using column chromatographic and ion exchange methods. The artificial fluorogenic substrate 4-methylumbelliferyl-β-N-acetylglucosaminide was used routinely to monitor enzyme activity. Tay-Sachs ganglioside N-acetylgalactosaminyl-(N-acetylneuraminosyl)-galactosyl-glucosylceramide was labelled in the N-acetylgalactosaminyl portion of the molecule with ^{14}C by an enzymatic synthetic scheme utilizing UDP-N-acetylgalactosamine-1-^{14}C. The asialo derivative of this compound was prepared by galactose oxidase treatment of N-acetylgalactosaminylgalactosyl glucosylceramide followed by reduction with tritiated sodium borohydride.

Major Findings: The complete purification of both forms of hexosaminidase has been accomplished. The enzymes have identical molecular weights and each is composed of four subunits of identical size. It is possible to convert hexosaminidase A into a "B-like" enzyme by heating it at pHs near neutrality. This "B-like" enzyme possessed identical electrophoretic mobility to the isolated hexosaminidase B and exhibited subunit interactions characteristics of the purified hexosaminidase B.

Both enzymes showed similar kinetic properties when artificial substrates were used to monitor activity. Hexosaminidase A and B possessed activity toward various lipidic substrates including the Tay-Sachs ganglioside in the presence of detergent.

Based on this examination of the properties of the purified enzymes, we have proposed that the two enzymes exist as conformational isomers of each other. A model for Tay-Sachs disease and the various variant forms was proposed based on the relative stability of these conformers and the prediction of a benign hexosaminidase A deciciency was made. This predition has subsequently been confirmed.

Significance: These studies represent the beginning of an understanding of the molecular pathology of this group of the sphingolipidoses. Based on these findings, we now may consider rational therapeutic measures for alleviating this disorder and assisting in the prenatal diagnosis of the disease. We also have a program for the detection of carriers and patients with Tay-Sachs disease.

Proposed Course: The project will be terminated because the principal investigator has accepted a position in another institute. We shall continue diagnostic testing for Tay-Sachs disease and genetic counseling. These activities will be subsumed under Project No. ZO1 NS 00815-15 DMN .

Keyword Descriptors:

Tay-Sachs Disease; Hexosaminidase A; Hexosaminidase B; ^{14}C-Labeled Tay-Sachs Ganglioside; ^{3}H-Labeled Asialo-Tay-Sachs Ganglioside.

Publications:

1. Tallman, J. F., Pentchev, P. G. and Brady, R. O. An enzymological approach to the sphingolipidoses. Enzyme 18: 136-149, 1974.

2. Tallman, J. F., The enzymology of Tay-Sachs disease and its variant forms. In: Buchwald, N. (Ed.): Brain Mechanisms in Mental Retardation, Brain Mechanisms Rev. pg. 379-399, 1975.

3. Tallman, J. F., Preparation and use of labelled glycoconjugates of the sphingolipids In: Montreuil, J. (Ed.): Actes du Colloque du CNRS sur les glycoconjugues 221, 993-997, 1975.

4. Tallman, J. F. and Brady, R. O.: In Rosenberg, A. and Schengrund, C.-L., (Eds.): The Biological Role of the Sialic Acids. in press.

5. Tallman, J. F., Brady, R. O., Quirk, J. M., Villalba, M. and Gal, A. E.: Isolation and relationship of human hexosaminidases. J. Biol. Chem. 249: 3489-3499, 1974.

6. Tallman, J. F., Brady, R. O., Navon, R., Padeh, B. Ganglioside catabolism in hexosaminidase A-deficient adults, Nature 252: 254-255, 1974.

7. Tallman, J. F. Hexosaminidases and ganglioside catabolism in the G_{M2}-gangliosidoses. Chem. Phys. Lipids 13: 292-304, 1974.

Project No. Z01 NS 01026-13 DMN

1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Treatment of Epilepsy--Clinical and Biochemical Study.

Previous Serial Number: NDS(I)-63 DMNB/CIT 1206(c)

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Michael Whyte, M.D. and E. Lehman, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	1.1
Other:	0.0

Project Description:

Objectives: During the past several years we have contributed to the treatment and long-range management of epilepsy in children. Our current studies are centered on the assessment of metabolism of anticonvulsant drugs in the patients whose seizures are particularly difficult to control. The second important aspect in the management of both children and adults with chronic epilepsy is prevention of excessive drowsiness and slowing of mental activity resulting from high doses of anticonvulsant medication.

Patient Material: 22 inpatients and 18 outpatients.

Methods Employed:

1. General medical, developmental and neurological investigations, including special laboratory tests.
2. Modified electroencephalographic studies.
3. Pneumoencephalography and arteriography, when indicated.
4. Quantitative tests for mental performance in control situation.
5. Determination of plasma partitioned lipids and urinary and serum amino acids, as well as assays of carbohydrate and endocrinological function.
6. Application of special therapeutic procedures including ketogenic diet and use of certain hormones (progesterone, corticosteroids).
7. Determination of anticonvulsant drugs in serum, CSF and urine during modified metabolic states and while on experimentally defined different doses of medication.
8. The determination of metabolites of the anticonvulsant drugs used.

Major Findings: Seventeen patients with epilepsy difficult to control were used to determine their drug metabolism. For this purpose we have developed special "profiles" for assessment of the metabolism of anticonvulsant drugs in individual patients while they are maintained on step-wise changed doses of the same medication. This permits determination of the best suited dosage of the combined medication for a particular patient.

The patients were divided into groups depending on the particular combination of anticonvulsant drugs they were taking. There have been reports in the literature that certain drugs given in combination can influence the speed of katabolic processes of other drugs, the notable example being induction of liver enzymes by phenobarbital.

1. In Group A patients receiving phenobarbital (PB) and diphenylhydantoin (DPH), we have demonstrated that DPH did not influence the metabolism of PB (similar slopes of regression lines); also during long standing administration of these two drugs, PB seemed to have little influence on the rate of DPH metabolism.

2. Group B patients were receiving a combination of three drugs: primidone (PRM), DPH and PB. The main findings here included the following: about one-third of PRM was metabolized to PB; about one-third was promptly (within 1-3 hours) excreted in the urine; and the remainder was metabolized into phenylethylmalonamide (PEMA). Of particular importance was our demonstration of the renal threshold for PRM; doubling or even tripling of the dose of this drug did not raise its plasma level, the greatest portion of PRM being promptly excreted by the kidneys. This finding should mitigate administration of excessively large doses of this drug to patients with epilepsy. Moreover, there is a considerable individual variability of this drug's metabolism. Children in particular are very resistant to the increase of plasma PRM levels. Here, construction of the profile of drug metabolism in individual patients is very important. We have not observed improvement of seizure control following further increase in the dose of PRM once blood PRM level reached the plateau values.

3. The following were precise ratios of serum to cerebrospinal fluid levels of PB, DPH and PRM:

	Number of Determinant	Ratio of Serum to CSF Levels
PB	22	5.6:1
DPH	10	2.2:1
PRM	17	1.1:1

These ratios reflect the existence of different blood-CSF barriers for the three drugs and are of importance in determining their therapeutic effects and central nervous system toxicity. These aspects will require further study.

4. Parallel with the study of drug metabolism in epileptic patients, we are developing a new method of testing changes in mental performance as related to the dose of anticonvulsant drugs. By repeated performance testing of patients on the same medication but on different dosages we have demonstrated that there is an optimal dose for each patient which is a compromise between permissible slowing of mental performance and acceptable frequency of seizures. Over sixty percent of patients investigated had a significant improvement in their mental performance without change in the frequency of seizures when their hospital admission dosage of medication was reduced by 30-50 percent. This was a startling finding.

Significance to Bio-Medical Research and the Program of the Institute: There are close to two million people in this country suffering from epilepsy. The anguish of the affected patients and their families and economic loss are enormous. Currently, the time has come to place treatment of epilepsy on a better scientific basis with chemical control; this should be associated with prevention of unnecessary impairment of mental performance by excessive medication.

Proposed Course of the Project: The modification of treatment of recurrent cerebral seizures and a better control of the type and dosages of the medication used by determination of balance studies of antiepileptic drugs will continue. There is a need for further improvement in the type of performance tasks used.

Keyword Descriptors:

Epilepsy; treatment of cerebral seizures; drug metabolism; mental performance.

Publications:

1. Fujitani, K., Dekaban, A.S. and Zimmerman, A.W.: Comparison of serum levels and urinary excretion of three major anticonvulsants between children and adults. Brain and Development. 6: 358-362, 1974.

2. Dekaban, A.S., Fujitani, K. and Constantopoulos, G.: The effects of different dosages of combined anticonvulsant drugs on their metabolism and their levels in body fluids. Clinical Neurology and Neurosurgery 3/4: 168-179, 1974.

3. Dekaban, A.S. and Lehman, E.: Effects of different dosages of anti-convulsant drugs on mental performance in patients with chronic epilepsy. Acta Neurol. Scand. (in press).

Project No. ZO1 NS 01309-10 DMN
1. Developmental & Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Metabolism and Role of Glycosphingolipids and Other Glycoconjugates in Malignancy and Heritable Disorders of Anabolism

Previous Serial Number: NDS(1)-66 DMN/EG 1309.

Principle Investigator: P. H. Fishman, Ph.D.

Other Investigators: R. O. Brady, M.D., R. O. Duffard, Ph.D., R. M.Bradley

Cooperating Units: Laboratory of Molecular Biology, National Institute of Neurological and Communicative Diseases and Stroke
Viral Leukemia and Lymphoma Branch, National Cancer Institute
Molecular Biology Section, Viral Carcinogenesis Branch, National Cancer Institute
Department of Neurology and Pediatrics, University of Maryland School of Medicine, Baltimore, Maryland.

Man Years:

Total:	2.1
Professional:	1.35
Other:	0.75

Project Description:

Objectives: To determine the mechanism which underlies the alterations of glycosphingolipid biosynthesis in neoplastic tissues; to investigate the function of membrane glycosphingolipids in the regulation of cell proliferation and in their involvement in cell morphology; to explore the regulation of glycosphingolipid biosynthesis during development and differentiation; these studies are being extended to other membrane glycoconjugates. Also emphasis will be placed on abnormal glycosphingolipid biosynthesis in other pathological conditions, with particular regard to heritable disorders of the nervous system.

Methods: The glycosphingolipid composition of normal and transformed cultured cells and normal and pathological tissue is determined by selective extraction of this class of lipids followed by separation of individual glycosphingolipids on thin-layer chromatograms. With the cultured cells, metabolism in vivo is determined by adding ^{14}C-or ^{3}H-labeled precursers such as galactose, N-acetylmannosamine and glucosa-

rat kidney cells (NRK) when transformed by either Ki MSV or WSV undergo the same change in ganglioside metabolism. Both virus-transformed cell lines are tumorigenic and have less ganglioside G_{M3} and reduced CMP-sialic acid: lactosylceramide sialyltransferase levels.

In addition, we are examining the possibilities that the organization of membrane glycoconjugates may be altered in WBALB cells or that the membrane glycoconjugates although present in WBALB cells may no longer be functional. We have previously shown that ganglioside G_{M1} is the membrane receptor for cholera toxin and that cholera toxin evokes its biological effects by binding to G_{M1} and then activating plasma membrane adenyl cyclase. If this is a reasonable model for the role of membrane glycoconjugates in the transmission of information across cell membranes, then an interruption could occur at a number of different points with dire consequences for the cell. Thus, WBALB cells may prove useful in elucidating the details of this hypothesis.

B. Studies on Role of Glycosphingolipids in Morphological Differentiation of Cultured Cells - These studies are in collaboration with the Laboratory of Molecular Biology, IR, NINCDS (ZO1 NS 01963-02 LMB). HeLa cells, a continuous human cell line derived from a cervical carcinoma, extend neurite-like processes and stop growing when cultured in the presence of certain short chain fatty acids such as butyrate. Prior to changes in morphology, there is an induction of CMP-sialic acid: lactosylceramide sialyltransferase activity (10-30 fold more than in control cells) and an increase in ganglioside G_{M3} content. There is no significant change in other glycosphingolipids or related glycosyltransferases (Simmons, Fishman, Freese and Brady, 1975, J. Cell Biol., in press) or in the activities of various glycophingolipid hydrolases (Tallman, Fishman and Henneberry, in preparation). Both sialyltransferase induction and morphological differentiation caused by butyrate can be reversed by the calcium ionophore, A23187 (Henneberry, Fishman and Freese, 1975, Cell, in press). In calcium free medium, the ionophore is ineffective. Calcium has been implicated in the regulation of microtubule assembly by other investigators; our results suggest that calcium may also be involved in the induction of ganglioside biosynthesis in butyrate-treated HeLa cells.

Butyrate also causes neurite formation in human neuroblastoma cells and changes in ganglioside composition. The enzymological basis of these changes is being investigated as well as the effects by butyrate on ganglioside metabolism and electrical activity in neuroblastoma-fibroblast hybrids. We are also exploring the biochemical response of different cell lines when induced to morphologically differentiate by various agents. Morphological changes are induced in HeLa only by butyrate (or the related fatty acids propionate and pentanoate) but not by removal of serum or cyclic AMP. Neurite formation is initiated by all three in neuroblastoma cells; it will be important to determine whether similar or different membrane changes occur in response to these various agents.

mine to the culture medium and isolating the labeled glycosphingolipids. Catabolism is measured in vitro by assaying cell and tissue extracts for glycohydrolase activities with specifically labeled (^{14}C and ^{3}H) glycosphingolipids prepared in our laboratory. Biosynthesis in vitro is determined by measuring the activities of the various glycosyltransferases involved in glycosphingolipid formation. Activities of glycosyltransferases located in the cell surface are detected on intact cells by modifications of the in vitro enzyme assays. Surface glycoconjugates are analyzed by selective oxidation with galactose oxidase or periodate and subsequent reduction with sodium borotritide before and after removal of surface sialic acid from intact cells with neuraminidase. Membrane glycoproteins of cultured cells are analyzed by adding ^{14}C- or ^{3}H-labeled precursors (L-fucose or D-glucosamine) to the culture medium. Surface labeled glycoproteins are removed from intact cells by selective digestion with trypsin and converted to glycopeptides by protease digestion. Glycopeptides are separated by gel filtration column chromatography; glycopeptides from different cell lines such as normal (^{3}H-labeled) and transformed (^{14}C labelled) are compared by co-chromatography and double-labelled isotope counting techniques.

Major Findings:

A. Studies on Membrane Glycoconjugates from RNA Tumor Virus-Transformed Cells - These studies are done in collaboration with the Molecular Biology Section, Viral Carcinogenesis Branch, National Cancer Institute (NCI-4940-8) when Balb 3T3 cells, an established contact-inhibited non-tumorigenic mouse embryo line, are transformed by the Kirsten sarcoma virus (Ki MSV), a murine RNA tumor virus, the tumorigenic cells (KBALB) are deficient in gangliosides G_{M1} and G_{D1a} and UDP-galactose: G_{M2} galactosyltransferase activity, an enzyme required for the synthesis of these gangliosides. Levels of other glycosyltransferases are similar to those in the control Balb 3T3 cells. This same defect was observed in several other Ki MSV-transformed subclones. Presence of Rauscher leukemia virus (RLuV), a helper virus required for sarcoma virus replication, had no effect on ganglioside biosynthesis. KBALB cells also have a different glycopeptide pattern from Balb 3T3 when pronase-digested surface glycoproteins are analyzed by gel filtration column chromatography. There is an increase in earlier eluting material from the transformed cells. Differences in glycopeptide pattern between normal and KBALB cells are minimized by prior digestion of the glycopeptides with neuraminidase. Thus the differences are related in large part to changes in sialic acid content.

In contrast, Balb 3T3 cells transformed by Wooley Monkey sarcoma virus (WSV), a primate RNA tumor virus, do not show these changes in gangliosides and sialoglycopeptides. Although the WBALB cells behave like KBALB cells in tissue culture (i.e., loss of contact-inhibition of growth and and anchorage dependence) their tumorigenicity has not yet been tested. This point is very important in order to correlate membrane changes with malignant potential. In this regard we have observed that normal newborn

Significance: These studies are providing information on the function of membrane glycosphingolipids and the regulation of their biosynthesis. An alteration in glycosphingolipid biosynthesis can be observed in certain tumorigenic cells, during morphological differentiation, and in an inherited metabolic disease. An understanding of how these membrane changes occur on a molecular basis and how they relate to altered cellular behavior may increase our knowledge of normal cellular growth and differentiation as well as of pathological conditions such as cancer and neurological diseases.

Proposed Course: The project will be continued with emphasis placed on the functional aspects of membrane glycosphingolipids and glycoproteins. It is important to understand the relationship between these membrane components and the abnormal behavior exhibited by transformed cells both in culture and in vivo. We are initiating studies with hybrid cells derived from normal and transformed cell fusions and with cells doubley transformed by two different tumor viruses in order to explore this relationship. We will pursue our studies on the role that membrane gangliosides might play in microtubule assembly and morphological differentiation in cultured cells especially neuroblastoma and related cells of neurological origin. We also hope to investigate other metabolic diseases that are a consequence of deficient glycosphingolipid biosynthesis as such studies are invaluable in understanding the normal function of these compounds.

Keyword Descriptors:

Cultured cells; enzyme induction; gangliosides; glycosphingolipids; glycosyltransferases; membrane glycoconjugates; microtubules; morphological differentiation; RNA tumor viruses; transformation; anabolic disorders of the nervous system.

Honors and Awards:

None.

Publications:

1. Brady, R. O., and Fishman, P. H.: Alterations of Complex Lipid Metabolism in Tumorigenic DNA and RNA Virus-Transformed Cell Lines. In Schultz, J. and Block, R. (Eds.): Membrane Transformations in Neoplasia. New York, Academic Press, 1974, pp. 275-294.

2. Brady, R. O., and Fishman, P. H.: Alterations in the Pattern and Synthesis of Gangliosides in Tumorigenic Virus Transformed Cells. In Clarkson, B. and Baserga, R. (Eds.): Cold Spring Harbor Conference on Cell Proliferation. Vol. 1, Control of Proliferation in Animal Cells. Cold Spring Harbor, N. Y., 1974, pp. 505-515.

3. Brady, R. O., and Fishman, P. H.: Biosynthesis of Glycolipids in Virus-Transformed Cells. Biochim. Biophys. Acta 355: 121-148,1974.

4. Brady, R. O., and Fishman, P. H.: Membranes of Transformed Mammalian Cells. In Fox, C. F. (Ed.): MTP International Reveiws of Science, Biochemistry Series I, Vol. 2. Biochemistry of Cell Walls and Membranes, London, Butterworth, 1974, pp. 61-96.

5. Coleman, P. L., Fishman, P. H., Brady, R. O., and Todaro, G. J.: Altered Ganglioside Biosynthesis in Mouse Cell Cultures Following Transformation with Chemical Carcinogens and X-irradiation. J. Biol Chem. 250: 55-60, 1975.

6. Fishman, P. H.: Normal and Abnormal Biosynthesis of Gangliosides. Chem. Phys. Lipids 13: 305-326, 1974.

7. Fishman, P. H., Max, S. R., Tallman, J. F., Brady, R. O., Maclaren, N. K., and Cornblath, M.: Deficient Ganglioside Biosynthesis: A Novel Human Sphingolipidosis. Science 187: 68-70, 1975.

8. Fishman, P. H., Simmons, J. L., Brady, R. O., and Freese, E.: Induction of Glycolipid Biosynthesis by Sodium Butyrate in HeLa Cells. Biochem. Biophys. Res. Commun. 59: 292-299, 1974.

9. Hollenberg, M.D., Fishman, P. H., Bennet, V., and Cuatrecasas, P.: Cholera Toxin and Cell Growth: Role of Membrane Gangliosides. Proc. Natl. Acad. Sci. USA 71: 4224-4228, 1974.

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11. Brady, R. O., and Fishman, P. H.: Alterations of Galactosaminyl and Galactosyl Transferases in Cultured Mammalian Cells and In Vivo. In Martonosi, A. (Ed.): Membrane-bound Enzymes. New York, Plenum Press, 1975, in press.

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Project No. ZO1 NS 01457-09 DMN
1. Developmental & Metabolic Neurology Branch
2. Neurochemical Methodology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Chemical Synthesis of Radioactive Sphingolipids.

Previous Serial Number: NDS(1)-67 DMN/NM 1457

Principal Investigator: A. E. Gal, Ph.D.

Other Investigators: F. J. Fash.

Cooperating Units: None.

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description:

Objectives: To prepare sphingolipids labeled with radioactive isotopes. These 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. An interesting 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 ceramide trihexoside. 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 in the detection, identification and isolation of enzymes connected to lipid storage diseases. Also studies related to qualitative and quantitative determination of enzymes in animal or human tissues necessitate these labelled substrates. Prenatal diagnoses are of rising importance. These labelled compounds play a key role in these diagnostic procedures. As a therapeutic approach, this branch initiated replacement therapy by the administration of the missing enzyme in hereditary diseases. The monitoring of the enzyme levels during and after this therapeutic procedure was done by the use of these radioactive substrates. It would be also of great interest to develop new methods which would allow to prepare relatively easily and inexpensively these compounds for the use of clinicians and for researchers who are not connected to large research centers.

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 by chemical oxidation will be explored. 3. Work will continue on the development of the technique: labelling of functional group exchange.

Keyword Descriptors:

Sphingolipids; Lipid Storage Diseases; Synthesis of Radioactive Lipids; ^{14}C-Labelled Cerebrosides; ^{3}H-Labelled Sphingosine; ^{3}H-Labelled Asialo Gangliosides; ^{3}H-Labelled Ceramide Trihexoside; Tritium Gas Exposure; Functional Group Exchange.

Honors and Awards:

None.

Publications:

See Project Nos. ZO1 NS 00815-15 DMN and ZO1 NS 00816-15 DMN.

Project No. Z01 NS 01523-08 DMN
1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Neurological, General Medical and Biochemical Aspects of Hurler, Hunter, Sanfilippo and Scheie Diseases: Basic Pathogenesis, Enzymology and Therapeutic Trials.

Previous Serial Number: NDS(I)-68 DMNB/CIT 1523(c)

Principal Investigators: Anatole S. Dekaban, M.D., Ph.D. and George Constantopoulos, Ph.D.

Other Investigators: Norio Sakuragawa, M.D. and Dennis Cain, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	1.1
Other:	0.0

Project Description:

Objectives: The disorders of the metabolism of glycosaminoglycans (GAG) are usually associated with severe dysfunction of the nervous system as well as of liver, spleen, heart and other tissues. Within the past ten years, several genetically different mucopolysaccharidoses (MPS) were identified and our Unit contributed significantly in delineating certain entities and in modifying the course of these diseases. The interest in MPS is additionally increased by the fact that the derangement of the CNS relates to the excessive accumulation of lipid-staining substance in the neurons (leading eventually to their destruction), while liver, spleen, skin, kidneys and other organs store GAG.

Patient Material: 8 inpatients and 12 outpatients.

Methods Employed:
1. General medical, developmental and neurological examinations including special tests as required.
2. Isolation, separation and determination of the composition of various GAG in the urine, serum and cerebrospinal fluid.
3. Determination of the molecular weight distribution in the isolated GAG.
4. Establishment of skin fibroblast tissue cultures for the purpose of histochemical studies and chemical determination of GAG.

5. Utilization of tissues from organs obtained by biopsy for determination of acid mucopolysaccharide composition and enzyme studies.
6. Extraction of tissue from organs obtained at autopsy for large scale recovery of the stored GAG and lipids.
7. Production of biologically labelled ^{35}S heparitin sulfate utilizing skin fibroblasts in the tissue culture from patients with Sanfilippo A variant. This labelled substrate will be then used for determination of respective enzymatic activity in leukocytes of patients and unaffected controls.

Major Findings:

1. This was an appropriate time for comprehensive assessment of different types of mucopolysaccharidoses (MPS) by clinical and biochemical criteria. Over several years we have investigated an uncommonly large number of patients with MPS--a total of 46. Results of this study will be helpful in our ongoing approach to investigation of enzyme deficiencies in different types of MPS and in conducting therapeutic approaches.

Glycosaminoglycans (GAG) were isolated and aided in characterization of the disease in 11 patients belonging to MPS-I (Hurler), 8 with MPS-II (Hunter), 16 with MPS-III (Sanfilippo type A or B), 9 with MPS-V (Scheie), 1 with MPS-VI (Maroteaux-Lamy) and 1 unclassified.

All 46 patients excreted in their urine excessive amounts of dermatan sulfate, heparan sulfate or both. In addition, patients of certain types excreted excessive amounts of chondroitin sulfates A and/or C. There was a strong trend in each type of the disease towards the same carbazole-orcinol ratio, glucosamine-galactosamine ratio and glycosaminoglycan composition.

Molecular weight distribution of the urinary glycosaminoglycans by gel filtration from Sephadex G-200 is characteristic for each different type of mucopolysaccharidosis and is distinguished from normal controls and patients without mucopolysaccharidosis. Preparation of elution diagrams from Sephadex G-200 allows an estimation of the composition of the glycosaminoglycans.

Practically all heparan sulfate and a sizable part of dermatan sulfate from the urinary glycosaminoglycans of all these patients have been highly degraded. In all the patients in which the specific enzyme defect was demonstrated, the assignment of the type of mucopolysaccharidosis, on the basis of the elution diagrams, was correct.

Patients with mucopolysaccharidosis Type V displayed two conspicuously different types of elution patterns, suggesting heterogeneity. Indeed, only a portion of these patients showed α-L-iduronidase deficiency. Carriers had normal urinary glycosaminoglycans output and composition and exhibited normal elution diagrams.

2. In order to facilitate the determination of enzyme differences in various types of MPS we have used radioactive isotope for labelling of the

natural substrate. The fibroblast cultures derived from the patients with Sanfilippo Type A, produced GAG labelled with ^{35}S. The material was separated by ion exchange chromatography. The 1.0 molar eluate was then recovered by ethanol precipitation. GAG determination was then carried out by four different electrophoretic systems. We have found that heparan sulfate accumulates only in the pulse-chased Sanfilippo fibroblasts. N-sulfate degradation revealed 80% of the label to be N-sulfated. Enzyme assays performed with both normal and pathological fibroblasts and leukocytes revealed that patients with the Type A Sanfilippo variant are unable to degrade the labelled substrate derived from Sanfilippo fibroblasts, in contrast to the normal cells.

Significance to Bio-Medical Research and the Program of the Institute: Correlation of the clinical findings and especially of mental performance with the biochemical and basic chemical determinations brings us closer to the understanding of the genetic variability and of the pathogenesis of different types of MPS. When coupled with previous and present therapeutic trials, the long-range goal of management or elimination of this disorder of inborn errors of metabolism will be eventually approached.

Proposed Course of the Project: Our experience and contributions to the understanding of mucopolysaccharidoses are very considerable and this project will be continued.

Keyword Descriptors:

Mucopolysaccharidoses; pathogenesis of inborn errors of metabolism; therapeutic trials in mucopolysaccharidoses; enzyme assays.

Publications:

1. Constantopoulos, G. and Dekaban, A.S.: Chemical definition of mucopolysaccharidoses. Clinica Chimica Acta. 59: 321-336, 1975.

Project No. Z01 NS 01655-07 DMN
1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of the Composition and Biosynthesis of Cerebral Proteins in Experimental Animals and Man

Previous Serial Number: NDS(I)-69 DMNB/CIT 1655

Principal Investigator: Dennis F. Cain, Ph.D.

Other Investigators: Anatole S. Dekaban, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.8
Other:	0.0

Project Description:

Objectives: Brain proteins differ from those of other tissues particularly in the elevated levels of the acidic proteins. They are also rapidly synthesized and degraded - a phenomenon without a functional correlate.

An understanding of the role of these specialized aspects of protein metabolism in normal nervous tissue requires determination of the identity, composition and relative rates of synthesis and degradation, as well as the localization of individual proteins within various cell types and subcellular organelles. This information is particularly important in the case of the acidic proteins, some of which are known to be brain-specific. Acidic proteins in other tissues have been shown to exert a regulatory function in RNA and protein metabolism. The possibility of interaction of acidic proteins in nervous tissue with neurotransmitters and neuropharmacological agents is of especial significance in neural tissue.

The application of appropriate extraction and separation procedures will allow the determination of both the spectrum and the variability in the composition of the soluble proteins in normal human brain. Thus, changes in the proteins associated with neurological diseases may be examined. The purpose of such studies is to screen normal and pathological surgical and necropsy specimens for the presence or absence of particular protein

fractions which may reflect changes in cell structures and cell populations characteristic of various pathological states.

Methods Employed: Various protein fractions are extracted and purified by salt fractionation, isoelectric precipitation, gel filtration, ion exchange, chromatography, etc. These proteins are subsequently analyzed by electrophoresis and isoelectric focusing in a soluble acrylamide gel system. The specific purified protein fractions are also characterized by amino acid analysis, sedimentation and immunological procedures.

Evolving techniques for the separation of subcellular as well as whole cell units will continue to be evaluated for purposes of localization of the individual protein fractions. Tissues from specific areas of the brain and pathological specimens containing a predominant cell type will be expecially valuable for this purpose.

Material: Brains and other organs from the patients obtained by biopsy or at autopsy are used.

Major Findings: Previously, we have demonstrated the presence of large amounts of two particular protein fractions (Band 15 and Band 20), and the marked decrease in another (Band 5) by electrophoretic separation of extracts of certain pathological brain biopsy specimens. One of these proteins (Band 15) has now been isolated in nearly pure form from two brain specimens by a combination of salt fractionation, dialysis and gel exclusion chromatography. The protein has a molecular weight in excess of one million as determined by gel filtration. On dissociation and reduction, it is converted to subunits of molecular weight of 45,000. The protein has an isoelectric point of 5.8. Analysis of sialic acid indicate about 1 mole of sialic per subunit. Amino acid analysis shows a high content (27-30%) of glutamic and aspartic acids.

We have prepared antiserum to the band 15 protein in rabbits. With the higher sensitivity of the antiserum, we have shown the protein to be absent in the other human organs examined. With the antibody, it has also been possible to demonstrate the presence of small amounts of this protein in normal brain tissue obtained during therapeutic surgery, whereas in some post-mortem brain specimens, we have not been able to demonstrate this protein, suggesting the possibility of isoelectric precipitation, aggregation, or autolysis. Using the antiserum, we have shown the presence of cross-reacting protein in the CSF of a patient months after surgery for glioma.

Significance to Bio-Medical Research and the Program of the Institute: There is some suggestive and still indirect evidence that specific cerebral proteins may be involved in the processes of memory and intellectual functions. Correlation between stimulatory events and the products of synthesis are lacking. There are large numbers of subjects with undifferentiated type of mental retardation who defy all investigative approaches

in clarifying the underlying abnormality. Because of several technical difficulties, the brain proteins have not been thus far explored in these and many types of other patients. The availability to us of surgical and post-mortem tissue of a number of hitherto obscure neurological diseases provides us with unique opportunity to study cerebral proteins in these patients.

Proposed Course of the Project: D. Cain left our Branch and because of ceiling in employment we were not allowed to replace him; consequently, this project is being discontinued.

Keyword Descriptors:

Brain proteins; cerebral gliomas; cerebral lipidosis; isolation and identification of proteins.

Publications:

1. Cain, D.F., Ball, E.D. and Dekaban, A.S.: Proteins in human brain tissue obtained during surgical procedures. J. Neurochem. 23: 561-568, 1974.

Project No. ZO1 NS 01808-06 DMN
1. Developmental & Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Glycoproteins of Myelin in Development and Disease.

Previous Serial Number: NDS(1)-70 DMN/EG 1808

Principal Investigator: R. H. Quarles, Ph.D.

Other Investigators: R. O. Brady, M.D., J. L. Everly, Ph.D., J.-M. Matthieu, M.D., J. Poduslo, Ph.D.

Cooperating Units: Section on Cellular Neuropathology, LNNS, NINCDS.

Man Years:

Total:	3.3
Professional:	3.3

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. 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. 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.

Glycopeptides are prepared from delipidated myelin by exhaustive digestion with pronase. The soluble glycopeptides are then purified by gel filtration on Bio Gel P-10. Gas liquid chromatography and colorimetric procedures are used for quantitation of individual sugars in glycopeptides and glycoproteins. Labeling of surface glycoproeins on the intact rat spinal cord is accomplished with galactose oxidase treatment and [^{3}H] sodium borohydride reduction.

Major Findings: A major advance this year year has been the determination of the nature and quantity of individual sugars in the glycoproteins of both central and peripheral myelin. These data were obtained by proteolytic conversion of the glycoproteins to glycopeptides which were subsequently purified by gel filtration. The total glycoprotein-carbohydrate in PNS myelin is about 5-fold higher than in CNS myelin. This is consistent with our previous findings that the major protein of PNS myelin is a glycoprotein, whereas the myelin-associated glycoproteins of CNS myelin are quantitatively minor components. The glycopeptides prepared from PNS myelin are eluted from Bio-Gel P-10 as a single sharp peak and contain fucose, mannose, galactose, N-acetylglucosamine, and sialic acid. The glycopeptides of CNS myelin contain the same sugars as those from PNS myelin, but are separated into two fractions by gel filtration on Bio-Gel P-10. Most of the galactose and sialic acid are in the fraction of larger molecular weight, and the smaller fraction contains primarily mannose and N-acetylglucosamine. The glycopeptides from both peripheral and central myelin are sulfated.

A procedure for the complete purification of the major glycoprotein of CNS myelin has been achieved. The procedure involves solvent fractionation, preparative polyacrylamide gel electrophoresis, and affinity chromatography on Concanavalin-A-Sepharose. As reported last year, a preparation which is about 50% pure can be achieved by solvent fractionation and preparative gel electrophoresis alone. Although, the use of Con-A-Sepharose yields some glycoprotein which is completely separated from other myelin proteins, it also subfractionates the glycoprotein into several fractions presumably because of microheterogeneity in the oligosaccharide moieties. Therefore, the pure glycoprotein is obtained in low yield and may not be representative of all the glycoprotein molecules in the myelin. For these reasons, immunological experiments are being carried out with the partially purified glycoprotein fraction, which is free of basic protein, proteolipid and the major Wolfgram protein. Antiserum to this fraction has been prepared in rabbits and gives four precipitin lines by immunodiffusion.

Our regional and developmental studies of CNS myelin subfractions have been completed. The results support our earlier conclusions from whole adult brain. Basic protein and proteolipid are the major proteins of compact multilammellar myelin which fractionates in the light fraction. In contrast, the glycoprotein, 2'3'-cyclic nucleotide 3'-phosphohydrolase, and some of the other high molecular weight proteins are most concentrated in the heavy fraction which contains loose myelin and membranes which are transitional between myelin and the oligodendroglial surface membrane.

Such a localization for the glycoprotein is also indicated by the use of galactose oxidase and [^{3}H] sodium borohydride reduction as a surface probe for glycoproteins. Thus, the glycoprotein was labeled when this probe was applied to the intact spinal cord indicating that the glycoprotein is at least partially localized on external surface of the myelin sheath or oligodendrocyte.

Last year we reported a developmental change in the electrophoretic mobility of the major CNS glycoprotein. As myelin matures, the apparent molecular weight of the glycoprotein on SDS-gels decreases. This change probably reflects a change in its carbohydrate residues. Consistent with this interpretation is the finding that there are also developmental differences in the fucose-labeled glycopeptides prepared from the glycoprotein. The glycopeptides prepared from the immature glycoprotein are enriched in higher molecular weight components. However, experiments in which the partially purified glycoprotein was incubated with neuraminidase indicate that the higher apparent molecular for the glycoprotein of immature myelin is not due to a higher sialic acid content. The change of the glycoprotein from higher to lower apparent molecular weight is delayed in hypothyroid and Cu-deficient rats in which the deposition of myelin is delayed. The glycoprotein in the heavy and medium subfractions of normal 16-day-old rats has a higher apparent molecular weight than that in the light subfraction, suggesting that some of the membranes in the heavier subfractions are at an earlier stage of maturation. Recently, we have shown that the developmental change of the glycoprotein seen in whole rat brain also occurs in the optic nerve.

Copper deficiency induced by a low copper diet in 3 generations of rats was associated with significant reductions in the yield of myelin (56%), brain weight (8%), and body weight (43%) in f/2 generation rat pups nursed by their own copper deficient mothers. The composition of the purified myelin was not different from controls in the content of individual proteins, lipids, 2', 3'-cyclic nucleotide 3'-phosphohydrolase (CNP) activity, or G_{M1} ganglioside. Postnatal copper replacement by a foster mother produced a normal yield of myelin per g of brain, but failed to reverse the deficiency of brain and body growth or the neurological symptoms. After replacement in a copper-deficient mother's diet prior to conception, a subsequent litter showed correction of all abnormalities which were found in her previous litters.

Hexachloroplene intoxication of developing rats resulted in a decreased yield of normal myelin and the appearance of an abnormal subcellular fraction of degenerating myelin. The protein and lipid composition of the degenerating myelin fraction closely resembled that of normal myelin, excep that the major glycoprotein was almost completely missing.

Significance: The probable localization of the major myelin-associated glycoprotein of the CNS in loose myelin membranes and in oligodendroglial plasma 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 membranes. The apparent developmental change in the carbohydrate structure of the major glycoprotein might be involved in these interactions. Since we have analyzed the carbohydrate composition of mature CNS myelin, it will now be possible to use similar procedures to precisely define developmental changes in carbohydrate composition. Similar considerations apply to the carbohydrates on the major protein of peripheral myelin.

The results found during dietary copper deficiency in the rat suggest that copper is essential for myelin formation and general growth during critical periods in development. They also support the view that abnormalities in Menkes' kinky-hair disease might be reversed only by prenatal or early postnatal diagnosis and treatment.

The anomalies of myelin glycoproteins, found in abnormal myelinogenesis (Quaking mice, hypothyroidism, Cu-deficiency) and in myelin degeneration (hexachlorophene intoxication) suggest that these glycoproteins are essential for normal myelin formation and maintenance. 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 the myelin-associated glycoprotein could be directly involved in demyelinating diseases. 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.

Proposed Course: Analyses of glycopeptides prepared from central and peripheral myelin will be extended in order to more precisely define the structure of their oligosaccharide moieties. Such analyses will also be done on immature myelin in order to elucidate the nature of the developmental changes. Procedures for the purification of the major glycoprotein of CNS myelin will be modified in order to improve the yield. When moderate amounts of the pure glycoprotein can be obtained, determination of its amino acid and carbohydrate composition will be undertaken. Also specific antibodies to the pure glycoprotein will be prepared, and immunohistochemical techniques will be used to precisely localize the glycoprotein in myelin and oligodendroglial membranes.

The antisera which we prepared against the partially purified glycoprotein of the CNS will be tested for demyelinating activity in collaboration with Dr. Frederick Seil of Stanford University. These experiments are designed to determine if the demyelinating factor in the serum of multiple sclerosis patients and in animals affected by experimental allergic encephalomyelities (EAE) induced by whole tissue might be an antibody directed against the glycoprotein. It will also be important to determine if MS patients have antibodies or sensitized lymphocytes directed against the glycoprotein. Also, we are currently doing experiments to see if an autoimmune disease similar to chronic EAE or multiple sclerosis can be induced by injecting experimental animals with the partially purified glycoprotein.

Finally, a collaborative biochemical and morphological investigation of developing optic nerve has been begun with Drs. Harry Webster and Paul Reier of LNNS. We plan to correlate developmental changes in myelin proteins and glycoproteins with the structural maturation of myelin as revealed by conventional electron microscopy and freeze-fracture techniques.

Keyword Descriptors:

Myelin; glycoprotein; nervous system; myelinogenesis; development; oligodendrocyte; demyelinating diseases; multiple sclerosis; copper deficiency; hexachlorophene.

Honors and Awards:

None.

Publications:

1. Druse, M. J., Brady, R. O., and Quarles, R. H.: Metabolism of a myelin-associated glycoprotein in developing rat brain. Brain Res. 76: 423-434, 1974.

2. Matthieu, J.-M., Brady, R. O., and Quarles, R. H., Anomalies of Myelin-Associated Glycoproteins in Quaking Mice, J. Neurochem. 22: 291-296, 1974.

3. Matthieu, J.-M., Brady, R. O. and Quarles, R. H., Change in a myelin associated glycoprotein during development: Metabolic aspects, Brain Res. 86: 55-65, 1975.

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5. Matthieu, J.-M., Daniel, A., Quarles, R. H., and Brady, R. O.: Interactions of conconavilin A and other lectins with CNS myelin. Brain Res. 81: 348-353, 1974.

6. Matthieu, J.-M., Reier, P. J., and Sawchak: Proteins of rat brain myelin in neonatal hypothyroidism. Brain Res. 84: 443-451, 1975.

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8. Matthieu, J.M., Everly, J. L., Brady, R. O. and Quarles, R. H.: [^{35}S]Sulfate incorporation into myelin glycoproteins. II. Peripheral nervous tissue. Biochem. Biophys. Acta, in press.

9. Matthieu, J.-M., Quarles, R. H., Poduslo, J. F. and Brady, R. O. [^{35}S]Sulfate incorporation into myelin glycoproteins: I. Central nervous tissue. Biochem. Biophys. Acta, in press.

10. Quarles, R.H.: Glycoproteins in the nervous system In Brady, R. O. (Ed.): NINCDS 25th Anniversary Volume, Basic Neurosciences Section, New York, Raven Press, in press.

11. Zimmerman, A. W., Matthieu, J. M., Quarles, R. H., Brady, R. O. and Hsu, J.M., Hypomyelination in copper deficient rats: Effects of prenatal and postnatal copper replacement. Arch.Neurology, in press.

12. Zimmerman, A. W., Quarles, R. H., Webster, H. deF., Matthieu, J.M., and Brady, R. O.: Characterization and protein analysis of myelin subfractions in rat brain: developmental and regional comparisons. J. Neurochem. in press.

Project No. Z01 NS 02024-03 DMN
1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of Transient or Definitive Strokes in Patients with Increased Plasma Lipids and in Familial Hyperlipoproteinemia

Previous Serial Number: NDS(I)-73 DMNB/CIT 2024(c)

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Jan K. Steusing

Cooperating Units: In negotiating phase

Man Years:

Total:	0.9
Professional:	0.3
Other:	0.6

Project Description:

Objectives: The incidence of strokes in subjects with elevated plasma lipids is higher than in the general population. Cerebrovascular disease is one of the four most common causes of neurological morbidity and mortality. Of various factors predisposing to atherosclerosis is persistent elevation of plasma lipids (whether exogenous - dietary, or endogenous - hereditary hyperlipidemia) and abnormal patterns of lipoproteins. It is our intention to study the content and composition of partitioned lipids and lipoproteins in patients with transient or definitive strokes and in persons at high risk such as members of families with hyperlipidemia. Special dietary measures will be made available to these patients and arrangements will be made for their follow-up.

Material: Eighty-three patients with transient cerebral ischemic attacks or definitive strokes were examined in ambulatory setting and fasting blood drawn.

Methods Employed:
1. Detailed personal and family medical history including dietary habits.
2. General medical and neurological examinations.
3. Special examination of the retinal blood vessels and peripheral arteries.

4. Radiological, electroencephalographic, electrocardiographic, blood viscosity studies and usual routine laboratory surveys of blood, urine and CSF.
5. Special tests will include - when indicated for diagnostic or planned assessment of therapy - cerebral angiography and brain scan.
6. Determination of plasma partitioned lipids using established chemical procedures as employed by us in studies of high fat diet in children administered for treatment of epilepsy.
7. Determination of plasma lipoproteins by unidimentional paper electrophoresis, and when indicated by polyacrylamide gel electrophoresis and density gradient centrifugation.

Major Findings: This project is still in developmental stage and several new parameters of study will be considered. Blood viscosity and performance tests will be added. Each patient's profiles of serum partitioned lipids as well as profiles of lipoprotein are in progress. A total of 83 patients were examined and their fasting blood sample drawn. Sixty-seven of these patients were in a chronic phase of stroke, 16 had transient ischemic attacks or were in an acute phase of stroke. Plasma from all these patients was used for determination of partitional lipids and lipoproteins. The main findings include the following: 6 patients had definite hyperlipoproteinemia (4 type II and 2 type IV), 10 had plasma elevation of both cholesterol and triglycerides, 8 had elevation of triglycerides, 5 had elevation of cholesterol and 54 had normal plasma lipids. We need an additional 30-50 patients before conducting correlation between clinical and pathological findings and various types of lipids and lipoproteins.

Significance to Bio-Medical Research and the Program of the Institute: Since the relationship between the severity of atherosclerosis and cerebral symptoms is reasonably well established, it will be fruitful to evaluate the handling of high fat diet ingested by the subjects with marked differences in fat metabolism. This could lead to the dietary modifications during early life, and may provide one mechanism for reducing the incidence of cerebrovascular damage.

Proposed Course of the Project: Although still in a process of development and further expansion, a considerable progress has been made and this project will continue; we are in need of an additional chemist to accelerate this work.

Keyword Descriptors:

Plasma lipids; lipoproteins; cerebrovascular disease; special diets.

Publications:

1. Dekaban, A.S. and Brady, R.O.: Therapeutic approaches to selected disorders of inborn errors of metabolism with neurological involvement. International J. Neurol. (in press).

Project No. Z01 NS 02103-03 DMN
1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Investigations of Pathogenesis and Assessment of Therapeutic Trials in Fabry's Disease and Gaucher's Disease

Previous Serial Number: NDS(I)-73 DMNB/CIT 2103

Principal Investigators: Anatole S. Dekaban, M.D. and Roscoe O. Brady, M.D.

Other Investigators: J.F. Tallman, Ph.D., A.E. Gal, Ph.D., P.G. Pentchev, Ph.D. N. Sakuragawa, M.D. and J.M. Quirk

Cooperating Units: None

Man Years:

Total:	2.1
Professional:	1.2
Other:	0.9

Project Description:

Objectives: The underlying abnormality in Fabry's disease is deficiency of an enzyme, α-galactosyl hydrolase, which is necessary for katabolism of a glycosphingolipid: galactosyl-galactosyl-glucosyl ceramide. The disorder is transmitted as an X-linked trait and is characterized clinically by telangiectatic skin lesions (angiokeratoma corporis diffusum), corneal opacities, intractable burning pain in the extremities, progressive renal dysfunction and general fatigue. The life span is shortened, death occurring in early to mid-adult years usually from renal failure, cardiac, or cerebral complications.

The basic abnormality in Gaucher's disease is a deficient enzyme activity of β-glucosidase, which catalyzes removal of glucose from glucosyl ceramide. This results in excessive accumulation of this glycolipid in the cells of reticulo-endothelial system, leading to life threatening manifestations--hepatosplenomegaly, skeletal abnormality and neurological involvement.

Recently we have demonstrated (New Engl. J. Med. 289:9-14, 1973) that intravenous infusion of large volume of completely matched white blood cells or of purified enzyme--ceramide trihexoside to the patients with Fabry's disease can produce transient improvement in biochemical abnormality in a

form of a substantial decrease of excessively accumulating glycolipid. Similar results were obtained after infusions of glucocerebrosidase to two patients with Gaucher's disease (New Engl. J. Med. 291: 989-993, 1974).

In order to produce amelioration of the clinical abnormality, a repetitive long-term supply of the deficient enzymes to these patients would have to be provided. Organ transplantation (kidneys), successive infusion of blood products or of purified enzymes are very involved and expensive procedures and are still in an experimental phase. Proper selection of patients and establishment of specific indication for such therapeutic trials is now in order and in progress.

Material: 7 inpatients and 25 outpatient visits

Methods: Active enzymes, α-galactosidase and ceramidetrihexosidase are obtained from human placenta by extraction and fractionation with ammonium sulfate. Subsequently the enzyme is purified by passages on Sephadex G-200 columns, ion exchange chromatography including DEAE and Sephadex cation exchange columns. The final enzyme proteins are homogeneous and pure. The enzymes are suspended in albuminated saline and submitted to the NIH Pharmaceutical Development Service for study of sterility and pyrogenicity in order to obtain their clearance for intravenous administration to the patients.

Infusion of the enzyme will be preceded and followed by examination of the blood, liver and kidney tissues obtained by needle biopsy. This will permit determination of the quantitative change in the excessively accumulating unmetabolized substrate.

Medical literature from 1920 to 1973 was scrutinized for detailed case reports on Fabry's disease. The data thus obtained plus the data from 7 of our own patients was analyzed for age of onset of the disease, predominant clinical abnormality and complications.

Major Findings:

1. Intravenous infusions of purified glucocerebrosidase to two patients with Gaucher's disease caused a substantial decrease in the quantity of glucocerebroside in erythrocytes of these patients. Likewise there was a decrease of this glycolipid in the liver following enzyme infusion as compared to the preinfusion value. There was no obvious change in patients' clinical condition following enzyme infusion and actually this was not expected after only two consecutive infusions. The theoretical value of these trials to correct the biochemical errors are far reaching and further infusions are planned.

2. Clinical data on 80 patients with Fabry's disease were evaluated for age of onset, characteristics of life history of the disease and the complications. This was done in order to provide criteria for better selection of patients for enzyme replacement trials and lay down the indications. The mean age of onset of Fabry's disease in 48 males still

alive was 10.2 years and in 17 males who died, the mean age of onset was 8.1 years. This suggests that eventual enzyme replacement therapy to be effective should start early, before irreversible changes occur. The mean survival age of 23 patients who died was 38.7 years. Autopsy examination showed the presence of renal damage in all patients; the coexisting complications, included occlusive condition of coronary arteries, liver involvement and cerebrovascular disease.

Proposed Course of the Project: We shall undertake investigations of the long-term effects of enzyme replacement in patients with Fabry's disease and Gaucher's disease. Particular attention will be devoted to assessment of the clinical results of this therapy; the possibility of antibody formation by the recipients will be critically assessed.

Keyword Descriptors:

Fabry's disease; Gaucher's disease; enzyme infusion therapy; infusion of white blood cells in treatment of enzymatic defects; isolation and purification of enzymes.

Publications:

1. Dekaban, A.S., and Zelkowitz, M.: Fabry's disease: Evaluation of age of onset and natural history as preliminary steps to replacement therapy trials. Clinical Proceedings of the Children's Hospital National Medical Center. (in press)

2. See Project No. Z01 NS 00815 DMN No. 4.

Project No. Z01 NS 02128-01 DMN

1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Inborn Error of Copper Metabolism: Kinky Hair Disease. Quantitative Determination of the Absorptive Defect of Copper and Therapeutic Trials.

Previous Serial Number: None

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D. and Jan K. Steusing

Other Investigators: Roger Aamodt, Ph.D. and Warren Rumble, B.Sc.

Cooperating Units: Nuclear Medicine Department, Clinical Center, NIH

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.3

Project Description:

Objectives: Kinky hair disease (KHD) and Wilson's hepatolenticular degeneration (HLD) are the only two known genetically determined disorders of copper metabolism. KHD is inherited as an X-linked recessive trait and so far the condition has been sublethal. However, there is now hope that early institution of therapy may control the disease. The main clinical features of this new disease include epileptic attacks, mental deterioration, spasticity and brittle hair. The last feature resulting from abnormal crosslinking of keratin which is copper dependent. Pathological lesions include cerebral degeneration and defects of elastica interna of arteries. The main laboratory findings include very low plasma copper (less than 20% of normal), low ceruloplasmin (less than 25% normal) and low tissue copper. The underlying abnormality of the disease seems to be related to impaired absorption of copper from the gastrointestinal tract. This needs to be confirmed and the extent of the defect needs to be determined.

With this in mind we decided to study the turnover and absorption of labelled copper in patients with KHD, HLD and normal controls. Knowledge of the percentage of copper absorption by KHD patients will facilitate development of appropriate therapy.

Patient Material: 4 inpatients and 6 outpatient visits

Methods Employed:

1. General medical, neurological and developmental studies.
2. Determination of serum copper by dilution method utilizing atomic absorption spectrophotometer. Determination of ceruloplasmin using p-phenylenediamine method of Ravin.
3. Intravenous and at later date oral administration of ^{67}Cu (half-life 61.7 hours).
4. Daily measurement of total body ^{67}Cu radioactivity using 60 cm arc with two 8 x 4" NaI (TI) scintillation detectors and Packard Instrument 1024 Channel Pulse Height Analyzer. An injection or ingestion duplicate to be counted just prior to each measurement on the patient, and resulting values to be used to correct patient data for physical decay of ^{67}Cu.
5. Probe measurements to be made using a Nuclear-Chicago dual probe and a Hamner Electronics Co. Sealer.
6. Radioactivity of stools, urine and blood to be measured daily.

Major Findings:

1. Administration of ^{67}Cu by both intravenous and oral routes to the same subject permitted us a precise calculation of the percent absorption of labelled ^{67}Cu.

   (a) The measured gastrointestinal absorption of the labelled copper in patients with KHD ranged between 11 and 13% only.

   (b) The patients with KHD retained the injected or absorbed labelled copper 3-4 times longer than normal controls. This indicates great demand for copper and possible recycling of this element in the body.

   (c) The retained ^{67}Cu remained longer in the liver; the uptake of copper by the red blood cells was almost normal, indicating preferential utilization of the plasma copper, even though its level was low.

2. Kinky hair disease is a sublethal condition with the onset of symptoms under 3 months of age. The main clinical abnormalities consist of uncontrollable convulsions, signs of progressive cerebral degeneration with motor weakness and spasticity and characteristic pili torti. Death usually occurs under 3-5 years of age. Recently we have demonstrated the extent of absorptive defect of the copper from gastrointestinal tract and embarked on therapeutic trials of the affected patients. Conjugation of copper with chelating agents, amino acids or peptides failed to increase absorption of copper. However, we have succeeded in developing a therapeutic regimen which corrects the biochemical defect indefinitely and maintains the serum copper and ceruloplasmin at normal levels, ameliorating patient's clinical condition. Essentially the treatment consists of subcutaneous infusion of 1-2 mg of copper sulfate in 25-50 ml of saline (0.04 mg of $CuSO_4$/ml) given by slow drip over 1-2 hours. The infusions are given every 3-4

days in four alternate sites: two beneath the lower border of the scapula and two at the level of the eighth rib in the midscapular line. We were able to maintain this therapy during seven months with satisfactory results. The reason for this manner of administration is a high toxicity of copper salt to the tissue unless greatly diluted. Intravenous infusions of large volume of saline containing copper salt proved impractical in young infants and could not be maintained on ambulatory basis. Using the method of subcutaneous infusion of copper sulfate in two patients with KHD we were able to correct biochemical defect: plasma copper and ceruloplasmin were brought to normal values and maintained at this level for a long time. The clinical course of the patients was improved.

Significance to Bio-Medical Research and the Program of the Institute: Demonstration of the greatly decreased but not absolute lack of the absorption of oral copper will have important implications in devising therapy for the patients with KHD. We have already tried to administer orally conjugated copper with various chelating agents and polypeptides, so far this was without success but further approaches are indicated. We were successful in devising the parenteral therapy with copper sulfate in the form of large subcutaneous infusions. This permitted complete correction of the biochemical abnormality in the plasma by reversion of the copper and ceruloplasmin to normal levels.

Proposed Course of the Project: Further work is needed to bring about maintenance of clinical health with the present therapy and to develop an oral route of copper administration.

Keyword Descriptors:

Kinky hair disease; copper metabolism; hepatolenticular degeneration; treatment of genetic diseases.

Publications:

1. Dekaban, A.S. and Steusing, J.: Menkes' kinky hair disease treated with subcutaneous copper sulfate. Lancet Dec. 21: 1523, 1974.

2. Dekaban, A.S., Aamodt, R., Rumble, W.F., Johnson, G.S. and O'Reilly, S.: Kinky hair disease. Study of copper metabolism utilizing ^{67}Cu; some reference to Wilson's disease. Arch. Neurol. (in press).

Project No. Z01 NS 02129-01 DMN
1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical Characterization of Mucopolysaccharidosis Type VI (Marateaux-Lamy)

Previous Serial Number: None

Principal Investigator: George Constantopoulos, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: The objective of this study is the characterization of the urinary glycosaminoglycans (GAG) excessively excreted by a patient with mucopolysaccharidosis type VI (MPS-VI) also known as Marateaux-Lamy disease. This material presumably represents the substrate of the enzyme which is deficient in these patients. The solution of this problem may help in the diagnosis and it may increase our understanding of the absence of cerebral involvement--as compared to the patients with other types of mucopolysaccharidosis.

Material: Urine from a patient with type MPS-VI.

Methods Employed: Isolation and measurement of total GAG from urine; fractionation of the individual GAG by ion exchange chromatography on Dowex 1; further purification of dermatan sulfate and chondroitin sulfates by copper precipitation, and/or depolymerization with chondroitinase AC. Characterization of the disaccharides produced after incubation with chondroitinase AC and of the remaining GAG with chondroitinase ABC. Chemical analysis. Acetate electrophoresis.

Major Findings: None (new project)

Significance to Bio-Medical Research and the Program of the Institute: The understanding of biochemical abnormality in type VI MPS will be considerably aided by characterization of the material which is not metabolized and which affects adversely certain body organs and tissues but spares the brain. Other forms of MPS are usually associated with severe cerebral abnormalities. The study of genetic neurological disorders is the objective of this section and of the Institute.

Proposed Course of the Project: New project.

Keyword Descriptors:

Type VI mucopolysaccharidosis; isolation and characterization of glycosaminoglycans; biochemistry of glycosaminoglycans.

Publications: None

Project No. ZO1 NS 02162-01 DMN
1. Developmental & Metabolic Neurology Branch
2. Neurochemical Methodology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Synthesis of Compounds Analogous to Glycolipids

Previous Serial Number: None.

Principal Investigator: A. E. Gal, Ph.D.

Other Investigators: F. J. Fash.

Cooperating Units: None.

Man Years:

Total:	0.7
Professional:	0.4
Other:	0.3

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: 2-Hexadecanoylamino-4-nitrophenyl phosphorylcholine (HNP) was synthesized. This compound resembles sphingomyelin except that the long aliphatic chain of sphingosine is replaced by the aromatic benzene ring. The benzene ring has a nitrogroup attached to it and it provides an intensely colored nitrophenol derivative after enzymatic attack. It was found that this substance is a reliable substrate for the diagnosis of homozygotes and detection of heterozygous carriers of Niemann-Pick disease using extracts of liver or cultured skin fibroblasts.

Significance: The prototype compound has been proven and synthesized and its diagnostic usefulness has been established. It and other analogs which will now be synthesized are designed to replace radioactive substrates. This finding is a major breakthrough because the radiolabeled products are scarce, expensive, and not widely available. The chromophogenic 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 scanning techniques.

Proposed Course: Based on the basic idea established by this project, compounds will be synthesized with chromophoric moieties which hopefully will be specific for the detection of other enzyme deficiency disorders such as Krabbe's disease and Gaucher's disease.

Keyword Descriptors:

Synthesis of Chromogenic substrates; nitrophenol analogs; glycolipid analogs; lipid storage diseases; diagnostic reagents; Niemann-Pick disease, Gaucher's disease, Krabbe's disease.

Honors and Awards:

None.

Publications:

1. Gal, A. E., Brady, R. O., Hibbert, S. R. and Pentchev, P. G.: A practical chromogenic procedure for the detection of homozygotes and heterozygous carriers of Niemann-Pick disease. New Engl. J. Med., 1975, in press.

Project No. ZO1 NS 02163-01 DMN
1. Developmental & Metabolic Neurology Branch
2. Neurochemical Methodology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of Special Analytical Methods and Preparative Techniques to Investigate the Etiology and Therapy of the Sphingolipidoses.

Previous Serial Number: None.

Principal Investigator: A. E. Gal, Ph.D.

Other Investigators: F. J. Fash.

Cooperating Units: None.

Man Years:

Total:	0.7
Professional:	0.3
Other:	0.4

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 thefollowing 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 temporily 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 liquid-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 were 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 attached 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 could be established and through these procedures, evaluation of the therapeutic administration of enzymes 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.

Keyword Descriptors:

Enzyme purification; affinity chromatography; diagnosis of lipid storage diseases; gas chromatography; thin-layer chromatography; analytical methods at the microgram level. Determination of lipids in human tissues.

Honors and Awards:

None.

Publications:

See Project Nos. ZO1 NS 00815-15 DMN and ZO1 NS 00816-15 DMN.

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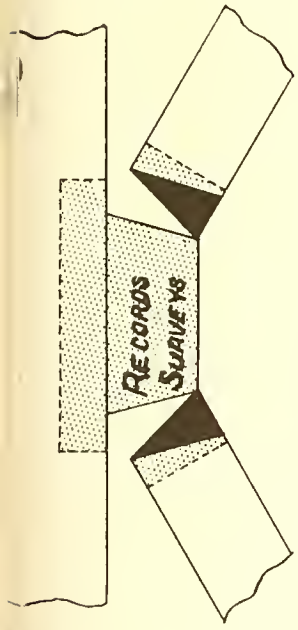

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ANNUAL REPORT
July 1, 1974 through June 30, 1975
Laboratory of Neuropathology and Neuroanatomical Sciences, IR
National Institute of Neurological and Communicative
Disorders and Stroke

Igor Klatzo, Chief

The main research efforts of the LNNS have continued to be directed towards elucidation of the basic pathological mechanisms operative in cerebrovascular disorders and demyelinating diseases. Among the former cerebral ischemia which is so prominently involved in various forms of stroke, constituted the main subject of our investigations.

Mongolian gerbils (Meriones unguiculatus), characterized by frequent anomalies of the circulus of Willis, offer special advantages as the experimental model for cerebral ischemia. Although the unilateral occlusion of the common carotid artery in the neck results in ischemic infarction of the corresponding hemisphere in approximately 30% of gerbils only, the simplicity of the procedure allows for a quick and easy processing of a large number of animals and for collecting statistically meaningful data based on many different parameters of experimentation.

For obtaining an overall dynamic profile of morphological changes and their relationship to the duration of ischemic and postischemic periods, light microscopy, although inferior to EM in providing cytological detail, presents obvious advantages and it was extensively used in the studies on Mongolian gerbils. Our observations revealed that the lesions develop during the ischemic period, as well as after re-establishment of the circulation, and this presents one facet of a "maturation" phenomenon which seems to be a general principle applicable to various parameters of ischemic injury. The rate of "maturation" of ischemic lesions in gerbils was directly related to the intensity (duration) of an ischemic insult, a lesser intensity resulting in slower development of the lesions. Thus, it took one week for a selective disintegration of the H2 sector of the hippocampus in carotid occlusions of short (8-15 minutes) duration, whereas similarly severe damage of this sector was observed in a few days following more prolonged occlusions. In another example, animals sacrificed almost immediately after 30 minutes occlusion showed practically no recognizable ischemic changes in the basal ganglia, whereas extensive, severe ischemic injury was observed in these structures in gerbils sacrificed after 20 hours release following occlusion of the same duration. The phenomenon of "maturation" was also recognizable in other parameters of ischemic injury. Thus, with regard to the behaviour of the blood-brain barrier (BBB), in shorter unilateral carotid occlusion (e.g. 15 minutes) the gerbils previously injected with Evans blue tracer never disclosed BBB damage. With one hour occlusion it took 20 hours for animals to show BBB changes, whereas with 6 hours occlusion all gerbils revealed Evans blue discoloration of the brain within one hour after release of the clip. Another instance of the "maturation" phenomenon can be deduced from carbohydrate and

biogenic amine assays in ischemic gerbils. Data for glycogen and for serotonin determinations revealed in animals with shorter occlusion periods peaks which were delayed but of similar magnitude to those that were reached in a relatively short time after more severe ischemic insults. With regard to glycogen, in sensitive (symptom-positive) animals the lowest level of this compound (250 micromoles/kg) was recorded in the ipsilateral hemisphere at a one-hour period of carotid occlusion, after which with longer duration of occlusion the glycogen levels climbed steadily reaching at 9 hours higher than starting point values. In "non-sensitive" (symptom-negative) animals, obviously much less affected by ischemia, the glycogen in the occluded hemisphere dropped to a similarly low level, but this occurred after 3 hours of ischemic occlusion. With regard to biogenic amines, serotonin was considerably elevated in the affected hemisphere when the gerbils were sacrificed after 6 hours of carotid occlusion. However, when the occlusion lasted only 15 minutes and then the clip was released, it took 20 hours for the serotonin to climb up to almost the same level as in animals sacrificed immediately after 6 hours of carotid occlusion.

Another finding brought out by our light microscopic observations on gerbils was the feature of selective vulnerability to ischemia displayed by certain topistic units and most clearly observed in the hippocampus. There, the earliest recognizable effect of ischemia was expressed by the accentuated Nissl staining of the neuronal processes, confined strictly to H2 sector in the gerbils sacrificed shortly after 4 minutes occlusion of the common carotid arteries bilaterally. In the animals which were allowed to live one hour after 4 minutes of bilateral occlusion, this change became much more prominent and it appeared to involve also the cortical neurons. The most striking topistic lesion was the "reactive change" which was confined to the H3 sector of the hippocampus. This change occurred only in animals which were subjected to relatively slight ischemic insult (7 minutes bilateral or 15 minutes of unilateral occlusion) and it was fully developed only after 20 hours of "maturation." At this stage it was characterized by a peripheral shift of the nucleus whereas the voluminous cytoplasm showed a central chromatolysis, the overall picture resembling that of "primäre Reizung" described by Nissl. The electron microscopic study on the "reactive change" revealed this region of the perikaryon filled with a dense accumulation of various organelles. Conspicuous among those were bizarrely shaped mitochrondria, membrane-bound lysosomal structures and unidentifiable, non-membrane bound particles. Histochemical observations on the "reactive change" showed intense activity within the cytoplasm of affected neurons with regard to such enzymes as various dehydrogenases and acid phosphatase. The animals subjected to the duration of ischemia which produces "reactive change" at 20 hours interval, when sacrificed one week later showed mostly excellent preservation of the H3 sector whereas the H2 sector revealed widespread disintegration of neurons. It is thus evident that the "reactive change" represents a cellular reaction to ischemia in which the neurons are capable of full recovery from an ischemic injury. Besides the hippocampus, in our studies on gerbils the feature of selective vulnerability was discernible also in a laminar injury of cortical neurons with special predilection for the third layer. This finding was conspicuous 20 hours after release of the clip in the gerbils subjected to one hour unilateral occlusion.

Our studies support an assumption that the basic pathomechanism of ischemia is related to the metabolic energy disturbance and therefore the sensitivity of various CNS cellular units to ischemia is influenced by their metabolic state. It is possible that in cerebral ischemia two modalities of ischemic injury are operative: 1) the fast one, - related to an acute energy crisis as expressed in precipitous drops in levels of glucose, glycogen and energy phosphates, associated with concomitant rise in lactate and pyruvate. Simultaneously affected may be energy-dependent osmotic regulation resulting in increased water uptake by various cellular compartments; 2) the slow one, - related to depletion of enzymic and structural protein reserves combined with inability to replenish them by nuclear or cytoplasmic synthesis. The latter type of ischemic injury may account for the described "maturation" phenomenon.

Unquestionably, one of the most serious complications of ischemia is the brain edema and its sequelae. Our studies indicate that the abnormal accumulation of fluid, which is the basic definition of edema, begins very promptly following an ischemic injury. The fluid accumulates predominantly in neurons and astrocytes, - neuronal mitochondria, astrocytic vascular processes and dendrites being especially susceptible to swelling. In light microscopic observations the initial cellular water uptake can be recognized as a vacuolization of the neuropile. The water distention affecting most of the cellular elements is very conspicuous in the later stages of ischemic injury. According to the classifications of brain edema, the edema occurring as a complication of cerebral ischemia should be classified as basically of a cytotoxic type. This assumption is supported by observations on the blood-brain barrier (BBB) which indicate that only in the later stages of ischemia, associated with rather severe tissue injury, is there a breakdown of the BBB which introduces a vasogenic component into the existing cytotoxic edema developed by intracellular imbibition of fluid.

Cerebral ischemia being a cerebrovascular disturbance is greatly influenced by the systemic blood pressure. In our studies on gerbils an acute hypotension, which was found to follow immediately the release of arterial occlusion, was closely associated with the appearance of a "no-reflow" phenomenon. The "no-reflow" phenomenon was evaluated by intravascular injections of carbon particle suspensions before the termination of experiments at different time intervals after carotid clip release. The appearance of a "no-reflow" phenomenon was of a transitory nature and its incidence was correlated closely with a drop in the levels of systemic blood pressure. It could not be demonstrated after periods longer than 15 minutes following release of occlusion during which time there was full recovery from the drop in blood pressure. Also indicating close relationship between systemic blood pressure and the "no-reflow" phenomenon was the fact that the latter could be abolished by administration of pharmacological agents such as epinephrine, which prevented a drop in blood pressure following release of an occlusion. It is then likely that an acute hypotension may lead in areas with presumably compromised vascular autoregulation to sludging, stasis and circulatory standstill which would have deleterious effects propagating further ischemic injury.

Our studies on gerbils revealed similarly damaging effects of hypertension on the brain tissue subjected to ischemia. The animals which were made hypertensive during the period of release of occlusion revealed consequently much more severe and earlier occurring damage to the BBB. Comparison between two groups of gerbils, which were all subjected to identical durations of occlusion and release but differed in one group having elevated blood pressure, showed that the histopathologic picture of ischemic injury was conspicuously more severe in the hypertensive group. The biochemical assays on these two groups revealed that in the hypertensive animals the lactate levels following release of occlusion kept rising, in contrast to the control normotensive group. It appears then that for brain tissue subjected to ischemia both systemic hypotension and hypertension may have very harmful effects and this should be of significance for the clinical management of stroke patients.

The clinically important question of neuronal recovery has been a controversial one, primarily due to the difficulty in ascertaining the functional state of neurons from static morphologic pictures. Nonetheless, the light microscopic observations on gerbils indicated that a considerable neuronal recovery from ischemia is possible. For example, the comparison between groups of animals all occluded for one hour but sacrificed at different intervals after release of the clip revealed the following: in all animals sacrificed 20 hours after release the cerebral cortex showed extensive neuronal changes. On the other hand, the majority of gerbils sacrificed one week later showed remarkably well preserved cortical neurons. Only occasional foci of cell disappearance, glial modules, or capillary proliferations testified that such areas had been affected by ischemia. Thus, this comparison suggests that many of the neurons which showed marked ischemic changes at 20 hours have by now completely recovered. Another instance of apparent neuronal recovery has been described above as a "reactive change" in the H3 neurons in gerbils occluded for short periods of time and sacrificed 20 hours later. An indication of neuronal ability to recover from ischemia is also provided by histochemical observations in gerbils demonstrating a strong enzymatic activity in neurons with pronounced ischemic changes, such as chromatolysis, cytoplasmic vacuolization or intense hyperchromasia. The intense enzymatic activity in these neurons clearly indicates their viability at the time of the termination of the experiment.

The recognition of intravital from post-mortem or artifactitious changes is of considerable importance in evaluation of ischemic neuronal changes in the light microscopy. Investigations using Baker's acid hematein method indicated that the characteristic staining is induced in normal neurons as they are transformed to "dark" neurons by post-mortem trauma to the unfixed brain, or in other words that it is nonspecific, or artifactitious in nature. Since this staining reaction, in addition to the others tested, gave similar results in "dark" and "ischemic" neurons, it is concluded that the development of the latter cell type is also the consequence of post-mortem trauma but to a neuron which had already been pathologically altered.

With regard to application of cortisone treatment in animals which were subjected to transection of various cranial nerves it was found that cortisone

not only depressed the incidence of reactive mitosis in microglial cells but also affected protein synthesis in neurons undergoing retrograde changes. This observation may be of potential significance for further study on the "reactive change" described in H3 sector of the hippocampus following ischemia which histologically greatly resembles the retrograde change observed after axonal transection.

Generally, our studies on pathophysiologic mechanisms of cerebral ischemia introduce an optismistic note that a considerable recovery of brain from ischemic injury can be achieved. This should provide encouragement for further elucidation of pathomechanisms involved with the hope of acquiring new approaches for successful clinical management of many stroke patients.

For elucidation of various pathological processes of cerebral vasculature, the studies on biology of cerebral endothelium are of great importance. Endothelial cells clearly constitute the cellular substrate which is responsible for the maintenance of homeostatic chemical environment of brain parenchyma. This is accomplished through various transport and blood-brain barrier functions. The study of cerebral endothelium was undertaken in vitro. It has been possible to develop a method by which a preferential growth of endothelium was stimulated in the cerebellar cultures. The studies revealed different features and behaviour of brain endothelium in comparison with endothelium grown in skin, bone and muscle cultures. The differences observed applied to the presence of various enzymes and to the uptake of H3 glucose analogues. Various transport phenomena were investigated under conditions of ischemia, hypoxia, hypercapnia and hypocapnia by using the double isotope technique of Oldendorf. A significantly increased facilitated transport of glucose analogues was demonstrated in ischemic gerbils. With regard to the effects of oxygen toleration and pCO_2 tension in the rabbit brain, our previous studies have shown a decreased brain uptake of 2-deoxy-D-glucose and 3-O-methyl-D-glucose in severe hypoxia and hypercapnia whereas an increased uptake of these substances was seen in hypocapnia. Presently, similar studies on the transport of amino acids indicate that the brain uptake of tested radiolabeled essential amino acids is different from the uptake of labeled glucose analogues and nonessential amino acids in hypercapnia but not in hypoxia.

The understanding of cerebrovascular pathology is greatly dependent on elucidation of various aspects of carbohydrate metabolism of the brain. These investigations carried out primarily in the Section on Cellular Neurochemistry have shown the following: The activities and properties of glycogen synthase and glycogen phosphorylase have been examined in C-6 astrocytoma and C-1300 neuroblastoma cells in culture. The interconversions of the active forms and the inactive forms have been investigated under various conditions. It has been demonstrated that in cell cultures, these interconversions are regulated by two mechanisms; first, the available supply of glucose, and second, the intracellular concentrations of cyclic AMP. When glucose concentrations are high either in the medium or in the cell, the amount of the glycogen degrading enzyme (phosphorylase) in the active form is low, the proportion of the active form of the glycogen synthesizing enzyme (synthase) is high, and glycogen accumulates in the cell. As the glucose in the medium is metabolized and thus decreases in concentration, the phosphorylase is activated and the synthase

deactivated. The regulation of the glycogen-related metabolic events by glucose concentration is similar in the C-6 astrocytoma cells and C-1300 neuroblastoma cells in culture.

Insulin added to C-6 cultures resulted in decreased phosphorylase a, increased synthase a, and a small increase in glycogen. This effect is dependent on glucose concentration and, perhaps, small decreases in cyclic AMP concentration.

The intracellular concentrations of cyclic AMP of these cells in culture can be increased by pharmacological or hormonal manipulation. In the astrocytoma cells, addition of norepinephrine, or isobutyl methyl xanthine (IBMX) causes a several fold increase in cyclic AMP. Norepinephrine activate adenyl cyclase which forms cyclic AMP, while IBMX inhibits the degradative enzyme, phosphodiesterase. The elevation in cyclic AMP results in increased active phosphorylase, decreased active synthase, and a fall in intracellular glycogen concentration. The C-1300 neuroblastoma cells do not respond to norepinephrine, but do respond to prostaglandin E or adenosine and IBMX showing elevated cyclic AMP levels, with consequent enzyme and glycogen changes as described for the C-6 cells.

Other studies with astrocytoma cells in culture have shown that norepinephrine will induce cyclic AMP phosphodiesterase. The induction has been shown to be mediated by cyclic AMP via the β receptor and to require new protein synthesis.

The kinetics of glucose uptake, and pyruvate and lactate efflux and reuptake have been examined in the astrocytoma cells in culture. The cells show two transport systems for glucose with Km's of 1.2 and 7.1 mM. The excretion of both pyruvate and lactate from the cells depends on the medium glucose content, the medium pH and the intracellular pyruvate and lactate concentrations. Long-term growth studies demonstrated that once the cells had utilized all the medium glucose, they were capable of taking up and metabolizing first pyruvate followed by lactate.

The effect of thiamine deficiency on the intermediary metabolism of the cells has also been studied. Thiamine deficiency in the presence or absence of the antimetabolite pyrithiamine decreased the growth rate of both cell lines, although the neuroblastoma cells were more sensitive. In addition, both the intracellular and excreted levels of pyruvate and lactate increased, indicating a block at the level of pyruvate dehydrogenase. These results suggest that the cell lines can serve as a model for some of the biochemical changes occurring in the nervous system with thiamine deficiency.

Other cell lines have been used to study the regulation of the NAD+/NADH ratio and its relation to the metabolic pattern of normal and virally transformed tumor cell lines. The fibroblasts have exhibited extreme sensitivity to the concentration of glucose in the medium. Removal of glucose increases the NAD+/NADH ratio 3 to 4 fold within 5 minutes, followed by a gradual decline in ATP. Readdition of glucose restores the redox ratio within 5 seconds. No other sugars can substitute for glucose completely for

growth, and the redox state of cells grown on other sugars varies greatly. The NAD+/NADH ratio in glucose-grown cells is 2 to 3, whereas when galactose is the energy source, the ratio is 10 to 15. There are striking changes in cell morphology accompanying these differences.

Biochemical changes have been investigated in gerbil brain made ischemic by ligature of the left common carotid artery. In such animals, the right hemisphere serves as control. ATP, P-creatine, glycogen and glucose decrease in the ischemic cerebral hemisphere. Cyclic AMP increases to a maximum 2 hours after ligation and then decreases. Gamma-amino butyric acid increases 5-fold in the ischemic brain. Other putative transmitters dopamine, norepinephrine and 5-hydroxytryptamine, decrease after ligation. All metabolites return to control values one hour after release of the ligature. No changes in enzyme activity were observed.

In another series of experiments, the role of cyclic nucleotides in the central nervous system during convulsive disorders is being investigated. Cyclic AMP is increased during the tonic extension phase of convulsion following electroconvulsive shock in mice. Cyclic GMP increases during the postictal depressive phase. Hypothermic animals showed much the same response except that the time course was prolonged. By using hypothermic animals it was possible to correlate the elevation in cyclic AMP with increases in phosphorylase a and decreases in synthase a. The administration of central nervous system depressants to mice (phenobarbital, papaverine and Mg SO_4) significantly decreased cerebellar cyclic GMP. Anticonvulsant agents, diphenyl hydantoin, clonazepam and dipropyl acetic acid decreased cerebellar cyclic GMP and increased the threshold for convulsant activity of isoniazid, pentylenetetrazol and theophylline. The latter three agents increased cyclic AMP when given to unprotected animals in convulsive doses. Cyclic AMP levels were not affected in the cerebellum by convulsive or anticonvulsant agents. Thus cyclic AMP and cyclic GMP appears to be independently regulated in vivo. Cyclic GMP apparently serves as a molecular indication of CNS excitability, increasing following administration of convulsive agents and decreasing in depressed states.

With regard to our studies on demyelinating diseases, a major goal in current research on the pathophysiology of multiple sclerosis (MS) is to develop model systems for studying the cellular mechanisms of demyelination. In the research program of the Section on Cellular Neuropathology on CNS myelin, most of the effort has been devoted to exploring the usefulness of Xenopus tadpole optic nerves as an in vivo model of a myelinated CNS tract. An important advantage of these nerves is that they are easier to expose to demyelinating agents and to study morphologically than CNS tracts of mammalian experimental animals. In this rather simple system that still has a higher level of organization than CNS tissue cultures, it is hoped to produce and study areas of demyelination that resemble MS plaques. This year, the CNS myelin model system project included three sets of experiments utilizing tadpole optic nerves. In the first, groups of tadpoles received subcutaneous injections of unconcentrated CSF from 18 patients; after 48 hours, myelin lesions were counted in whole mounts of their optic nerves. Compared to controls, counts of myelin lesions were significantly elevated in the CSFs

from four patients with multiple sclerosis. In other tests of these CSFs, the demyelinating effect was not altered by the addition of complement, was thermostabile, and seemed to parallel the severity of the patients' demyelination. In these few tests, there was no correlation between demyelinating activity and the gamma globulin or total protein content of the CSFs. In the second set of experiments, the above injection procedure and whole mount technique were used to test the demyelinating activity of sera from rabbits and guinea pigs with experimental allergic encaphalomyelitis (EAE). Some sera, particularly those from rabbits, produced higher myelin lesion counts than controls but the increase was not statistically significant. While using this tadpole optic nerve system to study factors in CSF or serum that may produce CNS demyelination, it was important to study the distribution of subcutaneously injected macromolecules. Therefore, in the third set of experiments, the penetration of macromolecular tracers into the optic nerve parenchyma after subcutaneous injection was examined. Within 3 hours, ferritin, horseradish peroxidase, and fluorescein labeled human IgG diffused between astrocytic processes forming the nerve's glial sheath and were found in extracellular spaces surrounding myelinated fibers. Thus, if the demyelinating effect of CSF or serum is related to macromolecules such as globulins, it was shown that they quickly reach CNS myelinated fibers in tadpole optic nerves after subcutaneous injection. The tests of EAE sera suggest that though present, the concentration of macromolecular demyelinating factors that surrounds these myelinated nerve fibers may still be too low to produce demyelination. Clearly, it is much higher in myelinated CNS tissue cultures where demyelination by sera from rabbits with EAE has been repeatedly demonstrated. Thus, other ways of testing CSF and sera are currently being explored in order to enhance their demyelinating activity in tadpole optic nerves.

The Section on Neurocytology is involved in three main pursuits: (A) the search for ways of circumventing the blood-brain barrier, (B) the relationship of glia to neurons, and (C) the changes within the cell membrane of smooth muscle and perineurium under tension.

With regard to circumvention of the BBB, it has been recently found that when endothelial cells of cerebral vessels shrink in response to the intravascular infusion of hyperosmotic urea, the tight junctions responsible for the BBB to dye and protein are opened. Hypertension also causes a leakage of the Evans blue tracer. It has been attempted to elucidate the mechanism of this latter opening, using horseradish peroxidase (HRP), a more sensitive tracer than Evans blue. When Aramine is given intravenously or a bolus of saline is infused quickly (0.6ml/second) into one carotid artery, HRP crosses cerebral vessels within two minutes. The barrier opens in a random, spotty fashion during a rise of at least 60 mm Hg above resting systolic pressure and is reversible within 1 to 2 hours after Aramine. The entire brains of over 40 rats have been sectioned and the ring exudates within each of six brain regions counted. The greatest number of leaks occurs in the cerebrum, the least in the medulla. There are about 5 time more exudates in the bolus opening than in the opening with Aramine. It is hypothesized, though not yet proven, that the barrier opening is due to distortion of the endothelial junctions upon stretching of the vessels during an increased flow through

their lumens. Thus, even a modest rise in systemic blood pressure causes a rapid, transient opening. It is conceivable that the infusion of contrast media during cerebral angiography could do the same.

We are also following the entry of blood-borne peroxidase (HRP) into the brain from the periphery along intracellular routes. When HRP (5-10mg) is injected into the tail vein of mice and other rodents it enters the brain via three routes: 1. The circumventricular organ, such as the median eminence and area postrema which have leaky fenestrated vessels. HRP floods the extracellular clefts of these regions and is pinocytosed by glial and neuronal cells within them. 2. The axons of neurosecretory nuclei (supraoptic, paraventricular and arcuate) which end on fenestrated capillaries in the median eminence and neurohypophysis. We have just shown that HRP is picked up by the axons of these neurohemal cells and transported within their axons to their cell bodies. 3. The axons of cranial nerves, both sensory and motor, which end near vessels that are not fenestrated but which have open junctions and brisk vesicular transport. This is the first demonstration that after the intravenous injection of 60-100mg HRP, the axon terminals of cranial nerves can incorporate protein derived from the normal circulation and not injected or iontophoresed into their target organ. Cranial nerve nuclei III, IV, V, VI, VII, X and XII become labeled within 10 to 24 hours after injection into the blood. Thus, there is an intracellular route as well as an extracellular one from blood to brain. Nerves VII and XII have been ligated to test this hypothesis and, primarily, to differentiate the organelles involved in protein uptake directly by the cell body from those involved in retrograde transport from axon terminals. The fate of organelles on the distal side of the ligatures is also being followed. The implications of the intracellular route are highly important with respect to the entry of virus and macromolecules circulating in the blood.

With regard to the relationship of glia to neurons in the cerebral parenchyma of vertebrates, many regions, usually subependymal, contain parallel rows of glial sheets between axons. A similar type of organization is observed in the ventral nerve cord of crayfish. The nerve cord is easily accessible and may be cut with minimum damage to surrounding connective tissue. The remodeling of glial cells has been followed 3 to 5 weeks after sectioning the cord. In freeze-fracture replicas, the inner faces of glial membranes are displayed as broad vistas of parallel sheets indented by many small depressions. These correspond to short, straight transcellular channels, numbering 16 per μ^2 in intact cords and about the same (13 per μ^2) in regenerating cords. When the normal cord is immersed in peroxidase, the channels and peri-axonal clefts are quickly penetrated. The trans-glial channels thus act as "short-cuts" for the diffusion of large molecules to the axonal surface.

The cell membrane of smooth muscle and perineurium under tension has been studied in the mollusc Aplysia in which the connective linking cerebral and abdominal ganglia is surrounded by a sheath containing numerous isolated smooth muscle cells. Their sarcolemma, like that of vertebrates, is indented to form many small pits which, despite the designation of "pinocytotic," do not pinch off and migrate upon prolonged immersion of the connective in

peroxidase. In Aplysia, the fractured faces of the pits are studded by large particles. In connectives stretched to 3 times their normal length, the pits are flattened so that the cleavage plane follows the membrane more than it does the cytoplasm. The resulting fracture displays many more large particles belonging to the pits than in the resting connective. Similar pits, without large particles, indent the cell membrane of vertebrate vascular smooth muscle and sciatic nerve perineurium. It is likely that here too, the pits are involved in extensibility of the cell membrane rather than pinocytosis.

In the Section on Functional Neuroanatomy, a major research effort is concerned with the structural basis of synaptic transmission. This project seeks to clarify the exact location and mechanism of synaptic transmission in the central and peripheral nervous system. It is carried out by examining synapses with the electron microscope and by determining the influence of various functional states on their structure. Much effort in the last year has been invested in development of better techniques for studying synapses. These efforts have resulted in a method for freezing synapses in various fleeting functional states without any of the chemical fixatives commonly used and then examining them by the freeze-fracture technique. Also, a method has been developed in collaboration with Dr. Dubois-Dalcq for localizing proteins on surfaces of membranes which will be of use in localizing receptors or toxin binding sites at synapses.

Our past work on synapses has shown that local recycling of synaptic vesicles replaces those lost during synaptic activity. In the last year we have examined this mechanism more carefully using the freeze-fracture technique. These studies have shown that synaptic vesicles can be discharged only at specific points on the synaptic membrane and that these points are determined by specific structures within the membrane. We have also been able to produce direct evidence that synaptic vesicles discharge their contents by fusing with the synaptic membrane, a process well known in other secretory cells as exocytosis.

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, although more types of synapse must be examined before this conclusion is certain. Study of the central nervous system has also showed that one class of synaptic connection is lacking in the forebrain of one strain of mouse, even though both components for this synapse are present and able to make synapses with other types of neuronal processes. Thus, formation of this synaptic type may be under genetic control.

One of the most immediately practical aspects of the present study is that it defines the normal structure of synapses in a variety of functional states. This knowledge will permit destruction of normal from pathological and resting from active synapses with the electron microscope. In structural studies of epileptic brains it should now be possible to distinguish normally active from pathologically active synapses. Similarly, in diseases involving peripheral nerve-muscle synapses at neuromuscular junctions, it would be

possible to distinguish pathological states from changes resulting from increased or decreased activity.

The finding that different chemical types of synapses might be distinguished 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.

The other major project in the Section on Functional Neuroanatomy refers to permeability of cellular layers in the vertebrate nervous system. The electron microscope is used to determine the cellular basis of the blood-brain barrier (BBB) to proteins. Our previous studies showed the location of the BBB within blood vessel walls in the parenchyma of the brain. Tight junctions between endothelial cells were shown to make an important contribution to this barrier. More recently we have tried to establish the basis of the BBB in the meninges and other borders of the brain. In particular, the ependyma over the hypothalamus and the meninges were carefully examined and papers on our findings submitted for publication.

Unlike most regions of the brain, the median eminence lacks a BBB at the walls of its blood vessels. However, it is isolated from the cerebrospinal fluid (CSF) by a specialized ependyma which acts as a barrier between its parenchyma and the overlying CSF. Similarly, the dural covering of the brain lacks a BBB but embedded in the underlying arachnoid is a specialized layer of cells which provides a barrier between the dura and the CSF. This barrier layer of the arachnoid is missing at arachnoid villae where CSF is reabsorbed. The conclusion which emerges from these studies is that the brain and CSF is separated from the blood by a continuous layer of cells which act as a barrier to proteins. This barrier layer is absent at only a few locations for specific purposes such as reabsorption of CSF.

More recent studies have used the freeze-fracture technique to permit a more detailed look at the limiting astroglial membrane of the brain where it faces the CSF. Although there is no barrier to proteins in this layer, a complex system of intercellular junctions and other specializations of the astrocytic membranes have been discovered. While this significance to the BBB system is not clear yet, this finding focuses attention on the astroglia as possibly having a subtle role in the blood-brain barrier system.

These ideas about the cellular basis of the BBB as well as the anatomical techniques used in these studies, afford a basis to design experimental studies of a variety of clinical disorders which depend on or produce disruption of the BBB.

Project No. Z01 NS 01884-05 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Brain Glycogen Synthetase

Previous Serial Number: SAME

Principal Investigator: Janet V. Passonneau, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0

Project Description·

Objectives: To determine the role of glycogen synthetase in glycogen formation in brain in normal and experimental states.

Methods Employed: Two methods are employed for the measurement of enzyme activity. The first is a coupled enzyme assay using pyruvate kinase and lactate dehydrogenase to measure uridine diphosphate which is formed in stoichiometric amounts with the glucose incorporated into glycogen from uridine diphosphoglucose. The second assay utilizes a radioisotope precursor, ^{14}C-uridine diphosphoglucose, and the incorporation of the radioactive glucose moiety into glycogen is measured.

Major Findings: The requirement for preincubation with glycogen for full activity of the purified brain enzymes was investigated. The enzyme-substrate complex was studied using sucrose-density gradients in the ultracentrifuge. The molar ratio of glycogen : enzyme indicated that the enzyme preferentially binds to low molecular weight polysaccharides.

Significance: The glycogen synthetase activity is dependent on the form in which it exists as well as the concentration of substrates and modifiers. The regulation of the activity in brain is of great importance to understand the accumulation of glycogen in anesthesia and trauma, and its depletion in early trauma and ischemia.

Proposed Course of Project: The investigation of the properties of purified glycogen synthase, both I and D forms, from pig brain has been completed.

Keyword Descriptors: Glycogen synthetase, brain in normal and experimental states, regulation of the activity of brain.

Honors and Awards: None

Publications:

Passonneau, J.V., Schwartz, J.P. and Rottenberg, D.A.: The partial purification and properties of pig brain glycogen synthase. J. Biol. Chem. 250: 2287-2292, 1975.

Project No. Z01 NS 01885-05 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The kinetics of brain glycogen

Previous Serial Number: SAME

Principal Investigator: Janet V. Passonneau, Ph.D.

Other Investigators: W. David Lust, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0

Project Description:

Objectives: To further explore factors in the turnover, accumulation, and disappearance of glycogen in the brain. To determine the effects of trauma, drugs, and altered physiological states such as hypothermia or drug administration on glycogen metabolism.

Methods Employed: A stab wound injury was made in one cerebral hemisphere of mouse brain and the contralateral side served as control. Glycogen content was measured at intervals after injury, as well as related metabolites and enzymes. These included glucose, glucose 6-P, uridine diphosphoglucose, cyclic AMP, glycogen phosphorylase and glycogen synthetase.

Major Findings: Following studies of glycogen turnover and the effects of injury on glycogen metabolism, attention was focussed on the alterations in cyclic 3',5'-AMP (cyclic AMP) concentrations in the cortex.

Stab wound injury produced a 7-fold elevation in cyclic AMP in the cortex of mouse brain within one minute. The increase in cyclic AMP was blocked by prior treatment of the animal with theophylline, chlorpromazine, trifluoperazine and diphenhydramine. Neither dichlorisoproterenol, pronethalol nor reserpine blocked the increase in cyclic AMP concentration due to injury. The results following administration of drugs suggest that the increases in cyclic AMP due to injury may be mediated by adenosine. Theophylline and phenothiazine derivatives have been shown previously to decrease adenosine-

mediated increases in cyclic AMP in brain slices. The absence of any effect after administration of dichlorisoproterenol or pronethalol suggests that the increase in cyclic AMP after injury is not influenced by catecholamine release. Hypothermia reduced the elevation in cyclic AMP in injured brain compared to normothermic mice.

Significance: The elevation in cyclic AMP following stab wound injury and the effect of drugs are consistent with adenosine release following ischemia and membrane depolarization. The mechanism of the release and the effect on glycogen metabolism were the subject of previous reports.

Proposed Course of Project: Further studies are being made on the effect of electroconvulsive shock, insulin, and experimental brain edema on the rate of incorporation of ^{14}C-glucose into brain glycogen and the subsequent loss of label.

Keyword Descriptors: Turnover, accumulation and disappearance of glycogen in the brain, stab wound injury, glycogen content, related metabolites, enzymes, cyclic AMP, elevation in cyclic AMP, mediated by adenosine, membrane depolarization.

Honors and Awards: None

Publications:

Passonneau, J.V. and Lauderdale, V.R.: A comparison of three methods of glycogen measurement in tissues. Anal. Biochem. 60: 405-412, 1974.

Watanabe, H. and Passonneau, J.V.: Cyclic 3',5'-AMP alterations in cerebral cortex following trauma. Arch. Neurol. 32: 181-184, 1975.

Project No. Z01 NS 01942-04 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The role of cyclic AMP and cyclic GMP in the central nervous system

Previous Serial Number: SAME

Principal Investigator: W. David Lust, Ph.D.

Other Investigators: Janet V. Passonneau, Ph.D.
Harvey J. Kupferberg, Ph.D.
Wayne D. Yonekawa, M.S.

Cooperating Units: C&FR, NINCDS

Man Years:

Total:	0.4
Professional:	0.4
Other:	0

Project Description:

Objectives: To determine if either pharmacological or physiological alterations of brain metabolism would affect the steady state levels of cyclic AMP and/or cyclic GMP in vivo.

Methods Employed: Mice were rapidly frozen in liquid nitrogen at the appropriate times following treatment. The brains were removed at -25°, weighed and extracted in perchloric acid. The neutralized PCA extracts were used in all subsequent metabolic measurements. Cyclic GMP was measured by an enzymic-cycling technique or by the immunoassay method of Steiner, and cyclic AMP was measured by a radioactive binding assay.

Electroconvulsive shock (ECS) was applied by corneal electrodes at an intensity of 50 mA for a duration of 0.2 sec. The electroshock produces a convulsive response manifested by a) a tonic extensor phase (0-15 sec), b) an intermittent clonic phase (15-30 sec) and c) a postictal depressive phase ($>$ 30 sec).

In the thermal experiments, the mice were rendered a) hypothermic (20°) by forcing them to swim for 1-2 min in a water back followed by a 10 min stay in a cold chamber, and b) hyperthermic (41°) by placing them under a

heat lamp. Rectal temperatures were monitored with a tele-thermometer.

Major Findings: The continuation of the work on electroconvulsive shock (ECS) and ischemia (produced by decapitation) confirmed our previous findings that the levels of cyclic AMP increase rapidly following both treatments in all areas of the brain tested. The levels of cyclic GMP in the cerebellum also increase almost 4-fold following ECS. The major difference between the cyclic nucleotide responses in the cerebellum is that the maximal change in cyclic AMP occurs at 15 seconds and precedes the cyclic GMP peak by approximately 45 seconds. Relating the cerebellar cyclic nucleotide responses to convulsive behavior, it is apparent that the major cyclic AMP changes occur during the excitable states (tonic, 0-15 seconds and clonic, 15-30 seconds), and cyclic GMP during the depressive stage (postictal, greater than 30 seconds). Although the levels of cyclic nucleotides drop rapidly following their maximal ECS-induced changes, the restoration of cyclic AMP and cyclic GMP to control levels was not reached by 240 seconds. In addition, the cyclic AMP response to ECS differs in the cerebral cortex and the cerebellum. The cyclic AMP response in the cerebral cortex is 2-3 fold greater than that in the cerebellum and the peak response occurs at 45 sec post-ECS or some 30 seconds after the cerebellar maximum. In contrast, the cyclic AMP increase following decapitation is greater in the cerebellum than in the cerebral cortex.

To determine what effect altered body temperature would have on cyclic nucleotides, mice were rendered hypothermic (20°) or hyperthermic (41°) as described in the methods. In hypothermic mice, the levels of cyclic AMP were lower than normothermic values in both the cerebellum and the cerebral cortex. The levels of cyclic GMP were not affected. Further, the cyclic AMP increases following either decapitation or ECS were significantly reduced. In hyperthermic mice, both cyclic nucleotides were unchanged in NIH mice; however, the concentrations of cyclic GMP increased 2-fold in the DBA-2N strain of mice. Hypothermia delays the cyclic AMP response to ECS in the cerebral cortex. Although the peak levels resulting from ECS are approximately the same in both hypothermic and normothermic mice, the hypothermic maximum occurs at 240 sec post-ECS, some 3 min after the normothermic peak. From the measurements of ATP, phosphocreatine, glucose and phosphorylase a, post-ECS, it appears (as is the case with cyclic AMP) that hypothermia only affects the time course and not the magnitude of these metabolic changes.

In pharmacological studies, phenobarbital, papaverine and $MgSO_4$ significantly decreased the levels of cerebellar cyclic GMP. Phenobarbital (100 mg/kg IP) lowered cyclic GMP within five minutes and the levels remained at 20% of control for up to 90 minutes. Of all the drugs tested, only chlorpromazine and trifluopromazine lowered cyclic AMP levels. Pretreatment with two drugs, amphetamine (10 mg/kg IP) which elevates GMP 2-fold, or phenobarbital (20 mg/kg IP) which decreases cyclic GMP 50%, had no effect on the ECS-induced increase in cyclic nucleotides, in spite of significant alterations in overt convulsive behavior.

The anticonvulsant agents, diphenylhydantoin, clonazepam (C) and dipropylacetic acid (DPA) decreased cerebellar cyclic GMP levels by more than 50%. Conversely, the convulsant agents, isoniazid (INH), pentylenetetrazol (PT-Z) and theophylline increased the cyclic GMP levels by at least 3-fold. And in combination, the convulsant-induced elevation of cyclic GMP was inhibited by the anticonvulsant agents. Specifically, DPA and C blocked the INH-induced increase in cyclic GMP, and C blocked the PT-Z-induced increase. Thus, these anticonvulsant agents were not only able to block the chemically-induced seizures, but also the increase in cerebellar cyclic GMP.

A time course following 400 mg/kg IP of DPA provides additional evidence for a relationship between anticonvulsant activity and cerebellar cyclic GMP levels. Anticonvulsant activity here was defined as the ability to abolish tonic extension following ECS. The levels of cyclic GMP were depressed at 1/2 and 1 hr after injection when more than 60% of the mice were protected against seizures. As anti-convulsant activity diminished at 2 and 4 hours, the cyclic GMP returned to control levels. So, the lowered cyclic GMP levels coincided temporally with the anticonvulsant activity.

Using 4 different fixation techniques, the levels of cyclic AMP were lowest in the microwave irradiated brain with increasing amounts in brains which were freeze-blown, frozen by immersion and frozen following decapitation. In comparison, the degree of anoxia as determined by the changes in ATP, phosphocreatine and lactate was lowest in the freeze-blown brain, with increasing anoxia in brains which were frozen by immersion, irradiated and frozen following decapitation.

Significance: Both the ECS and pharmacological data favor the argument that cyclic AMP and cyclic GMP are independently regulated in vivo. The possibility of a direct relationship between elevated cyclic GMP and depression seems remote, since pharmacologically-induced depression either reduced or had no effect on cyclic GMP levels. There is an increasing amount of evidence favoring an association between either lowered cyclic AMP or elevated cyclic GMP and CNS excitation; and between elevated cyclic AMP or lowered cyclic GMP and CNS depression. Thus, neural events following a number of neurotropic agents or such treatments as ECS or brain injury might be defined by the cyclic nucleotide profiles. From our studies with pharmacological agents, the levels of cerebellar cyclic GMP appear to reflect a behavioral spectrum from depression to convulsion; CNS depressants and anticonvulsant agents lower cyclic GMP while convulsant agents elevate cyclic GMP. Thus, cyclic GMP in the cerebellum may serve as a molecular indicator of the degree of CNS excitability, and therefore may be useful in our understanding of the mechanism of action of a variety of neurotropic drugs.

The ECS- and anoxia-induced changes in brain cyclic AMP exhibit regional variation. Thus, while the cerebellum is more sensitive to the stimulus of anoxia, the cerebral cortex is more responsive to the electrical stimulus. Although hypothermia does slow metabolic events in the brain, it is apparent

that only the temporal relationships and not the magnitude of response are affected.

Since cyclic AMP increases rapidly in the brain following decapitation, low cyclic AMP levels have been equated with rapid fixation. However, following microwave irradiation low levels of cyclic AMP were obtained although the irradiated brains were anoxic by other criteria. Of the fixation methods tested, freeze-blowing and freezing intact most closely approximate the in vivo state.

Proposed Course of Project: Presently, the primary emphasis is on the relationship of cerebellar cyclic GMP to the different types of anticonvulsant agents, and their different anticonvulsant activities (anti-pentylenetetrazol, anti-ECS, etc.). Also, more extensive studies on the seizure-dependent rise of cyclic nucleotides whether chemically-or electrically-induced are being undertaken in other regions as well as the cerebellum.

Keyword Descriptors: Cyclic AMP, cyclic GMP, central nervous system, electroconvulsive shock, ischemia, hypothermia, anticonvulsant agents, convulsant agents, microwave irradiated brain, freeze-blown brain, CNS depressants.

Honors and Awards: None

Publications:

Lust, W.D., Passonneau, J.V. and Veech, R.L.: Cyclic adenosine monophosphate, metabolites and phosphorylase in neural tissue: a comparison of fixation methods. Science 181: 280-282, 1973.

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1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Regulation of metabolism in glioma and neuroblastoma cell lines

Previous Serial Number: SAME

Principal Investigator: Joan P. Schwartz, Ph.D.

Other Investigators: W. D. Lust, Ph.D.
J. V. Passonneau, Ph.D.
S. K. Crites, B.S.
M. L. Nahrwold, M.D.

Cooperating Units: Anesthesia, CC

Man Years:

Total:	1.4
Professional:	1.2
Other:	0.2

Project Description:

Objectives: To investigate the regulation of metabolism in a glioma and a neuroblastoma cell line.

Methods Employed: The cells are being grown in a CO_2 incubator in the laboratory. Homogenates of the cells are analyzed for various metabolites, such as cyclic AMP, cyclic GMP, and pyridine nucleotides, as well as for enzymes such as the adenylate and guanylate cyclases, cAMP phosphodiesterase and glycogen synthetase and phosphorylase. All of these analytical methods have been used in the laboratory on whole brain and are thus easily adaptable for use with the cells. In addition, the effect of various agents on the morphology of the cells will be undertaken.

Major Findings: The glioma cells contain an adenylate cyclase which is responsive to catecholamines such as norepinephrine. Activation of the cyclase by norepinephrine elevates the intracellular cyclic AMP level, resulting in the breakdown of glycogen due to inactivation of the glycogen synthetase and activation of the phosphorylase (Browning, E.T., Schwartz, J.P., and Breckenridge, B.M.: Molec. Pharmacol. 10: 162, 1974). The expression of the norepinephrine receptor has been shown to require cell contact. Activity

of the cyclic AMP phosphodiesterase can also be regulated by growth of the cells in either dibutyryl cAMP or in bromo-deoxy-uridine an agent which causes dedifferentiation (Schwartz, J.P., Morris, N.R., and Breckenridge, B.M.: J. Biol. Chem. 248: 2699, 1973). Treatment of the glioma cells with norepinephrine not only results in short-term effects, but also causes an induction of the phosphodiesterase. The induction has been shown to be mediated by cyclic AMP via the β-receptor and to require new protein synthesis.

The effects of other hormones and drugs, such as depolarizing agents, on the metabolism of both cyclic AMP and cyclic GMP, have also been studied. In addition, both cell lines have been shown to contain individual phosphodiesterases specific for the 2 cyclic nucleotides, which can be independently induced.

In addition to cyclic AMP metabolism, a study of the energy metabolism of both cell lines has been carried out. The kinetics of glucose uptake, of glycogen breakdown, and of pyruvate and lactate efflux and uptake have been studied. The effect of thiamine deficiency, brought about by growth without thiamine or in the presence of the anti-thiamine drug pyrithiamine, on the above parameters has also been examined. In addition, the effects of the local anesthetic, tetracaine, on this system have been studied.

Significance: A study of the regulation of metabolism in the brain is complicated by the presence of several cell types and the inability to determine in which cells metabolic alterations are occurring. Such studies are facilitated by the use of the two distinct cell lines, the glioma (rat C-6 line) and the neuroblastoma (mouse C-1300 line), because regulation can be studied in each cell line separately. Until these cell lines became available, for example, it had not been realized that glial cells might be hormone-responsive. Such results suggest that the glial cells may have a greater involvement in brain function than the supportive one originally envisaged. Although these two cell lines are tumor cells, they offer a first approach to the problem of regulation of metabolism in the brain, which might be followed up in normal brain cells when the isolation techniques for glial and neuronal cells have been perfected.

Proposed Course of Project: A thorough investigation of the regulation of cyclic AMP and cyclic GMP levels, of other energy-related parameters, and of pyridine nucleotide ratios will be undertaken, in both cell lines. Studies on ion transport are also planned, as are morphological studies of the effects of various agents and growth conditions. A method of perfusion for the cells has been developed, and is being utilized in a study of the effects of anesthetics. Further studies related to the effect of thiamine deficiency on thiamine-dependent enzymes and the pentose shunt are planned.

Keyword Descriptors: Cyclic AMP, cyclic GMP, pyridine nucleotides, adenylate and guanylate cyclases, cAMP phosphodiesterase.

energy metabolism, thiamine deficiency, method of perfusion.

Honors and Awards: None

Publications:

Schwartz, J.P. and Passonneau, J.V.: Cyclic AMP-mediated induction of the cyclic AMP phosphodiesterase of C-6 glioma cells. Proc. Nat. Acad. Sci. USA: 71, 3844-3848, 1974.

Lust, W.D., Schwartz, J.P. and Passonneau, J.V.: Glycolytic metabolism in cultured cells of the nervous system. I. Glucose transport and metabolism in the C-6 glioma cell line. Molec. Cell. Biochem. (in press).

Schwartz, J.P., Lust, W.D., Lauderdale, V. and Passonneau, J.V.: Glycolytic metabolism in cultured cells of the nervous system. II. Regulation of pyruvate and lactate metabolism in the C-6 glioma cell line. Molec. Cell. Biochem. (in press).

Schwartz, J.P., Lust, W.D., Shirazawa, R. and Passonneau, J.V.: Glycolytic metabolism in cultured cells of the nervous system. III. The effects of thiamine deficiency and pyrithiamine on the C-6 glioma and C-1300 neuroblastoma cell lines. Molec. Cell. Biochem. (in press).

Project No. Z01 NS 02140-01 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Regulation of the NAD^+/NADH and metabolic pattern of normal and transformed fibroblasts

Previous Serial Number: NONE

Principal Investigator: Joan P. Schwartz, Ph.D.

Other Investigators: George S. Johnson, Ph.D.

Cooperating Units: Laboratory of Molecular Biology, NCI

Man Years:

Total:	0.2
Professional:	0.2
Other:	0

Project Description:

Objectives: To investigate the factors which regulate the NAD^+/NADH ratio and the metabolic pattern in normal and transformed cell lines.

Methods Employed: The cells are grown in tissue culture. Cell extracts are analyzed for various metabolites such as NAD^+, NADH, ATP and cyclic AMP by a variety of fluorometric and immunological methods.

Major Findings: The cells are extremely sensitive to the presence and concentration of glucose in the medium. Five minutes after removal of glucose, the NAD^+/NADH ratio increases 3-4-fold, followed by a much more gradual decline in ATP (20% fall in 2 hrs). Readdition of glucose restores the redox state within 5 seconds. No other sugar can substitute for glucose completely for growth: the redox state of cells grown on other sugars also ranges widely. For example, the NAD^+/NADH ratio in all cell lines tested was 2-3 in the presence of glucose, but 10-15 when galactose was substituted. There are no apparent changes in cyclic nucleotide metabolism related to these metabolic changes, but there are striking changes in cell morphology.

Significance: The pyridine nucleotides (NAD^+ and NADH) are involved in many of the oxidation-reduction reactions of a cell. The ratio of NAD^+ to NADH is therefore a basic indicator of the metabolic state and growth properties of the cells. The differences in the ratio found between normal and

tumor cells must therefore reflect the metabolic alteration in transformed cells. The ability to regulate this ratio in confluent transformed cells might thereby allow restoration to the growth and metabolic state of normal cells. Alteration of the glucose level or of the sugar source available may be one key to such regulation.

Proposed Course of Project: We plan to examine which enzymes and/or transport systems are involved in the overall metabolic changes seen in these studies.

Keyword Descriptors: NAD /NADH ratio, metabolic pattern, fluorometric and immunological methods, ATP, cyclic nucleotide metabolism, cell morphology.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02141-01 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Glycogen metabolism in glioma and neuroblastoma cell lines

Previous Serial Number: NONE

Principal Investigator: Janet V. Passonneau, Ph.D.

Other Investigators: W. D. Lust, Ph.D.
S. K. Crites, B.S.
J. P. Schwartz, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.7
Professional:	0.9
Other:	0.8

Project Description:

Objectives: To determine the factors regulating glycogen metabolism in glioma and neuroblastoma cell lines.

Methods Employed: Two cloned cell lines, C-6 astrocytoma cells (rat brain) and C-1300 neuroblastoma cells (mouse cord) are grown in a CO_2 incubator. Cells are rapidly fixed by freezing in liquid nitrogen to preserve the *in vivo* state. Homogenates of the cells are analyzed for total glycogen phosphorylase and total glycogen synthase, as well as the percent of those enzymes which are in the *a* or active form. Extracts of cells grown under identical conditions are analyzed for glycogen, glucose, glucose 6-P, uridine diphosphoglucose and cyclic 3',5'-AMP (cyclic AMP) content. Tne 2 cell lines have been investigated with regard to response to available energy sources in the medium, as well as the influence of norepinephrine, prostaglandins, phosphodiesterase inhibitors and insulin.

Major Findings: (1) Two independent mechanisms serve to control glycogen metabolism in both the glioma and neuroblastoma cell lines. (2) Both cell lines exhibit a response to available energy sources with regard to glycogen content, and the interconversion of the active forms of glycogen synthase and glycogen phosphorylase. (3) In addition, if cyclic AMP concentrations

are elevated by hormones of other effectors in the cell line, phosphorylase is converted largely to the active form, synthase to the inactive form, and glycogen stores are decreased.

The response to energy supply, primarily medium glucose, is comparable in both cell lines. When the cells are fed with medium containing 5 mM glucose, there is an immediate response with respect to glycogen, phosphorylase, and synthase. Within 5 minutes, the amount of phosphorylase in the active form is minimal, active synthase increases, and glycogen concentration is slightly increased. Glycogen stores continue to increase for about 3 hours under these conditions. At this time, when medium glucose has fallen to 2 mM, the phosphorylase is converted to the active form, synthase to the inactive form, and the glycogen content of the cells begins to decrease. All of these changes in the enzymes and glycogen occur without significant changes in cyclic AMP concentrations in the cells. The cyclic AMP concentration over a 24 hour period ranges from 10 - 20 picomoles/mg protein, and the minor fluctuations observed bear no relationship to the conversions of phosphorylase and synthase to the active and inactive forms.

Further evidence that these changes in enzyme forms are a response to energy source is demonstrated by the response of C-6 cells to insulin. If insulin is added to the culture when glucose in the medium is 3 mM or greater, the phosphorylase a is decreased and synthase a is increased. However, if glucose in the medium is 2 mM or less, the effect of insulin is decreased or absent.

If the cyclic AMP concentrations are elevated by treatment with exogenous agents, the interconversions of phosphorylase and synthase can be induced. In the case of the C-6 astrocytoma cells, treatment with the catecholamine, norepinephrine, for 45 min results in a 30-fold increase in cyclic AMP, while phosphorylase a increases from 12 to 45 percent, and synthase a decreases from 13 to 5 percent, and glycogen decreases from 60 to 40 nmoles/mg protein. The action of isobutyl methylxanthine (IBMX), a cyclic AMP phosphodiesterase inhibitor, was examined for effects on C-6 cells in culture. After 35 min incubation in the presence of IBMX, cyclic AMP was increased 2-fold, phosphorylase a was increased 7-fold, and synthase a was decreased to 50% of control values. Adenosine, an activator of adenyl cyclase, did not potentiate the effect of IBMX.

In the C-1300 neuroblastoma cells norepinephrine was without effect. The addition of IBMX or prostaglandin E_1 (5 μM) had similar effects: cyclic AMP was increased 2-fold, synthase a was decreased to 78% of control values and phosphorylase a was increased about 3-fold. The simultaneous addition of IBMX and prostaglandin E_1 resulted in a 10- to 25-fold increase in cyclic AMP, a 30% decrease in synthase a and a 4-fold increase in phosphorylase a.

Significance: A great deal of attention has been focussed on the hormonal control of glycogen concentration and turnover, primarily as a result of

changes in cyclic AMP concentration. We now have demonstrated that changes in glycogen concentration, as well as changes in the active forms of the enzymes responsible for glycogen synthesis and breakdown can occur as a result of changes in energy supply. In addition, hormones and other effectors can regulate glycogen metabolism through changes in cyclic AMP concentration. It has been possible to study these effects in 2 cell lines, one of which is glial and the other neuronal in origin. The responses of the cells to available energy supply is similar, while the responses to hormones and effectors are unique to each cell type.

Proposed Course of Project: Further studies are being made to elucidate more precisely the relationship between extra- and intracellular glucose concentration and the turnover of glycogen in these 2 cell lines. Preliminary report was made at the American Society of Neurochemistry meetings in Mexico City, March, 1975.

Keyword Descriptors: Regulating glycogen metabolism, total glycogen phosphorylase, total glycogen synthase, active form, independent mechanisms, available energy sources, cyclic AMP concentrations, isobutyl methylxanthine, prostaglandin E_1, hormonal control, insulin.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02142-01 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical changes during both ischemia and the recovery following ischemia.

Previous Serial Number: NONE

Principal Investigator: W. David Lust, Ph.D.

Other Investigators: Bogomir B. Mrsulja, M.D.
Branislava J. Mrsulja, M.D.
Janet V. Passonneau, Ph.D.
Joan P. Schwartz, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	1.2
Other	0

Project Description:

Objectives: To determine the alteration in enzymes, metabolites and putative neurotransmitters during long-term unilateral ischemia in the gerbil cerebral cortex; and, further, to monitor the recovery of these metabolites following a period of ischemia.

Methods Employed: Mongolian gerbils were anesthetized and the left common carotid artery was looped with a suture. As the gerbils emerged from the anesthesia, the artery was ligated. If the gerbils exhibited positive neurological symptoms, they were frozen in liquid nitrogen at various periods following ligation.

The cerebral cortex was removed at -20° and extracted in perchloric acid. The left hemisphere served as the ischemic side and the right as the control. ATP, P-creatine, glucose, glycogen, glutamate, citrate and gamma aminobutyric acid (GABA) were determined enzymically. Cyclic GMP was measured by immunoassay and cyclic AMP by the protein binding method. Dopamine, norepinephrine and 5-hydroxytryptamine were measured fluorometrically.

In the recovery studies, the carotid artery was occluded with a clip for

the appropriate time and then released. At various times following release, the gerbils were frozen and brains removed as described above.

Major Findings: By most of the criteria previously established in the decapitated mouse brains, the left cerebral cortex following the ligation of the left common carotid artery is in fact ischemic. From 1/2 to 6 hours after ligation, the ATP, p-creatine, glycogen and glucose were decreased by 60% or more and remained low for the entire period. The right or control cerebral cortex was essentially the same as a sham control up to 6 hours.

Cyclic AMP in the ischemic side increased 7-fold to a maximum at 2 hours and thereafter decreased to control values by 6 hours. GABA increased with time in the ischemic cerebral cortex to a maximum 5-fold greater than control at 6 hours. In addition, putative neurotransmitters, dopamine, norepinephrine and 5-hydroxytryptamine decreased with increasing time of ligation. The initial decrease in dopamine and norepinephrine occurred within 30 minutes while that for 5-hydroxytryptamine occurred only after 3 hours of ischemia.

In the recovery studies, the metabolites were measured 5 minutes, 1, 5 and 20 hours after either 1 or 3 hours of ischemia. All the metabolites measured were essentially back to control levels by 1 hour of recovery. Even though cyclic AMP was restored by 1 hour, this cyclic nucleotide increased an additional 5-fold over the already elevated cyclic AMP levels after 5 minutes of recovery.

Lastly, attempts to show changes in enzyme activity at various stages of ischemia were unsuccessful. Phosphodiesterase activity was unchanged not only in the cerebral cortex but in other affected regions of the brain.

Significance: The gerbil model for unilateral ischemia permits the investigation of the long term effects of ischemia. From the measurement of the energy metabolites, the ischemic state is established by 30 minutes and remains for as long as the artery is ligated. The lowering of cyclic GMP and the elevation of both cyclic AMP and GABA all indicate the depressed state of the cerebral cortex. The reason for the decrease of cyclic AMP following peak levels at 2 hours is not clear, but may indicate a secondary stage of ischemic damage.

The rapid recovery following 1 and 3 hours of ischemia in spite of histochemical evidence of brain damage suggests that a major portion of the cortical cells do remain viable even after 3 hours of ischemia. The large accumulation of cyclic AMP after 5 min of recovery coincides with other phenomenon. This increase in cyclic AMP does not occur after 5 minutes of occlusion and 5 minutes of recovery. Thus, this effect appears to be dependent on the duration of the ischemia before release.

Proposed Course of Project: The major emphasis is presently on the biochemical characteristics of recovery. In addition, the nature of the large

recovery-induced increase in cyclic AMP will be investigated more extensively.

Keyword Descriptors: Metabolites, putative neurotransmitters, long-term unilateral ischemia

Honors and Awards: None

Publications: None

Project No. Z01 NS 01995-03 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Morphological studies of CNS demyelination and remyelination in model systems

Previous Serial Number: Same

Principal Investigator: Henry deF. Webster, M.D.

Other Investigators: Paul J. Reier, Ph.D.
Takeshi Tabira, M.D.
Marian W. Kies, Ph.D.
Shirley H. Wray, M.D., Ph.D.

Cooperating Units: Section on Myelin Chemistry, LCM, NIMH
Department of Neurology, Harvard Medical School

Man Years:

Total:	4.1
Professional:	2.8
Other:	1.3

Project Description:

Objectives: Using Xenopus tadpole optic nerves as an in vivo model system of CNS myelinated fibers: 1) to study the penetration of subcutaneously injected macromolecules (ferritin, horseradish peroxidase, labelled serum IgG) into the optic nerve parenchyma; 2) to test the demyelinating activity of CSFs and sera from multiple sclerosis (MS) patients; and 3) to test the demyelinating activity of sera from guinea pigs and rabbits with experimental allergic encephalomyelitis (EAE).

These objectives were selected because we showed recently that Xenopus tadpole optic nerves are a useful model system for studying regeneration and remyelination of transected myelinated fibers in the CNS. Also, when these optic nerves were exposed by subcutaneous injection to low molecular weight myelinotoxic agents, demeylinating lesions developed rapidly and could be counted in whole mounts examined with a differential-interference microscope. Finally, when this injection procedure and whole mount technique were used to test the demyelinating activity of sera from MS patients and of sera from rabbits and guinea pigs ill with EAE, some positive results were obtained in preliminary experiments.

Methods Employed: 1) Single injections of ferritin and horseradish peroxidase (HRP) were given around the right optic nerve of tadpoles that were sacrificed 15 min.-48 hrs. later. Both nerves from each tadpole were fixed and prepared for electron microscopic examination. For fluorescein-conjugated human IgG, the injection procedure and intervals before sacrifice were similar. After brief fixation, frozen sections were cut and studied with a fluorescence microscope. 2) Coded samples of CSF from 18 patients were injected into groups of tadpoles and after 48 hrs., whole mounts of their optic nerves were prepared and randomized before counting the myelin lesions. 3) Myelin lesions were counted also in optic nerves of tadpoles that had been injected with coded samples of serum from guinea pigs and rabbits with EAE.

Major Findings: 1) Within 3 hrs., each of the tracers had penetrated the pial sheath and were found bilaterally within the optic nerve parenchyma. Passage of HRP and ferritin into the nerve occurred via a) extracellular clefts between adjacent astroglial endfeet at the glia limitans and b) vesicular uptake by astrocytic processes. These tracers tended to be more highly concentrated in the extracellular spaces between adjacent, radially directed astrocytic processes. This localization corresponded to the fluorescent thread-like network seen in the nerve following injection of the human IgG conjugate. 2) In tests to date, significant demyelinating activity has been found in CSF obtained from 4 chronic MS patients during an acute attack. This effect was not changed by adding a source of complement and was thermostabile. Finally, the number of optic nerve lesions observed appeared to reflect the severity of the patients' demyelination and was not proportional to the CSF levels of either gamma globulin or total protein. 3) Although some EAE sera (especially those from rabbits) produced higher lesion counts than control sera, the increase was not statistically significant. In an effort to enhance their demyelinating activity, some sera were concentrated fourfold before injection. This did not increase the number of lesions observed. Other ways of testing the sera in this system are being explored.

Significance to Biomedical Research and the Program of the Institute: Our results have shown that this in vivo model of a CNS myelinated tract is well suited for investigating many aspects of CNS myelin formation, maintenance, and breakdown. If macromolecules (including those present in serum and CSF) are injected perineurally, they penetrate the optic nerve's glial sheath and surround myelinated fibers. We have also demonstrated that unconcentrated CSF from some MS patients produces significant demyelination in this model system. However, many more experiments must be done before we can determine the usefulness of this type of test for multiple sclerosis and other human demyelinating diseases. In our system, some EAE sera have produced higher lesion counts than controls but the increase has not been statistically significant. Although penetration of serum proteins has been demonstrated, their concentration around optic nerve fibers may be below the threshold required for demyelination; other injection techniques are being tried. Also,

immature amphibian myelin may be resistant to demyelination by sera from mammals with EAE; this possibility is also being explored.

Proposed Course of Project: To be continued. The above findings have been presented at annual meetings of the American Association of Anatomists and the Society for Neuroscience.

Keyword Descriptors: Myelin, demyelination, Wallerian degeneration, regeneration, remyelination, experimental allergic encaphalomyelitis, multiple sclerosis, cerebrospinal fluid, ferritin, horseradish peroxidase, electron microscopy.

Honors and Awards: None

Publications:

Billings-Gagliardi, S., Webster, H. deF., and O'Connell, M. F.: In vivo and electron microscopic observations on Schwann cells in developing tadpole nerve fibers. Amer. J. Anat., 141: 375-392, 1974.

Reier, P. J. and Webster, H. deF.: Regeneration and remyelination of Xenopus tadpole optic nerve fibers following transection or crush. J. Neurocytol. 3: 591-618, 1974.

Webster, H. deF., Reier, P. J., Kies, M. W., and O'Connell, M. F.: A simple method for quantitative morphological studies of CNS demyelination: Whole mounts of tadpole optic nerves examined by differential-interference microscopy. Brain Res. 79: 132-138, 1974.

Reier, P. J., Tabira, T., and Webster, H. deF.: The penetration of fluorescent and electron dense tracers into Xenopus tadpole optic nerves following perineural injection. Brain Res., 1975 (in press).

Tabira, T., Webster, H. deF., and Wray, S. H.: Demyelinating activity of cerebrospinal fluid from multiple sclerosis patients tested in a new model system, the optic nerves of Xenopus tadpoles. Trans. ANA, 1975 (in press).

Project No. Z01 NS 01996-03 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Myelin membrane structure - morphological comparisons in intact CNS tissue and subcellular brain fractions

Previous Serial Number: Same

Principal Investigator: Henry deF. Webster, M.D.

Other Investigators: Jean-Marie Matthieu, M.D.
Paul J. Reier, Ph.D.
Richard H. Quarles, Ph.D.
A. W. Zimmerman, M.D.

Cooperating Units: Developmental and Metabolic Neurology Branch, NINCDS

Man Years:

Total:	0.4
Professional:	0.35
Other:	0.05

Project Description:

Objectives: 1) To correlate the electron microscopic appearance of myelin subfractions isolated from different CNS regions of developing rats with their protein composition. 2) To study the morphology of myelin and myelin related fractions isolated from the CNS of young rats intoxicated with hexachlorophene (HCP). 3) To study the myelin deficiency in mice with hereditary pituitary dwarfism.

Methods Employed: Following aldehyde fixation, myelin fractions were postfixed in a solution of potassium ferrocyanide before dehydration and embedding. For morphological comparisons of myelin structure in tissue, brains and optic nerves were fixed by perfusion with aldehydes before immersing myelinated regions in the above osmium solution. Sections of appropriate blocks were surveyed with the light microscope before cutting and staining thin sections for electron microscopic study.

Major Findings: 1) In electron micrographs of the heavy, medium, and light subfractions of myelin isolated from 16 day old rat brains, there were multilayered membrane whorls with the characteristic lamellar structure and

periodicity of myelin. However, in the heavy subfraction, the proportion of these whorls was lowest and it also contained many more smaller vesicular membrane profiles than either the whole myelin preparation or the medium and light subfractions. 2) In HCP intoxicated 22 day old rats, there was diffuse vacuolation in the CNS white matter. When electron micrographs of myelin fractions isolated from control and HCP intoxicated pups were compared, their appearance was essentially similar. Both contained multilayered myelin membrane whorls. In micrographs of the abnormal "floating" fraction that was isolated only from the brains of the HCP intoxicated pups, the membrane fragments were smaller; some had fewer layers with a periodicity different from that in normal myelin. 3) In 19 day old mice with hereditary pituitary dwarfism (nomozygone recessive mutant of Snell's dwarf strain) the corticospinal tract and anterior commissure were smaller than in controls. Also, in the dwarfs, these tracts contained fewer myelinated axons per unit area. However, the distribution of myelinated fibers based upon sheath thickness was normal.

Significance to Biomedical Research and the Program of the Institute: 1) Our developmental study of myelin subfractions provides additional evidence that CNS myelin contains glycoprotein that probably is associated with loose uncompacted membranes. These may be oligodendrocyte surface membranes during the initial stages of myelin formation or, alternatively, parts of more mature compact sheaths associated with oligodendrocyte cytoplasm such as the paranodal terminal loops. 2) We have shown that in HCP intoxication, glycoprotein is the only myelin constituent not found in the abnormal "floating" fraction. This is the first evidence identifying glycoprotein degradation as a possible initial biochemical event that precedes breakdown of myelin's compact layered structure. 3) Finally, our biochemical and morphological studies have better defined the CNS myelin abnormality associated with hypothyroidism. When present throughout development (as in hereditary pituitary dwarfism) our data show that myelination is significantly delayed. However, the myelin sheaths that are formed are normal in appearance and size. If the hypothyroid state is not induced until after birth (neonatal radiothyroidectomy) the retardation in myelin formation is less severe when assessed by similar morphological and biochemical techniques.

Proposed Course of Project: These findings have been presented at annual meetings of the American Society for Neurochemistry and the Society for Neuroscience. Other correlative morphological and biochemical studies of CNS myelin are in progress.

Keyword Descriptors: Myelin, demyelination, myelination, hypothyroidism, hypopituitarism, glycoprotein, hexachlorophene, biochemistry, electron microscopy

Honors and Awards: None

Publications:

Matthieu, J.-M., Quarles, R. H., Webster, H. deF., Hogan, E. L., and Brady, R. O.: Characterization of the fraction obtained from the CNS of jimpy mice by a procedure for myelin isolation. J. Neurochem. 23: 517-523, 1974.

Matthieu, J.-M., Zimmerman, A. W., Webster, H. deF., Ulsamer, A. G., Brady, R. O., and Quarles, R. H.: Hexachlorophene intoxication: Characterization of myelin and myelin related fractions in the rat during early postnatal development. Exp. Neurol. 45: 558-575, 1974.

Matthieu, J.-M., Reier, P. J., and Sawchak, J. A.: Proteins of rat brain myelin in neonatal hypothyroidism. Brain Res. 84: 443-451, 1975.

Reier, P. J., Matthieu, J.-M., and Zimmerman, A. W.: Myelin deficiency in hereditary pituitary dwarfism: A biochemical and morphological study. J. Neuropath. Exp. Neurol. 1975 (in press).

Zimmerman, A. W., Quarles, R. H., Webster, H. deF., Matthieu, J.-M., and Brady, R. O.: Characterization and protein analysis of myelin subfractions in rat brain: Developmental and regional comparisons. J. Neurochem. 1975 (in press).

Project No. Z01 NS 02082-02 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Human muscle spindles: Fine structure of the IA sensory endings

Previous Serial Number: Same

Principal Investigator: William R. Kennedy, M.D.

Other Investigators: Henry deF. Webster, M.D.

Cooperating Units: University of Minnesota School of Medicine

Man Years:

Total:	0.1
Professional:	0.1
Other:	0

Project Description:

Biopsied muscle spindles were fixed, embedded, and studied electron microscopically. The IA ending's contour, organelle content, and surface relationships with spindle muscle fibers have been characterized and correlated with some of the ending's functional properties.

Proposed Course of Project: This project has been completed and is terminated.

Keyword Descriptors: Human nerve endings, human muscle spindles, electron microscopy

Honors and Awards: None

Publications:

Kennedy, W. R., Poppele, R. E., and Webster, H. deF.: Human muscle spindles: Isolation from biopsy, physiological activity and fine structure. Trans. ANA 99: 126-129, 1974.

Kennedy, W. R., Webster, H. deF., Yoon, K. S., and Jean, D. H.: Human muscle spindles: Microfilaments in the group IA sensory nerve endings. Anat. Rec. 180: 521-532, 1974.

Kennedy, W. R., Webster, H. deF., and Sung, K.: Human muscle spindles: Fine structure of the primary sensory ending. J. Neurocytol.: 1975 (in press).

Project No. Z01 NS 01998-03 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Central biogenic amines in edema and ischemia

Previous Serial Number: Same

Principal Investigator: Jonathan Costa, M. D., Ph.D.

Other Investigators: Umeo Ito, M.D.
Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: Laboratory of Clinical Science, NIMH

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description:

Proposed Course of the Project: This project has been terminated with the departure of the principal investigator.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02093-02 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of pressure gradients within brain tissue during the formation of vasogenic brain edema

Previous Serial Number: Same

Principal Investigator: Hans J. Reulen, M.D.

Other Investigators: Igor Klatzo, M.D.
Robert S. Graham, B.S.

Cooperating Units: Department of Neurosurgery
University of Mainz, Mainz, West Germany

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: Experiments were performed in order to evaluate the concept that an increase in local interstitial fluid pressure may play a paramount role in the dynamics of the vasogenic type of edema.

Methods Employed: Assuming that the pressure measured by the cotton wick technique represents the hydrostatic interstitial tissue pressure, the tissue pressure was recorded with pressure transducers in the white matter adjacent and remote to a local cold injury in the opposite hemisphere and in the cisterna magna. The cold injury was inflicted to the right frontal pole in the anesthetized and artificially ventilated cats. Spreading of vasogenic edema was followed by intravenous injection of sodium fluorescein at the time of operation and of Evans blue 3 hours later. Animals were sacrificed 6 hours following the injury. Water content was measured in consecutive blocks dissected from a horizontal tissue slice taken at the level of the tip of the wick.

Major Findings: Normal interstitial fluid pressure (IFP) ranged 1-4 mm Hg. below the CSF pressure and responded well to respiratory pulsations and changes in $paCO_2$. The development of vasogenic brain edema was associated with an increase in local IFP in edematous white matter adjacent to the

lesion and with the occurrence of a variety of pressure gradients within the tissue and the CSF. The pressures were always highest adjacent to the lesion and fell gradually toward the remote areas. At the time of sacrifice, pressure differences between the corresponding recording sites in the injured and uninjured hemispheres amounted to 8-13 mm Hg. Tissue pressures in the edema territory were significantly higher than the CSFP, while the pressure in the distant and opposite normal areas remained below the CSFP. Comparison of local IFP and local tissue water content revealed a close relationship. The shape of this curve shows a rapid IFP increase above CSFP with small increases of edema fluid. Thereafter, additional increases in edema fluid influences the tissue pressure to a lesser extent. Since the major part of this fluid accumulation has been shown to be extracellular, this relationship seems to represent the pressure/volume relationship of the extracellular space.

Significance to Biomedical Research and the Program of the Institute: These experiments allow a new insight into the dynamics of vasogenic type of brain edema. Our studies have shown previously that the hemodynamic pressure provides a primary force for spreading exsudated serum contents and edema fluid through the brain tissue. The movement of edema fluid encounters the resistance of the interstitial compartment and this results in elevation of the IFP in the area of edema. It is thus apparent that two major factors in dynamics of vasogenic edema must be systolic blood pressure and the IFP. It follows that reduction or elevation of the latter may greatly influence the dynamics of edema process. This work obviously contributes to basic understanding of brain edema.

Proposed Course of Project: This project has been terminated with the departure of the principal investigator. The results of this work were presented at the Second International Symposium on Intracranial Pressure, Lund, Sweden, 1974.

Keyword Descriptors: Pressure gradients, vasogenic brain edema

Honors and Awards: None

Publications:

Reulen, H. J., Graham, R. S., and Klatzo, I.: Development of pressure gradients within brain tissue during the formation of vasogenic brain edema. Proceedings of the Second International Intracranial Pressure Symposium, Lund, Sweden, 1974.

Project No. Z01 NS 02104-02 LNNS
1. Laboratory of Neuropathology
and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Evaluation of the "no-reflow" (NR) phenomenon in Mongolian gerbils

Previous Serial Number: Same

Principal Investigator: Umeo Ito, M.D.

Other Investigators: Igor Klatzo, M.D.
Maria Spatz, M.D.
K. G. Go, M.D.

Cooperating Units: Department of Neurosurgery, University of Groningen
Groningen, The Netherlands

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description:

Proposed Course of the Project: This project has been terminated with the departure of the principal investigator.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02105-02 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Behavior of the blood-brain barrier and the development of edema in cerebral ischemia

Previous Serial Number: Same

Principal Investigator: Umeo Ito, M.D.

Other Investigators: K. G. Go, M.D.
Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: Department of Neurosurgery, University of Groningen, Groningen, The Netherlands

Man Years:

Total:	2.3
Professional:	1.7
Other:	0.6

Project Description:

Objectives: One of the complications of cerebral ischemia is the development of brain edema. Two main types of brain edema are the cytotoxic and vasogenic varieties which can be differentiated on the basis of the behavior of the blood-brain barrier (BBB). The objective of this study has been to elucidate the nature of ischemic brain edema by studying in a model of experimental cerebral ischemia the behavior of the BBB and the abnormal increase in brain tissue fluid, the essential criterion of any type of brain edema.

Methods Employed: Mongolian gerbils (Meriones unguiculatus) in which, due to anatomical irregularities of the circulus of Willis, an occlusion of the common carotid artery on the neck produces in about 30% of animals an ischemic injury in the ipsilateral cerebral hemisphere, were used as an experimental model for cerebral ischemia. In this project, the left common carotid artery was clipped on the neck for various periods of time. For evaluation of the BBB, Evans blue dye was injected intravenously as a tracer. The systemic arterial blood pressure was monitored by intrafemoral catheter connected with transducer. Abnormal increase of the brain tissue fluid was estimated by wet/dry weight ratios. After decapitation, the brains were

taken out and the separated hemispheres were weighed (fresh weight). After drying at 98°C for six days the samples were weighed again (dry weight). Histological changes were evaluated in brain tissue fixed by paraformaldehyde perfusion, embedded in the paraffin and stained with hematoxylin eosin and cresyl violet.

Major Findings: The estimation of wet/dry weight ratios revealed a progressive accumulation of the fluid in relation to the duration of carotid occlusion. Definite evidence of brain edema was apparent in animals sacrificed after 3 hours of carotid clipping which showed 7.34 $\pm$ 1.01 mean swelling percentage in the left hemisphere. In animals with 18 hours occlusion the swelling of the infarcted hemisphere amounted to 22%. The incidence of the BBB damage, evident by blue staining of the brain tissue, was related to the duration of ischemia and to the duration of release following carotid occludion. Thus, e.g., in gerbils subjected to 6 hours ischemia all animals showed the BBB damage 1 hour after release of occlusion. On the other hand, gerbils with 1 hour left carotid occlusion showed 100% incidence of the BBB damage only 20 hours following release of carotid clipping. Raising the systemic arterial blood pressure accelerated significantly the BBB damage; e.g., a 100% incidence of the BBB damage was evident in gerbils with 147.8 mean arterial blood pressure (MABP) within 15 minutes after release, whereas, in animals with the normal MABP (72.4) such incidence was present only after 5 hours of release of carotid occlusion. The histological changes characteristic of ischemic damage clearly preceded the BBB injury. They were first recognizable in animals sacrificed 30 minutes after carotid occlusion. They were progressively increasing during the period of release of carotid occlusion. This was in agreement with the observation that the histological picture of ischemic damage was similar in intensity in gerbils sacrificed immediately 6 hours after ischemia with that in animals with 1 hour occlusion and sacrificed 20 hours after release of the clipping.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the nature of ischemic brain edema is potentially of great value for the clinical management of stroke patients. The studies described above indicate that following ischemia there is a development of brain edema which is primarily of cytotoxic type. Only in later stages when there is damage to cerebral blood vessels and their increased permeability to BBB tracers, a leakage of serum components is adding a vasogenic component to overall picture of brain edema. These findings might be of importance for designing proper treatment of patients suffering from cerebral ischemia.

Proposed Course of the Project: The data from this work is being prepared for publication.

Keyword Descriptors: Blood-brain barrier, edema, cerebral ischemia

Honors and Awards: None

Publications:

Ito, U., Spatz, M., Walker, J. T., Jr., and Klatzo, I.: Experimental cerebral ischemia in Mongolian gerbils. I. Light microscopic observations. Acta Neuropath., 1975 (in press).

Project No. Z01 NS 02138-01 LNNS
1. Laboratory of Neuropathology
and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Light microscopic observations in experimental cerebral ischemia

Previous Serial Number: None

Principal Investigator: Umeo Ito, M.D.

Other Investigators: Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.5
Other:	0.0

Project Description:

Objectives: Light microscopic observations were carried out on Mongolian gerbils (Meriones unguiculatus) subjected to a partial cerebral ischemia by occlusion of the left common carotid artery on the neck. The simplicity of the procedure allows for processing large groups of animals and collecting information on dynamic features of ischemic brain lesions.

Methods Employed: The gerbils were operated under light anesthesia and the left common carotid artery was occluded at the neck with the Heiffetz aneurysm clip for various periods of time. The animals were sacrificed by perfusion in groups according to occlusion time and the time elapsed after release of the clip. After fixation, coronal blocks of the brains were embedded in paraffin and the sections stained with the hematoxylin and eosin and cresyl violet stains.

Major Findings: The histopathologic changes following carotid occlusion in the sensitive animals were related to the intensity (duration) of the ischemic insult and to the time elapsed following the release of the occlusion. The ischemic lesions appear to progress after re-establishment of the circulation and this presents one facet of a "maturation" phenomenon which seems to be a general principle applicable to various parameters of ischemic

injury. The rate of "maturation" of the lesions is related to the intensity of the ischemic insult, a lesser intensity resulting in longer development of lesions. Otherwise, the changes were either focal or diffuse in character. The former were assumed to be directly related to a vascular involvement; among the latter the topistic distribution of the hippocampal changes suggested a feature of selective vulnerability. An indirect indication of neuronal recovery was surmised from observations on animals sacrificed after different periods following occlusions of the same duration. Also capable of recovery was a "reactive change" observed in the H3 neurons of the hippocampus. This change was characterized by central chromatolysis and resembled the "primäre Reizung" of Nissl.

Significance to Biomedical Research and the Program of the Institute: The described "maturation" phenomenon seems to be a general principle applicable to various parameters of ischemic injury and it may play an important part in interpretation of clinical situations where deterioration of a clinical condition and emergence of positive isotope scanning are observed after certain periods of latency. The evidence of neuronal recovery from ischemic injury is of importance for designing procedures which would further stimulate such recovery and thus be of potential significance for clinical treatment of cerebral ischemia.

Proposed Course of the Project: This project has been completed. The results have been submitted for publication in Acta Neuropathologica.

Keyword Descriptors: Cerebral ischemia, Mongolian gerbils, light microscopy

Honors and Awards: None

Publications: None

Project No. Z01 NS 02150-01 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Electronmicroscopic studies of the hippocampus in gerbils subjected to cerebral ischemia

Previous Serial Number: None

Principal Investigator: Jose J. Bubis, M.D.

Other Investigators: Tsukasa Fujimoto, M.D.
Branislava J. Mrsulja, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	1.35
Professional:	1.25
Others:	.10

Project Description:

Objectives: In a previous study (Ito, U., Spatz, M., Walker, Jr., J. T., and Klatzo, I.: Experimental cerebral ischemia in Mongolian gerbils: I. Light microscopic observations. Acta Neuropath., 1975, in press.) a marked neuronal reaction in H2 sector of Ammon's Horn was observed in gerbils subjected to 15 minutes of common carotid artery clipping and 20 hours release. This lesion was characterized by prominent cells with eccentric nuclei, central cytoplasmic eosinophilia and either complete absence or peripheral cytoplasmic presence of Nissl bodies. These light microscopic changes are morphologically similar to some cases of neuronal chromatolysis. The present study was designated to investigate the morphologic and histoenzymatic changes of this ischemic lesion by electronmicroscopy.

Methods Employed: Gerbils were subjected to unilateral or bilateral clamping of the carotid arteries for periods ranging from 7 to 15 minutes. Twenty hours after release of the clamp, the animals were perfused through the heart with 15 ml of balanced salt solution followed by 1% paraformaldehyde, 1.25% glutaraldehyde solution, and then by a 4% paraformaldehyde, 5% glutaraldehyde fixing solution. Three hours afterwards the brain was removed, the selected areas of hippocampus were cut and processed for Araldite or Epon embedding.

For histochemistry only the 1% paraformaldehyde, 1.25% glutaraldehyde fixing solution was used, the brain was removed 30 minutes after perfusion, and 50μ thick sections from selected hippocampal regions were incubated for acid phosphatase, thiamine pyrophosphatase and endogenous peroxidase and then processed for electronmicroscopy.

Major Findings: Light microscopic observations of the resin embedded tissue showed the affected neurons as rounded cells with the characteristic eccentric nucleus, central cytoplasmic dark granules and some vacuoles.

Under the electron microscope, the eccentric nucleus had a kidney-like shape with its concavity facing the center of the cell. Irregular and short cisternae of the rough endoplasmic reticulum and also membrane-free ribosomes were seen at the periphery of the cell. The central part of the cytoplasm was devoid of endoplasmic reticulum and it was filled with mitochondria of bizarre shape and of different sizes, with numerous lysosome-like structures, with several vacuoles and with small (60-80 nm) electron-dense inclusions of ragged contour and not membrane bound, these inclusions may represent glycogen deposits. The Golgi apparatus was either absent or was seen in the form of irregularly arranged cisternae or of a group of vesicles. Thiamine pyrophosphatase reaction was seen in some short cisternae or vesicles.

Acid phosphatase activity was present in many of the lysosome-like structures suggesting the formation of secondary lysosomes and autophagosomes. No normal GERL (Novikoff) was seen in the affected neurons.

Significance to Biomedical Research and the Program of the Institute: The structural and functional elucidation of cerebral lesions caused by ischemia of short duration is of great clinical importance for the basic understanding of cerebrovascular disease process as well as its prevention and possible therapy.

Proposed Course of Project: These findings correlated with radioautographic studies of protein synthesis could throw light on two main problems: 1) Function of the Golgi apparatus with its disorganized morphology, and 2) function of the remaining endoplasmic reticulum. Further studies and different release time of clamped animals are necessary to determine whether these neurons may represent a postischemic regenerative phase.

Keyword Descriptors: Electron microscopy, hippocampus, ischemia, lysosomes, Golgi apparatus, chromatolysis, acid phosphatase, thiamine pyrophosphatase

Honors and Awards: None

Publications: None

Project No. Z01 NS 02174-01 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Histochemical investigation of the Mongolian gerbil's brain during unilateral ischemia

Previous Serial Number: None

Principal Investigator: Branislava J. Mrsulja, M.D.

Other Investigators: Igor Klatzo, M.D.
Maria Spatz, M.D.

Cooperating Units: None

Man Years:

Total:	.85
Professional:	.75
Other:	.01

Project Description:

Objectives: The Mongolian gerbils possess a high susceptibility for the development of cerebral infarction following unilateral ligation of the common carotid artery due to frequent absence arterial communication between the cerebral and vertebral system. The contralateral hemisphere then serves as a control (Kahn, K., Neurol. 22: 510-515, 1972). The purpose of present investigation was to study ischemic effect on the various enzymes activities in the brain histochemically.

Methods Employed: The histochemical investigation was performed on several groups of Mongolian gerbils which were subjected to unilateral ischemia of 30 and 60 minutes, 2, 3, 6, and 9 hours. Fresh frozen sections were used for the histochemical enzyme evaluation of the ischemic and control hemisphere. The following enzymes were investigated: respiratory enzymes, succinic (SDH), lactic (L-DH), glutamic (G-DH), glucose-6-phosphate (G-6PDH) dehydrogenases were demonstrated by Nitro BT-methods. Cytochrome oxidase (COX) activity was studied according to Burstone's p-Aminodiphenylamine method. Sections heated at 60°C prior to incubation served as controls. L-leucyl-β-napthylamide was used as a substrate according to the Gomori's modified method by Burstone and Fold. Alkaline and acid nonspecific phosphatase activities were assessed by Burstone's napthol AS-phosphate methods using napthol AS-TR as a substrate for both enzymes but with different pH of the incubating medium. Total

glycogen-phosphorylase activity was demonstrated by Takeuchi and Kuriaki. The brain sections were incubated at 37°C in Navikoff's and Goldfisher's (1961) medium for thiamine pyrophosphatase (TPP-ase).

The evaluation of glycogen, the brain tissue was fixed by perfusion with paraformaldehyde embedded in paraffin and stained according to the cold Schiff's method, preceded by one hour of incubation in 5 per cent Dimedon (5,5-Dimethyl-1,3-Cyclohexadion) solution (Bulmer, D., Stain Tech. 34: 95, 1959).

Major Findings: The first detectable changes in the activities of all dehydrogenase was seen as a slight decrease in the staining intensity of ischemic hemisphere in animals subjected to 2 hours ischemia. The loss of the dehydrogenase activities was parallel with the duration of ischemia. The greatest diminution of the demonstrable enzymatic staining product was seen after 9 hours of ischemia. The slight decrease of nonspecific acid monophosphatase activity was also not seen prior to 2 hours ischemia. It manifested itself by slight decrease of stainable neuronal cytoplasm granule in H_2 and H_3 sector of the Ammon's Horn and thalamus. However at the periphery of the ischemic lesion (in the thalamo-hypothalamic region) a higher more diffuse (nongranular) cytoplasmic activity of the acid phosphatase was seen than on the contralateral control side. In later stages of ischemia, a loss of enzymatic activity was seen in the severely damaged region while the surrounding areas showed an increase activity of the acid phosphatase. No significant change in the activity of alkaline phosphatase were demonstrated in ischemic regions at any time. Cytochemical studies of the Golgi apparatus revealed no abnormalities earlier than after three hours. At this stage of ischemia, the cortex, hippocampus and thalamus showed very slight decrease of the staining intensity. In many of these places the neurons were completely devoid of enzyme activity. In addition to this, there was an unusual increase in the number of neurons with diffuse or granular cytoplasmic and nuclear staining. In zones of advanced necrosis, diminished staining of the blood vessels and glia were seen in the TPP-ase preparations. Total phosphorylase was the only one enzyme which was changed after 1 hour of ischemia. Animals killed within 1 hour of ischemia showed a diminution of phosphorylase activity prominent on the ischemic side. By 6 and 9 hours of ischemia phosphorylase activity was completely suppressed. Necrotic areas revealed markedly diminished or complete loss of total phosphorylase activity while the rest of the hemisphere exhibited hyperactivity of the same enzyme and increased glycogen content, especially close to the necrotic areas. Cytologically the increased glycogen and enzymatic activity were situated mainly within the astrocytes and in the neuropil.

Significance to Biomedical Research and the Program of the Institute: The histochemical evaluation of several groups of enzymes after various periods of brain ischemia will be helpful in assessing the extent of ischemic effect in cerebral metabolism. Such studies are very important for the understanding of the ischemic process: development, arrest, and possible prevention.

Proposed Course of Project: Other enzymes like ATT-ase and carbonic anhydrase will be evaluated in this model.

Keyword Descriptors: Mongolian gerbils, dehydrogenases, alkaline, acid, nonspecific phosphatase activities, total glycogen-phosphorylase, thiamine, pyrophosphatase

Honors and Awards: None

Publications: None

Project No. Z01 NS 02175-01 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Histochemical observation on Mongolian gerbils' brains during and after regional ischemia

Previous Serial Number: None

Principal Investigator: Branislava J. Mrsulja, M.D.

Other Investigators: Igor Klatzo, M.D.
Maria Spatz, M.D.
Umeo Ito, M.D.

Cooperating Units: None

Man Years:

Total:	.85
Professional:	.75
Other:	.01

Project Description:

Objectives: In previous studies we have observed a "maturation phenomena" of cerebral lesions in unilateral ischemia of gerbils. The rate of "maturation" of the ischemic lesions was directly related to the intensity (duration) of ischemic insult (Ito, U., Spatz, M., Walker, J. T., Jr., and Klatzo, I.: Experimental cerebral ischemia in Mongolian gerbils. I. Light microscopic observations. Acta Neuropath., 1975, in press). The investigation reported here was designated to evaluate and possibly elucidate the mechanism responsible for this phenomena.

Methods Employed: Regional cerebral ischemia was produced in Mongolian gerbils by clipping of the common carotid artery (unilateral or bilateral) for various periods of time. Animals with neurological signs of infarction were sacrificed either immediately after the end of the ischemic period (7.5, 15, 60 and 180 minutes), or after 1, 5, 20 hours, 1 and 4 weeks of recirculation. The following enzymes were investigated: respiratory enzymes succinic (SDH), lactic (LDH), glutamic (G-DH), glucose-6-phosphate (G-6PDH); dehydrogenases were demonstrated by Nitro BT-methods. Cytochrome oxidase (COX) activity was studied according to Burstone's p-aminodiphenyl-anine method. Sections heated at 60°C prior to incubation served as controls

L-leucyl-β-naphtylamide was used as a substrate according to the Gomori's modified method by Burstone and Fold. Alkaline and acid nonspecific phosphatase activities were assessed by Burstone's naphthol AS-phosphate methods, using naphthol AS-TR as a substrate for both enzymes, but with different pH of the incubating medium. Total glycogen-phosphorylase activity was demonstrated by Takeuchi and Kuriari. The brain sections were incubated at 37°C in Navikoff's and Goldfisher's (1961) medium for thiamine pyrophosphatase (TPP-ase). For glycogen demonstration the brain tissue was fixed by perfusion with paraformaldehyde embedded in paraffin and stained according to the cold Schiff's method, preceded by 1 hour of incubation in 5% Dimedon (5,5-Dimethyl-1,3-cyclohexadion) solution (Bulmer, D., Stain Tech. 34: 95, 1959).

Major Findings: The histochemical regional ischemic changes have been directly related to the duration of the blood supply restriction to the brain and the following period of re-established circulation. However, individual variation in the activity of all enzymes examined were also found in the group of animals subjected to the same duration of experimental ischemia. The experimental animals were divided into two distinct groups according to the histochemical enzymatic observations: (1) short period of ischemia (7.5 and 15 minutes) with various recovery periods: At the end of ischemic insult and after 1 hour of common carotid artery clip release, no differences in enzymatic activities were seen between ischemic and non-ischemic Ammon's horn. Twenty hours after restoration of cerebral circulation, the oxidative mitochondrial enzymes S-DH, L-DH, G-DH, G-6-DH and C-OH were markedly increased in the pyramidal cells of sabiculum and H3 sector of the hippocampus. The proteolytic enzyme aminopeptidase and the Golgi's marker TPP-ase were also increased in the same part of Ammon's horn. Furthermore, the activity of acid phosphatase was seen as an increased diffuse and homogeneous reaction in the neuronal cytoplasm of the affected ischemic side, as compared to the cytoplasmic granular reaction on the control side. One week after the ischemic insult all of the above mentioned enzymes were increased as compared to controls, but to a lesser degree than after 20 hours of clip release. In addition, the H2 sector showed focally increased activity of all above mentioned enzymes. (2) Long period of ischemia (1 and 3 hours) with various recovery periods: The activity of all investigated enzymes in the brain was decreased at each of the examined periods of re-established cerebral circulation. The loss of activity was progressive and directly related to the duration of the restored circulation. The difference in the intensity of the observed activity between the ischemic and non-ischemic hemisphere was slight at 1 hour but extremely marked at 1 week after the ischemic insult. However, the group of animals subjected to 1 hour carotid artery occlusion and 1 week release show a characteristic exception to this rule; namely, an increased activity of lysosomal and proteolytic enzymes.

Significance to Biomedical Research and the Program of the Institute: Upon completion of this study, it will be possible to ascertain the relationship of the intensity (duration) of ischemic insult to the recovery of cerebral lesions. The basic evaluation of cerebrocellular recovery potential is of

great prognostic and therapeutic importance, clinically.

Proposed Course of the Project: In this study an attempt will be made to determine the time of first appearance and disappearance of enzymatic changes observed during and after recovery of short and long-term ischemia.

Keyword Descriptors: Mongolian gerbils, cytochrome oxidase, dehydrogenases, alkaline, acid nonspecific phosphatase, glycogen phosphorylase, thiamine pyrophosphatase.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02176-01 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Action of cerebral ischemia on decreased levels of catechol and indol amine metabolites produced by pargyline

Previous Serial Number: None

Principal Investigator: Bogomir B. Mrsulja, M.D.

Other Investigators: Branislava J. Mrsulja, M.D.
Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	.08
Professional:	.08
Other:	0

Project Description:

Objectives: The catechol and indol amines are thought to play an important role in cerebral function and their relationship to neuropathology is also pointed out. In cerebral ischemia we found the changes of dopamine, norepinephrine and serotonin and here we are reporting about the changes of their main metabolites, homovamillic acid (HVA), 3-methoxy-4-hydroxy-phenyl-ethylglycol-sulphate (MOPEG-SO_4), and 5-hydroxyindolacetic acid (5-HIAA), respectively.

Methods Employed: Experiments were conducted on Mongolian gerbils. Unilateral cerebral ischemia was produced by ligation of one common carotid artery of the neck. Following ligature, the animals with neurological signs of cerebral infarction were sacrificed 30 minutes, 1, 2, 3 and 6 hours after onset of ischemia. Also, gerbils received pargyline (75 mg/kg) 30 minutes prior to treatment with probenecid (200 mg/kg) or production of ischemia. In this experiment, animals were sacrificed 2 hours thereafter. In all animals in ischemic and control hemisphere, concentrations of HVA, MOPEG-SO_4 and 5-HIAA were analyzed spectrophotofluorometrically.

Major Findings: Cerebral ischemia of 30 minutes duration significantly increases the level of HVA, while it has no influence on MOPEG-SO_4 and 5-HIAA

levels. MOPEG-SO_4 level was not changed 1 hour after onset of ischemia, while that of 5-HIAA was increased. However, MOPEG-SO_4 level was increased after 2 hours of ischemia. It took about 2 hours for catecholamine metabolites to reach the highest values, and 1 hour for 5-HIAA. Thereafter, these levels remained unchanged. In gerbils' brains, inhibitor of monoamine oxidase, pargyline, significantly decreased the levels of HVA, MOPEG-SO_4 and 5-HIAA. This decrease could be prevented by: (1) blockage of active out-transport of metabolites from the brain of CSF by means of probenecid or, (2) by the exposure of the animals to ischemia. It appears that ischemia has the same influence on pargyline-treated animals as probenecid.

Significance to Biomedical Research and the Program of the Institute: In addition to the well known increase of lactate in ischemia which influences the pH of nervous tissue, retention of organic acids, metabolites of catechol and indol amines might also be a factor involved in producing tissue acidosis. This retention in ischemia is probably due to the altered out-transport of HVA, MOPEG-SO_4 and 5-HIAA from the tissue and CSF.

Proposed Course of the Project: The pharmacological effect on catechol and indol metabolites will be investigated in the recovery period following cerebral ischemia.

Keyword Descriptors: Cerebral ischemia, catechol and indol amine metabolites

Honors and Awards: None

Publications: None

Project No. Z01 NS 02177-01 LNNS
1. Laboratory of Neuropathology
and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Application of steady-state kinetics to the study of catecholamine and serotonin turnover in cerebral ischemia

Previous Serial Number: None

Principal Investigator: Bogomir B. Mrsulja, M.D.

Other Investigators: Branislava J. Mrsulja, M.D.
Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	.08
Professional:	.08
Other:	0

Project Description:

Objectives: Gerbils exhibiting cerebral infarction showed in cerebral cortex decreased levels of dopamine (DA), norepinephrine (NOR), and serotonin (5-HT) and increased levels of homovamillic acid (HVA), 3-methoxy-4-hydroxy-phenylethylglycol-sulphate (MOPEG-SO_4) and 5-hydroxy-indolacctic acid (5-HIAA) metabolites of monoamines, respectively. In order to clarify behavior or biogenic amines and their main metabolites in ischemia, we applied pharmacologic measurement of amine turnover and steady-state kinetics studies.

Methods Employed: All analyses were conducted on Mongolian gerbils showing the neurological signs of cerebral infarction. Two to four hours prior to the common carotic artery occlusion, the following modulators of biogenic amine metabolism were injected i.p.: pargyline (75 mg/kg), alfa-methyl-p-tyrosine (AMPT, 400 mg/kg), p-chlorphenylalanine (PCPA, 500 mg/kg), probenecid (200 mg/kg), pyrogallol (200 mg/kg), and 2-3 hours after occlusion the levels of DA, NOR, 5-HT, HVA, MOPEG-SO_4 and 5-HIAA were measured fluorometrically. Steady-state kinetics study was applied to calculate the turnover rate and turnover time of biogenic amines in ischemia.

Major Findings: Our results suggest that cerebral ischemia in gerbils is accompanied by: (1) decreased rate of DA, NOR and 5-HT synthesis in

cerebral cortex as indicated by: (a) decreased accumulation of NOR and 5-HT after inhibition of monoamine oxidase by means of pargyline, and (b) further reduction of DA, NOR and 5-HT after inhibition of tyrosine hydroxylase and tryptophan hydroxylase by means of AMPT and PCPA, respectively. (2) An altered active out-transport of HVA, MOPEG-SO_4 and 5-HIAA from the tissue or CSF as indicated by: (a) increased accumulation of HVA, MOPEG-SO_4 and 5-HIAA in ischemia following the inhibition of active transport by means of probenecid, and (b) accumulation of HVA and MOPEG-SO_4 in ischemia following inhibition of catecholamine synthesis by means of AMPT and accumulation of 5-HIAA after inhibition of 5-HT synthesis by means of PCPA. (3) Possible potentiated O-methylation as indicated by increased levels of DA and NOR following inhibition of catechol-O-methyl-transferase by means of pyrogallol.

Significance to Biomedical Research and the Program of the Institute: Our results suggest synaptosomal release and probably depletion of biogenic amines during ischemia, inhibition of synthesis and degradation of these monoamines and alteration of active transport of their main metabolites from the brain tissue. Such behavior of catechol and indol amines and their metabolites in ischemia might be responsible or might cause further ischemic injury in surrounding brain tissue primarily not affected. At the same time, data implicates the possibility of using drugs which can modify the levels or action of brain monoamines and at least the extension of ischemic brain damage.

Proposed Course of the Project: Further studies will be concerned with chemical modification of brain monoamine levels and possible prevention of catechol and indol metabolite accumulation.

Keyword Descriptors: Cerebral infarction, biogenic amines, turnover

Honors and Awards: None

Publications: None

Project No. Z01 NS 02178-01 LNNS
1. Laboratory of Neuropathology
and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Behavior of biogenic amines in and following cerebral ischemia

Previous Serial Number: None

Principal Investigator: Bogomir B. Mrsulja, M.D.

Other Investigators: Branislava J. Mrsulja, M.D.
Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	.08
Professional:	.08
Other:	0

Project Description:

Objectives: The molecular basis of the vulnerability of brain in ischemia is not completely understood. When brain becomes severely ischemic as a consequence of thrombi, emboli or the vasospasm that can follow hemorrhage or trauma, it too probably "leaks" various intercellular constituents that normally are stored in high concentrations. The resulting inappropriate loss of several of these compounds; the monoamine neurotransmitters, norepinephrine, dopamine, and serotonin may exacerbate the pathophysiological changes caused by initial ischemia. In this report, we are presenting the behavior of biogenic amines in and following ischemia of various periods of duration.

Methods Employed: Mongolian gerbils were subjected to left common carotic occlusion and those with neurological signs of cerebral infarction were sacrificed: (a) 15 minutes, 1, 3 and 6 hours after onset of ischemia, or (b) 1 and 20 hours and 1 week after ischemia lasting 15 minutes, 1, 3 and 6 hours, respectively. Brain dopamine (DA), norepinephrine (NOR) and serotonin (5-HT) in ischemic and control brain hemispheres were assayed fluorometrically by the method of Cox and Perhach (1973). Histochemical studies of NOR and 5-HT in ischemia were done using the freeze-dried fluorescence method of Falk and Hillarp (1966) as described by Olsson and

Ungerstedt (1970).

Major Findings: In 15 minute ischemic animals, no changes were found in the levels of DA, NOR and 5-HT. One hour after onset of ischemia, 5-HT level was significantly increased in ischemic hemispheres, and was not further changed up to 6 hours duration of ischemia. The levels of DA and NOR were reduced 1 hour after the occlusion was established and the reduction of these concentrations were proportional to the duration of ischemia. In recovery after ischemia, one hour after 15 minute occlusion, as well as one hour after one hour occlusion, 5-HT level was significantly higher than it was during ischemia, while in gerbils which were ischemic for longer periods, 5-HT levels were significantly lower than in ischemia. Twenty hours after the clip was removed, 5-HT level in animals which were ischemic only for 15 minutes reached the peak and was higher than one hour after the clip was removed in the same experimental group of animals. On the other hand, 5-HT level in animals clipped for 6 hours was not different from the level of the control hemisphere. The behavior of NOR in recovery in gerbils which were occluded for shorter periods (15 and 60 minutes) is different from the behavior of this catecholamine in animals which were ischemic for longer periods (3 and 6 hours). Reduced NOR level in gerbils which were ischemic for longer periods recovered after the ischemia. On the other hand, in gerbils which were ischemic for the shorter periods, NOR content showed biphasical behavior. One hour after the clip was removed NOR was higher than in controls; 20 hours after ischemia NOR level was lower than in controls. During recovery periods, DA levels were reduced proportionally to the time of duration of ischemia. However, recovery of DA concentration was more pronounced in animals which were ischemic for the longer periods.

Significance to Biomedical Research and the Program of the Institute: These findings showed the involvement of monoamines in biochemistry of brain ischemia. In addition, data, especially that of serotonin, confirmed the "maturation phenomenon." Complex alteration in biogenic amines metabolites after ischemia of short time may be expected in the period which follows the ischemic episode.

Proposed Course of the Project: The follow-up investigations will attempt to elucidate the sources responsible for the decreased brain content of catecholamines and decreased levels of serotonin in cerebral ischemia.

Keyword Descriptors: Biogenic amines, cerebral ischemia, recovery

Honors and Awards: None

Publications: None

Project No. Z01 NS 02179-01 LNNS
1. Laboratory of Neuropathology
and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Carbohydrates in brain during and following experimental unilateral ischemia

Previous Serial Number: None

Principal Investigator: Bogomir B. Mrsulja, M.D.

Other Investigators: Umeo Ito, M.D.
Branislava J. Mrsulja, M.D.
Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	.08
Professional:	.08
Other:	0

Project Description:

Objectives: The Mongolian gerbils possess a high susceptibility for the development of cerebral infarction following unilateral ligation of the common carotic artery due to frequent absence of arterial communication between the cerebral and vertebral system (Levine, S. and Payan, H., Exp. Neurol. 16: 225, 1966). The project described here was designed to evaluate the effect of ischemia on carbohydrate (glucose, glycogen, lactate and pyruvate) metabolism. In addition, the possibility of recovery of these metabolites following ischemia was approached.

Methods Employed: Mature Mongolian gerbils were subjected to left common carotic artery clipping for 5, 15, 30 minutes, 1, 3, 5 and 9 hours. The left common carotic artery was also clipped for one hour and then released; the animals were sacrificed 1, 5 and 20 hours and 1 week after the release of the common carotic occlusion. All the animals with unilateral carotid artery occlusion were divided into two groups: with and without cerebral symptoms (Kahn, K., Neurol. 22: 510, 1972). The whole animals were rapidly frozen in liquid nitrogen and the following brain regions of left and right (control) hemispheres were dissected respectively: cortex,

caudate, thalamus, and hippocampus. In cases of 1, 3, 5 and 9 hours of cerebral ischemia and in recovery, the brain was divided into left and right hemispheres of symptoms positive and symptoms negative animals since 1 hour of carotic occlusion induced profound changes in all investigated brain structures. Contents of glucose, lactate, pyruvate and glycogen were determined by enzymic methods spectrophotometically. Glycogen content was also verified histochemically.

Major Findings: In symptoms positive groups of gerbils significant decrease of glucose and glycogen and increase of lactate and pyruvate was obtained 5 minutes after onset of ischemia in all brain structures of the hemisphere ipsilateral to the occlusion. The lowest content of glycogen was obtained 1 hour after carotic artery occlusion. Lactate and pyruvate concentrations increased and that of glucose decreased with duration of ischemia. Nine hours after onset of ischemia glycogen content was much higher than in the controls. Accumulated glycogen was histochemically observed at the same time in areas bordering the infarction. In groups of gerbils without neurological signs of infarction, no changes were found after 5 minutes of clipping. On the other hand, the findings similar to the symptoms positive animals, but less pronounced, were found in caudate after 15 minutes, in thalamus after 30 minutes, and in cortex and hippocampus after 60 minutes duration of occlusion. The lowest concentrations of glycogen and glucose, and the highest of lactate and pyruvate were obtained 3 hours after occlusion. The values obtained 9 hours after clipping were in the range of controls. In recovery from one hour ischemia, in symptoms negative gerbils, levels of glucose, lactate, pyruvate and glycogen returned to the control values during 1 hour. In symptoms positive animals, levels of glucose, pyruvate and glycogen were not different from the controls 1 hour after the occlusion was removed. Lactate was significantly elevated even after 20 hours of recovery. Glycogen content was higher at that time and was elevated even 1 week after the release.

Significance to Biomedical Research and the Program of the Institute: The results support the possibility of a "maturation phenomenon" which seems to be a general principle applicable to various parameters in ischemic injury. The rate of maturation of the lesions is directly related to the intensity (duration) of an ischemic insult, a lesser intensity resulting in slower development of lesions. Data for glycogen particularly supported this phenomenon. Ischemia of 1 hour duration shows accumulation of glycogen 20 hours after the clip was removed; the phenomenon observed after 9 hours of ischemia. The principle of "maturation" may be operative in clinical situations, and it could explain deterioration of the clinical conditions, in particular, after variable latent periods following ischemic insult.

Proposed Course of the Project: This model system will be useful in studying the effect of ischemia on biochemistry of the brain.

Keyword Descriptors: Carbohydrates, cerebral infarction, recovery

Honors and Awards: None

Publications: None

Project No. Z01 NS 02180-01 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Interference of increased systemic blood pressure with the recovery of carbohydrates in brain after ischemic injury

Previous Serial Number: None

Principal Investigator: Bogomir B. Mrsulja, M.D.

Other Investigators: Umeo Ito, M.D.
Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	.07
Professional:	.07
Other:	0

Project Description:

Objectives: In previous studies we demonstrated that hypertensive periods account for a greatly increased incidence of blood-brain barrier damage, and it produced more severe histopathologic lesions than the ones observed in the corresponding normotensive animals. These investigations are the continuation study of influence of increased systemic blood pressure on the recovery of carbohydrates following the ischemic injury.

Methods Employed: Mature Mongolian gerbils were subjected to the common carotic artery clipping and the animals with the neurological signs of cerebral infarction (Kahn, K., Neurol. 22: 510, 1972) were: (a) sacrificed immediately after 1 hour clipping; (b) clipped for 1 hour and then the occlusion was released for 1 hour; (c) clipped for 1 hour, re-anesthetized with sodium pentobarbital and sacrificed 1 hour after clip was removed; and (d) treated as the previous group, but during the release period systemic blood pressure was maintained at 150 mm Hg. All animals were killed by stirring the whole animal into liquid nitrogen and left (ischemic) and right (control) hemispheres were dissected and analyzed for glucose, glycogen, lactate and pyruvate using the enzymic spectrophotometric method.

Major Findings: Glucose and glycogen were found decreased, while lactate

and pyruvate increased after 1 hour of ischemic damage and except for lactate, returned to the control levels 1 hour following the ischemic episode. The recovery of metabolites was stimulated in the ischemic animals when they were under the influence of anesthesia during the recovery period. Increased systemic blood pressure did not alter the levels of carbohydrates in the control hemisphere contralateral to the occlusion. On the other hand, the recovery of glycogen, lactate and pyruvate was depressed, while that of glucose stimulated when the systemic blood pressure was increased during 1 hour recovery period and maintained at 150 mm Hg.

Significance to Biomedical Research and the Program of the Institute: The results reported here show that obviously the post-ischemic elevation of the systemic blood pressure during the period of re-established circulation has a deleterious effect on the ischemically affected brain tissue. Even though the findings presented here can't be completely equated with the cerebral ischemia in man, they might be of great clinical significance in considering the appropriate therapy.

Proposed Course of the Project: Other parameters of cerebral metabolism and transport will be investigated in this model of cerebral ischemia complicated by increased systemic blood pressure.

Keyword Descriptors: Carbohydrates, ischemic injury, increased systemic blood pressure, recovery

Honors and Awards: None

Publications: None

Project No. Z01 NS 01066-12 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The specificity of neuronal changes in cerebral infarcts

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.5
Professional:	0.3
Other:	1.2

Project Description:

Objectives: To establish the identity of pathologic neuronal changes.

Methods Employed: To use Baker's acid hematein method for demonstration of phospholipid as a supplement to routine histologic methods for the study of changes in experimentally produced lesions and brains exposed to post-mortem autolysis.

Major Findings: Normal immersion-fixed cat and rat brains, as the consequence of post-mortem trauma, contained numerous neurons which by conventional techniques were identified as "dark" neurons; these neurons stained with acid hematein. When formation of "dark" neurons was prevented by perfusion fixation, neurons with affinity for acid hematein did not develop.

Embolic lesions in perfused fixed monkey brains contained neurons displaying "ischemic" neuronal changes with affinity for acid hematein and "severe" neuronal changes without such affinity. The latter cell change was characterized by severe cytoplasmic vacuolation while the nucleus was preserved.

After post-mortem autolysis, the immersion-fixed brains of cats and rats displayed "dark" neurons which had remained unaffected by autolysis for up to 72 hours. Other neurons had undergone changes of cytoplasm which in a few instances mimicked the "severe" neuronal change but with nuclear pyknosis. Changes simulating those of "ischemic" neurons were not observed. In embolic lesions produced in monkeys, "ischemic" neurons resisted the effect of post-mortem autolysis for 24 hours.

Compression of the cerebral cortex in the cat resulted in focal cortical damage with formation of "ischemic" and "severe" neuronal changes: "dark" neurons were numerous in the tissue surrounding the foci. The different pathologic cell types displayed the same tinctorial qualities as those in embolic lesions. In addition, subtle changes in the form of loss of Nissl substance were evident in small neurons throughout the brain on the compressed side, and these neurons were not stained with acid hematein.

Significance: With respect to the phospholipid staining method with acid hematein, it is concluded that the reaction is induced in normal neurons as they are transformed to "dark" neurons by post-mortem trauma to the unfixed brain, or in other words that is is nonspecific, or artifactitious, in nature.

Since this staining reaction, in addition to the others tested, gave similar results in "dark" and "ischemic" neurons, it is concluded that the development of the latter cell type is also the consequence of post-mortem trauma but to a neuron which had already been pathologically altered.

After scrutiny of the material with compression of the brain, neurons with dispersal of Nissl substance were considered to represent acute reaction to impaired blood flow with oxygen deprivation. The absence of affinity for acid hematein in these neurons is further evidence that, in contrast to the "ischemic" neurons, they have not been affected by post-mortem trauma incurred during removal of the brain. At this stage, the manifestation of irreversibly damaged neurons is uncertain.

Proposed Course of Project: To perform experiments in different animals under such conditions that fixation of the tissue by perfusion can be successfully accomplished and to identify in such tissues the sequence of changes in irreversibly damaged neurons.

To test the usefulness of rabbits for such experiments after a search for the cause of complicating factors, such as spontaneous fat embolism, has been completed.

Project No. Z01 NS 01066-12 LNNS

To identify the various normal and pathologic neuronal types in experimental material fixed by perfusion for a comparison with those in human material fixed by immersion.

Keyword Descriptors: Nerve cell, pathology.

Honors and Awards: None

Publications:

Cammermeyer, J.: The effect of postmortem trauma on neuronal cell types stained histochemically for phospholipids. Exp. Neurol. 46: 616-633, 1975.

Cammermeyer, J.: Histochemical phospholipid reaction in ischemic neurons as an indication of exposure to postmortem trauma. Exp. Neurol. (in press).

Project No. Z01 NS 01449-09 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The effect of cortisone on the retrograde neuronal changes

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.2
Professional:	0.3
Other:	0.9

Project Description:

Objectives: To determine what effect cortisone treatment may have on the recovery phases of motor neurons subjected to transection of their axons.

Methods Employed: Cranial motor nerves were severed in different animal species, and after varying intervals the animals were sacrificed and fixed by perfusion in deep anesthesia.

Major Findings: Preliminary studies revealed that cortisone treatment not only depressed the incidence of reactive mitosis in microglial cells but also affected protein synthesis in neurons undergoing retrograde changes and glycogen deposition in normal neurons.

After severance of the axon, an acute reduction in glycogen took place in the glycogen-containing neurons.

Significance: In view of the frequent use of cortisone, which is known to impair peripheral regeneration after severance of the nerve. it is of importance to determine the fate of the central portion of the nerve after many months of survival. These results might eventually help to formulate guidelines for clinical therapy.

Proposed Course of Project: Long term study of animals subjected to transection of cranial nerves after treatment with cortisone.

Keyword Descriptors: Nerve cell, cortisone, axon severance, mitosis, glycogen.

Honors and Awards: None

Publications:

Cammermeyer, J.: The effect of cortisone treatment and reoperation on reactive changes in the facial nucleus after axotomy. Brain Res. (in press).

Project No. Z01 NS 01676-07 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A comparative study of the area postrema

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.5
Professional:	0.1
Other:	0.4

Project Description:

Objectives: (1) To determine the effect of chronic meningitis on the topography of the roof of the fourth ventricle, and (2) to determine the effect of chronic trypan blue treatment on the area postrema in cat and monkey.

Methods Employed: Light microscope techniques.

Major Findings: (1) Defects in the roof of the fourth ventricle vary with species.

(2) The sensitivity of cats and monkeys to apomorphine is depressed by chronic treatment with trypan blue; no histologic changes were detected in a preliminary study of serial sections of the brain stem. Iron is deposited in considerable amounts in the area postrema, more so in the rhesus monkey than in the cynomolgus monkey.

Significance: (1) The validity of the statement that the formation of holes in the roof of the fourth ventricle is very marked in man suffering from chronic meningitis with hydrocephalus will be tested by study of the dog, which frequently

suffers from severe hydrocephalus. The formation of holes in the roof of the fourth ventricle as a means to maintain adequate cerebrospinal circulation is still disputed; a study of dog material should clarify current uncertainties about cerebrospinal fluid physiology under pathologic conditions.

(2) Since in the normal animal, retching and vomiting are supposed to be induced by apomorphine via chemoreceptors in the intact area postrema, the fortuitous demonstration that cats treated with trypan blue have a lowered sensitivity to apomorphine could be indicative of damage to the area postrema. The absence of microscopically discernible changes may cast doubts on the theory that this region acts as a chemoreceptive area for the vomiting reaction; however, other histologic methods may shed new light on the mechanism involved in the present experimental modal.

Proposed Course of Project: (1) Histologic preparation of brains from dogs suffering from hydrocephalus. (2) Completed.

Keyword Descriptors: Area postrema, trypan blue, foramen of Magendie

Honors and Awards: None

Publications:

Cammermeyer, J.: Depression of retching-vomiting reflex in cats and monkeys after chronic trypan blue treatment. In: VIIth International Congress of Neuropathology, Budapest, September 1974. Budapest, Akademiai Kiado, 1975 (in press).

Project No. Z01 NS 02002-03 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mast cells in the brain

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: Jorge Luis Ribas, D.V.M.

Cooperating Units: Department of Neurophysiology, Walter Reed Army Institute of Research, Department of the Army, Washington, D. C. 20012

Man Years

Total:	0.6
Professional:	0.1
Other:	0.5

Project Description:

Objectives: To determine the significance of denucleation of mast cells in the central nervous system.

Methods Employed: Examination of microscopic sections from the brains of various animals with the aid of a specific staining technique for mast cells.

Major Findings: The appearance of mast cells varies greatly; pallor, as an expression of loss of biologically active material, is often associated with loss of the nucleus.

Significance: Since mast cells contain a variety of substances - heparin, histamine, serotonin, dopamine, etc. - which are known to act on coagulability and viscosity of blood, diameter and permeability of blood vessels and neuronal function, and since these cells occur in many parts of the brain but in varying numbers, it is assumed that their presence depends on functional requirements. If this is the case, they may play a role in controlling blood flow and neuronal interaction in the

brain. The microscopic demonstration of pallor and nuclear loss may suggest that mast cells disintegrate in situ as their functional capacity is exhausted.

Proposed Course of Project: The specificity of the histologic demonstration of nuclear loss is being investigated in serial sections of different animal species with a varying content of mast cells.

In another investigation, started in collaboration with Dr. J. L. Ribas, the purpose is to determine whether the number and morphologic characteristics of mast cells can be affected experimentally.

Keyword Descriptors: Mast cells, brain, denucleation.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02003-03 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The thalamo-choroidal body

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: To subject the taenia thalami in cat to an ultramicroscopic survey because of its prominence in this species.

Methods Employed: The variety of elements in this region have been identified by conventional light microscopic techniques.

Major Findings: A region rich in blood vessels and collagenous fibers, nerve cells, supporting glial elements, plasma cells and mast cells occupies the taenia thalami of the cat.

Significance: On the basis of its complex structure, it has been speculated that this region may serve a chemoreceptive or baroreceptive function.

Proposed Course of Project: To examine the submicroscopic composition of the thalamo-choroidal body.

Keyword Descriptors: Taenia thalami, electron microscopy

Honors and Awards: None

Publications: None

Project No. Z01 NS 02004-03 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The pathogenesis of myelopathies

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: To elucidate the mechanism of spinal cord lesions.

Methods Employed: For an orientation of the characteristics of edema within the spinal cord, the volume of each segment of the spinal cord is measured prior to and after immersion in water for varying lengths of time.

Major Findings: (1) When the spinal cord is immersed in fluids, the degree of swelling varies with tonicity of the solute as well as with region.

(2) Splitting of the surrounding membranes demonstrated a far greater swelling which in the intact spinal cord is restricted by the membranes.

Significance: In some preliminary reviews, it was found that the human spinal cord becomes firmer during swelling because of the restrictive action of the superficial membranes, a reaction also noticeable in the cat spinal cord.

Since resistance to swelling by the unyielding membranes results in abnormal blood circulation, edema can cause spinal cord lesions. Knowledge of the role of edema in the pathogenesis of spinal cord diseases is important for the establishment of guidelines for treatment and prevention of spinal cord edema occurring as a complication to a number of human diseases, such as trauma, viral infection (poliomyelitis), allergic manifestations (multiple sclerosis), cancer, etc. The results of post-mortem experiments purporting to mimic intravital processes will serve as a foundation for additional experimental investigations.

Proposed Course of Project: To define criteria whereby morphologic evidence of compromised blood flow can be determined.

Keyword Descriptors: Spinal cord, volume, edema

Honors and Awards: None

Publications: None

Project No. Z01 NS 02005-03 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Heredopathia atactica polyneuritiformis (Refsum's disease)

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To summarize the pathologic changes in heredopathia atactica polyneuritiformis (Refsum's disease).

Methods Employed: Routine histopathologic techniques.

Major Findings: (1) Accumulation of fat in brain, liver and kidney; (2) atrophy with formation of "onion bulbs" in peripheral nerves and loss of motor nerve cells in spinal cord; (3) loss of myelin sheaths in certain fiber tracts in the medulla oblongata and the dorsal column of the spinal cord; and (4) loss of nerve cells in the inferior olivary nucleus.

Significance: In the first descriptions of the changes in the brain and the peripheral nerves, the principal investigator (Nord. Med. 29: 617, 1946; addendum in Refsum, Acta Psychiat. Neur., Suppl. 38: 1-303, 1946; J. Neuropath. Exp. Neur. 15: 340-361, 1956) predicted that the fundamental lesion was a defect in lipid metabolism, a suggestion which was confirmed in investigations by German biochemists, Klenk and Kahlke (Hoppe Selyers Z. Physiol. Chem. 333: 133-139, 1963) many years later.

From a review of 15 publications and of the microscopic material from 7 of these cases, it was concluded that (a) the deposition of fat in various parts is of secondary nature, associated with the level of fat or phytanic acid in the blood, (b) peripheral nerve lesions are non-specific, (c) changes in the brain can be prevented by dietary regimen, and (d) the metabolic abnormality resulting in excess phytanic acid in blood and organs does not seriously affect neurons.

The histologic material from the last Norwegian case, deceased 1972, was reviewed jointly with Professor Dr. Aagot Christie Löken during a visit to her laboratory at the Rikshospitalet Institute for Pathology, Oslo, Norway, January 1975. While a severe atherosclerosis with terminal cerebral hemorrhage was present in the brain, no changes referable to the metabolic lipid abnormality were detected. This would suggest that when the patients have maintained a strict dietary regimen, the malady can be arrested or retarded in its progression, or in other words, the malady has a more favorable prognosis than previously anticipated.

Proposed Course of Project: Completed.

Keyword Descriptors: Heredopathia atactica polyneuritiformis, neuropathology

Honors and Awards: None

Publications: None

Project No. Z01 NS 02143-01 LNNS
1. Laboratory of Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Volumetric changes of brains during histologic preparation

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To measure the volume of brains from small animals in order to test the effect of fixation and dehydration.

Methods Employed: An apparatus was designed for direct measurement of small brains utilizing the same principle as laid down earlier in the design of an apparatus for volumetric measurement of segments of the intact spinal cord. Simultaneous measurement of the brain weight permitted calculation of the specific gravity.

Major Findings: When brains of animals of different species were fixed by perfusion first with saline and then with Bouin's solution, their specific gravity remained constant.

The volume of the Bouin-fixed brains was unaffected by flow, rate, amount or temperature of the fixative.

Significance: For standardization of the histologic preparation of the brain, it is important that volume of tissues be controlled. Constancy in volume reflects adequacy of the preparatory technique, a requirement for estimating the status

of the neurons. The results obtained may provide a baseline for assessing the effect of experimentally produced damage or edema in the brain.

Proposed Course of Project: To compare the changes in volume of brains during the process of alcohol dehydration after fixation by immersion and by perfusion.

Keyword Descriptors: Brain volume, method, comparative

Honors and Awards: None

Publications: None

Project No. Z01 NS 01231-11 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Organization of synaptic connections in the mammalian brain

During FY 1975 this project was incorporated with Serial No. Z01 NS 01881-05 LNNS.

Project No. Z01 NS 01442-09 LNNS
1. Laboratory of Neuropathology and and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Permeability of cellular layers in the vertebrate nervous system

Previous Serial Number: Same

Principal Investigator: Thomas S. Reese, M.D.

Other Investigators: Milton W. Brightman, Ph.D.
Dennis M. D. Landis, M.D.
Sachio Nabeshima, M.D.

Cooperating Units: Laboratory of Experimental Pathology, NIAMDD

Man Years:

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description:

Objectives: Determination of the pathways taken by colloids and large molecular weight solutes to cross the various cellular layers associated with the nervous system. Currently being studied are: (1) ependyma covering the median eminence and area postrema; (2) arachnoid-dural membrane; (3) arachnoid villae.

Methods Employed: Various methods are used to prepare brains of laboratory animals for examination with the electron microscope. The cytochemical method of Karnovsky is used to localize purified horseradish peroxidase (MW 40,000) or microperoxidases (MW less than 2000) after they are injected into the blood or cerebrospinal fluid. Junctions between cells bordering these compartments are examined with the electromicroscope after applying special stains for intercellular junctions. The freeze-fracture technique has also been used to determine the structure and deployment of these junctions.

Major Findings: Peroxidase injected into the cerebrospinal fluid of the subarachnoid space in the mouse is able to pass between cells of the pia-arachnoid layer, cross the basement lamina of the brain, and pass between the astrocytic processes forming the limiting surface of the brain to reach typically open interstitial spaces of the brain. However, peroxidase does

not cross from ventricular cerebrospinal fluid into the median eminence and area postrema. The basis of this selective barrier is a system of tight junctions within the specialized epithelial cells lining the ventricular surface over these regions.

A specialized layer of cells joined by tight junctions at the outer border of the arachnoid also prevents peroxidase from reaching the subdural space from the subarachnoid space. Also, we have given our attention to the arachnoid villae where this arachnoid barrier layer becomes discontinuous at its apposition with the endothelium of the superior sagittal sinus. Study of sinus endothelium utilizing tilt microscopy and serial sections has revealed a system of minute pores which could be responsible for bulk drainage of cerebrospinal fluid. We are now attempting to determine whether these pores might also be the basis for the valvular action of the arachnoid villae. Another ramification of our studies of the meninges is to examine the meningeal barriers after acute infection with E coli. One effect of the infection is that the tight junctions between barrier cells open permitting leukocytes from the dura to migrate into the subarachnoid space.

Application of the freeze-fracture technique has demonstrated that membranes of astrocytic processes facing blood vessels and cerebrospinal fluid have a peculiar internal structure characterized by the presence of assemblies of specialized subunits, presumably membrane proteins. This finding suggests that these glial membranes might have special permeabilities or pumping mechanisms which could be part of the blood-brain barrier.

Significance to Biomedical Research and the Program of the Institute: We are determining the permeability to small proteins of special cellular layers associated with blood vessels and meninges of the blood vessels and brain surfaces. 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 (blood-brain barrier and blood-cerebrospinal fluid barriers). By applying this knowledge it is possible to determine the changes in cell and cell membrane structure which are responsible for pathological changes in the brain barrier system, such as those which occur during acute experimental meningitis. Also of interest is that our recent data indicates 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 first of a series of papers on the arachnoid membrane has been submitted to the Journal of Comparative Neurology. Subsequent papers in this series are in preparation. A definitive paper on the brain barriers at the median eminence is in press (Brightman, M.W., Prescott, L., Reese, T.S.: Intercellar junctions in specialized ependyma. In Scott, D.E. and Kobayashi, M. (Eds.) Proceedings of the Second International Symposium on Brain-Endocrine Interaction. S. Karger AG, Switzerland, 1975) and this phase of the project is completed. Work on the arachnoid villae has been held in abeyance this year.

Project No. Z01 NS 01442-09 LNNS

Keyword Descriptors: arachnoid villae, astrocytes, blood-brain barrier, blood-cerebrospinal fluid barrier, central nervous system, electronmicroscopy, ependyma, freeze-fracture, median eminence, meninges

Honors and Awards: None

Publications: Nabeshima, S., Reese, T.S., Landis, D.M.D. and Brightman, M.W.: Junctions in the meninges and marginal glia. J. Comp. Neurol., 1975. In press.

Project No. Z01 NS 01684-07 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Structure and function of close intercellular (gap) junctions

During FY 1975 this project was incorporated with Project No. Z01 NS 01881-05 LNNS.

Project No. Z01 NS 01881-05 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Structural basis of synaptic transmission

Previous Serial Number: Same and incorporating Project No. Z01 NS 01231-11 LNNS and Project No. Z01 NS 01684-07 LNNS

Principal Investigator: Thomas S. Reese, M.D.

Other Investigators: Christopher Brandon, Ph.D.
Monique Dubois-Dalcq, M.D.
Dennis M. D. Landis, M.D.
Avery D. Nelson, Ph.D.
Edward L. White, Ph.D.

Cooperating Units: Laboratory of Experimental Pathology, NIAMDD and Infectious Diseases Branch, NINCDS

Man Years:

Total:	4.2
Professional:	2.9
Other:	1.3

Project Description:

Objectives: Synapses are sites where electrical signals pass between neurons or between neurons and muscles cells. This project seeks to clarify the exact location and mechanism of synaptic transmission in the central and peripheral nervous system.

Methods Employed: Synapses are prepared for examination with the electronmicroscope by a variety of methods. Central nervous system synapses are studied in perfusion fixed brains either in serial thin sections or with the freeze-fracture technique. Tissue to be used for freeze-fracturing is sometimes prepared by fresh freezing in a special apparatus, designed in our laboratory, in order to avoid use of any chemical fixative. The mouse olfactory bulb and cerebellum are the principal areas used for these studies. Peripheral synapses are studied at the frog neuromuscular junction and at the frog sympathetic ganglion. Use of these preparations permits a variety of experiments to be performed prior to preparation for electron microscopy.
1. Frog sartorius nerve-muscle preparations are exposed to altered ionic environments or stimulated at various intervals until they reach a desired functional state. Then they are immediately fixed and prepared for examina-

tion with the electron microscope. Tissue to be freeze-fractured is usually fresh frozen in order to capture the fleeting events associated with synaptic vesicle discharge. Similar experiments are performed in solutions containing horseradish peroxidase and the horseradish peroxidase subsequently localized with the electron microscope. 2. Similar experiments are performed on preganglionic autonomic synapses in the frog.

Major Findings: Central Nervous System Synapses: Major new findings concern the genetic control of the development of certain synaptic patterns and differentiation of the postsynaptic membranes of excitatory and inhibitory synapses.

In Balb/c mice a certain type of synapse was found to be lacking in the olfactory bulb even though the other three types of synapse in this region of the olfactory cortex were present in normal numbers. Furthermore, the normal complement of axons which would have produced these synapses were present so that the Balb/c mouse apparently lacks the genetic information to form or maintain a particular synaptic connection. This is the first demonstration that formation of particular synaptic connections differs in different strains of mice.

The postsynaptic membranes of a variety of known excitatory and inhibitory synapses from olfactory bulb and cerebellum were examined with the freeze-fracture technique. Excitatory synapses were found to have an array of particles embedded in the postsynaptic membrane coextensive with the synaptic junction. Similar particles were lacking or not specifically clustered at the synaptic junction of inhibitory synapses. Fresh-freezing the brain without any chemical fixation, revealed that similar distributions of particles are found. Two experimental manipulations were used prior to freezing which caused the particles dispersed over cell membranes to cluster in small groups. However, the distribution of particles at synapses were not affected by these manipulations which suggests that they are anchored in the membrane by some mechanism which is of interest for further study.

Peripheral Synapses: The typical appearance of these synapses represents a resting or equilibrium state. With intense electrical stimulation and transmitter release approaching exhaustion, the intracellular organelles of the presynaptic terminal undergo well-defined, reversible changes. These include mitochondrial swelling and migration to new regions, depletion of synaptic vesicles with coincident enlargement of the presynaptic membrane surface, and finally formation of coated vesicles and Golgi-apparatus-like bodies, apparently as intermediate states in the recycling of membrane back into synaptic vesicles. When similar experiments are done in solutions containing horseradish peroxidase, the tracer progressively fills various membrane forms inside the synapse, thus tracing directly the recycling of membrane from coated vesicles to synaptic vesicles and back to surface membrane concomitant with transmitter release. Identical results were obtained with autonomic synapses from the sympathetic ganglion indicating that new synaptic vesicles here are also formed by recycling of membranes.

The freeze-fracture technique produced new information about the stage of this membrane cycle where synaptic vesicles fuse with the presynaptic membrane. Since this technique exposes large expanses of membrane, it was possible to show in both frog sympathetic ganglion and neuromuscular synapses that vesicles fuse with specific regions of the postsynaptic membrane when and only when transmitter is being discharged. This result shows that transmitter discharge from synaptic vesicles is by means of exocytosis. Also, it shows that only those vesicles attached to the presynaptic fuzz are releasable and therefore that it is likely that these vesicles contain the readily releasable pool of transmitter. This conclusion is supported by our finding in sympathetic ganglion synapses, that the synaptic vesicles lined up next to the presynaptic specialization are specifically depleted by short bursts of nerve stimulation.

At fixed neuromuscular synapses there are, however, many more vesicles caught during exocytosis than would be predicted from the rate of discharge of transmitter quanta. This discrepancy was thought to be caused by the action of the chemical fixative and therefore an effort was made to prepare tissue by freezing it directly without any fixative or cryoprotective agents. This method is now working and it is clear that the number of quanta being discharged can approximate the number of synaptic vesicles undergoing exocytosis. This approach, when applied in a quantitative manner, will provide direct proof of the "vesicle hypothesis" which states that neurotransmitter is discharged from synaptic vesicles.

The structure of the postsynaptic membrane at these peripheral, cholinergic, excitatory synapses differed from both types of central nervous system synapse. Specific aggregates of particles were found on the inner rather than the outer half of the split membrane. Thus, the postsynaptic membrane of each chemical type of synapse might have a specific organization discernible with the freeze-fracture technique. If this is so, the freeze-fracture technique will give us information on the organization and deployment in the postsynaptic membrane of different types of receptor-associated ionic channels.

Gap junctions, a special type of synaptic junction which produces direct electrical coupling between neurons, can be found readily with the freeze-fracture technique which is, to date, the most sensitive method of locating them. They are absent at the peripheral synaptic sites we have examined and at most synaptic sites in the central nervous system but are found at synapses between cell bodies and dendrites in the olfactory bulb. The results of this freeze-fracture study minimizes the importance of gap junctions in signalling between neurons at many locations in the central nervous system.

A major effort has been made in the last year to develop new techniques for examining the cell surface at a molecular level. These efforts have been successful and have been applied to a study viral budding in tissue culture in collaboration with Dr. Monique Dubois, Infectious Diseases Branch, NINCDS (Project No. Z01 NS 02034-03 ID). This subject was selected for our initial studies of surface labelling techniques both because of its intrinsic importance and because it gave a rapid reliable preparation with which to develop the

techniques. These techniques are currently being used to look at the distribution of cholinergic receptors on muscle cells in culture and will be used for future studies on the distribution of receptors in developing synapses.

Significance to Biomedical Research and the Program of the Institute: Much effort in neurological research has been directed to mapping the axonal tracts connecting different areas of cerebral grey matter. One part of our study of synapses is directed toward understanding what occurs at the termination of these tracts. We have shown previously that complex synaptic patterns exist in various regions of the brain, each subserving specific types of physiological activity. This year we have completed a study of genetic and environmental factors controlling development and maintenance of certain synaptic patterns. The knowledge that genetic diseases could be expressed by specific changes in the organization of these systems is helpful in understanding the neurological symptoms produced by these diseases.

Different arrangements of postsynaptic membrane appear to be characteristic of different chemical types of synapses, although more direct evidence about the nature of these membrane particles is needed. Knowledge about the chemical basis of synaptic interaction in different regions of the central nervous system is helpful in understanding the mechanisms of action of drugs on different regions of the brain.

Work on peripheral synapses contributes to an understanding of the role of membranes in the storage and release of transmitter at synapses and shows that the mechanism for release of neurotransmitters is similar to that employed for release of glandular secretions. Also, this work helps to broaden the general anatomical criteria for identifying synapses, thereby aiding future attempts to define the existence and location of functional contacts in the brain, and most important, permitting attempts to define their physiological state in normal and pathological brain samples. In particular, the rapid freezing technique allows synapses to be examined very close to their natural state and permits visualization of very rapid and functionally significant changes in structure. Armed with the new knowledge from these approaches, it should be possible to distinguish normally active from pathologically active synapses in a variety of pathological conditions, including samples from epileptogenic foci.

Proposed Course of Project: The work on genetic control of synaptic connections in the olfactory cortex has been published and is completed. Descriptions of the structure of the pre- and postsynaptic membranes of various central nervous system synapses have been published in the last year. The work on rapid freezing of frog neuromuscular synapses, and the freeze-fracture study of the frog sympathetic ganglion will be finished and submitted for publication during the next year. These data have been presented by invitation at symposia at the annual meeting of the Biophysics Society, the annual meeting of the Society for Neuroscience, a meeting of the New York Academy of Sciences International Conference on Carriers and Channels in Biological Systems, a Neurosciences Research Program Workshop, and will be discussed at a Cold Spring

Harbor Symposium on the synapse this summer. Future plans are to use the freeze-fracture technique and scanning microscopy to examine the deployment of vesicle release sites and postsynaptic specializations in synapses developing in tissue culture.

Keyword Descriptors: cerebellum, electronmicroscopy, freeze-fracture, membrane structure, neuromuscular junctions, olfactory bulb, receptors, secretion, synapses, synaptic development, synaptic organization, synaptic vesicles

Honors and Awards: None

Publications:

White, E.L.: Synaptic organization of the mammalian olfactory glomerulus: new findings including an intraspecific variation. Brain Res. 60: 299-313, 1973.

Heuser, J.E. and Reese, T.S.: Morphology of synaptic vesicle discharge and reformation at the frog neuromuscular junction. In Bennett, M.V.L. (Ed.): The Synapse, New York, Raven Press, 1974, pp. 59-77.

Landis, D.M.D., Reese, T.S. and Raviola, E.: Differences in membrane structure between excitatory and inhibitory components of the reciprocal synapse in the olfactory bulb. J. Comp. Neurol. 155: 67-92, 1974.

Landis, D.M.D. and Reese, T.S.: Differences in membrane structure between excitatory and inhibitory synapses in the cerebellar cortex. J. Comp. Neurol. 155: 93-126, 1974.

Project No. Z01 NS 01880-05 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Hyperosmolar and $HgCl_2$ effect on the brain uptake of ^{14}C glucose analogues

Previous Serial Number: Same

Principal Investigator: Maria Spatz, M.D.

Other Investigators: Igor Klatzo, M.D.
Zbigniew M. Rap, M.D.
Stanley I. Rapoport, M.D.

Cooperating Units: Polish Academy of Sciences, Warsaw, Poland
Laboratory of Neurophysiology, NIMH

Man Years:

Total:	1.37
Professional:	1.12
Other:	.25

Project Description:

Objectives: In previous studies, we demonstrated an increased facilitated transport of glucose analogues from blood to brain following intracarotid perfusion of hypertonic solutions. This study was a continuation of the same project in order to (1) elucidate the relationship of the increased brain uptake of glucose to the condition of the blood flow in the cerebral hemisphere, and (2) to study the effect of $HgCl_2$ BBB injury on the brain uptake of glucose analogues.

Methods Employed: Young adult rabbits were used for these experiments. An intracarotid cannula was inserted in one of the common carotid arteries, the external carotid artery was ligated in order to perfuse the hypertonic urea or $HgCl_2$ directly into the internal carotid artery. The concentration of the urea perfusate was two or three molar and the concentrations of $HgCl_2$ was .01 mM or .08 mM.

For the estimating of blood flow, ^{14}C antipyrine was injected into the femoral vein ten minutes after hypertonic urea perfusion. The animals were sacrificed by decapitation and the section of brain tissue frozen quickly and

subsequently cut in a cryostat and processed for radioautography.

The uptake of ^{14}C 2-deoxy-D-glucose into the brain was analyzed 10 minutes after $HgCl_2$ perfusion by using a modified double isotope technique of Oldendorf. The self-inhibition of ^{14}C 2-deoxy-D-glucose was determined with simultaneous injection of cold 2-deoxy-D-glucose with the labeled tracer. The brain uptake index was calculated per 100 mg of brain tissue.

$$BUI = \frac{\text{Tissue } ^{14}C \ / \ \text{Tissue } ^{3}H}{\text{Injectate } ^{14}C \ / \ \text{Injectate } ^{14}C} \times 100$$

Major Findings: The ^{14}C antipyrine radioautography of the brains perfused with hypertonic urea revealed an equal distribution of ^{14}C antipyrine in both the perfused and unperfused cerebral hemisphere. Thus, no direct relationship of the increased BUI for ^{14}C glucose analogues to an altered blood flow could be established by this method.

Intracarotid perfusions of .01 mM $HgCl_2$ decreased BUI for ^{14}C 2-deoxy-D-glucose to the level of maximally inhibited controls injected with cold 2-deoxy-D-glucose probably due to inhibition of the facilitated transport of ^{14}C 2-deoxy-D-glucose into the brain (BUI 16.3 $\pm$ .05 S.E., N=4, 14.35 $\pm$ 1.85 S.E., N=5, respectively). Passive diffusion of ^{14}C 2-deoxy-D-glucose occurred also with $HgCl_2$ perfusate of high concentration (.08 mM) although the BUI was below (26.8 $\pm$ 3.06 S.E., N=5) the one in controls (48.83 $\pm$ .83 S.E., N=10).

Significance to Biomedical Research and the Program of the Institute: The investigation of glucose transport across an altered blood-brain barrier (BBB) such as following hyperosmotic and metabolic injury is of great interest and importance from the pathophysiological point of view of the BBB. (A) Since glucose is one of the most significant nutrients of the brain, the broadening of the knowledge concerning its passage from blood to brain in health and disease is of major importance. (B) It may help in elucidating the pathogenesis of many neurological disorders. (C) It may offer an understanding and a possibility to select the best therapeutic approach to given disease.

Proposed Course of Project: This model may be useful in the investigation of hypertonic effects on amino acids and biogenic amines transport from blood to brain.

Keyword Descriptors: Hypertonic solutions, blood flow, $HgCl_2$ BBB injury, brain uptake of glucose analogues

Honors and Awards: None

Publications:

Spatz, M., Rap, Z. M., Rapoport, S. I., and Klatzo, I.: Effects of hypertonic solutions and of $HgCl_2$ on the uptake of ^{14}C glucose analogues by rabbit brain. Neuropath. Appl. Neurobiol. 1975 (in press).

Project No. Z01 NS 01999-03 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Brain uptake of ^{14}C 3-O-methyl-D-glucose and ^{14}C sucrose in ischemic gerbils

Previous Serial Number: Same

Principal Investigator: Maria Spatz, M.D.

Other Investigators: I. Klatzo, M.D.
K. G. Go, M.D.

Cooperating Units: Department of Neurosurgery, University of Groningen
Groningen, The Netherlands

Man Years:

Total:	1.25
Professional:	.75
Other:	.50

Project Description:

Objectives: The Mongolian gerbils possess a high susceptibility for the development of cerebral infarction following unilateral ligation of the common carotid artery due to frequent absence of arterial communication between the cerebral and vertebral system (Levine, S. and Payan, H., Exp. Neurol. 16: 255-262, 1966, and Kahn, K., Neurol. 22: 510-515, 1972). The project described here was designed to evaluate the effect of ischemia on glucose transport from blood to brain as one of the multidisciplinary approaches for the pathophysiological investigations of cerebrovascular disorders. Recently we demonstrated an increased brain uptake of ^{14}C 2-deoxy-D-glucose after carotid arterial occlusion for various periods of time. This augmented ^{14}C 2-deoxy-D-glucose brain uptake was self-inhibited with cold 2-deoxy-D-glucose in all animals except for gerbils with cerebral symptoms subjected to 18 hours of carotid artery occlusion. The present investigation is a continuation study of ischemic effect on the transport of glucose and sucrose into the brain.

Methods Employed: Several groups of gerbils were subjected to left common carotid artery clipping for various periods of time and the release of the clip for five minutes. Thereafter, a double isotope technique was used for measuring the differential uptake of tracers injected as a mixture intra-carotidally 15 seconds prior to decapitation. In this method $^{3}H_2O$ was used

as a reference with which the uptake of ^{14}C labeled 3-O-methyl-D-glucose or ^{14}C sucrose was compared. For the inhibition studies the injectate contained 2 mM phlorizin (cold) with the labeled 3-O-methyl-D-glucose. The brain uptake index (BUI) for the test substance was calculated as follows:

$$BUI = \frac{\text{Tissue } ^{14}C \text{ / Tissue } ^{3}H}{\text{Injectate } ^{14}C \text{ / Injectate } ^{3}H} \times 100$$

Major Findings: An increased brain uptake of ^{14}C 3-O-methyl-D-glucose was demonstrated in both sensitive (with cerebral damage) and nonsensitive (without cerebral damage) gerbils after 3, 6, and 18 hours of common carotid artery occlusion (BUI 65.47 $\pm$ 6.19 S.E., 60.75 $\pm$ 2.73 S.E., 66.84 $\pm$ 2.51 S.E. respectively, controls 43.71 $\pm$ 1.09 S.E.). In controls and ischemic gerbils cross inhibition of ^{14}C 3-O-methyl-D-glucose was obtained with simultaneous injection of unlabeled 2 mM phlorizin. A difference in the degree of inhibition was found between the sensitive and nonsensitive gerbils following 18 hours of carotid artery occlusion. The 3-O-methyl-D-glucose brain uptake was 43.77 $\pm$ 5.21 S.E., (N=5) in the sensitive, 24.31 $\pm$ .81 S.E., (N=6) in the nonsensitive, and 25.91 $\pm$ 1.42 S.E., (N=5) in control animals.

The BUI for ^{14}C sucrose was the same in the ischemic (BUI 7.5 $\pm$ .32 S.E., N=10) as in the control gerbils (BUI 7.72 $\pm$ .52 S.E., N=5) except for 18 hours ischemic sensitive gerbils which showed an increase in ^{14}C sucrose brain uptake (BUI 19.76 $\pm$ 3.9 S.E., N=3).

The cross inhibition of the increased ^{14}C 3-O-methyl-D-glucose brain uptake of the brain non-metabolizable hexose support our previous contention that the transfer of glucose analogues took place by facilitated transport in the sensitive and nonsensitive gerbils except for 18 hours sensitive (with cerebral damage) animals in which passive diffusion occurred, too. This assumption is supported by the normal ^{14}C sucrose passage at 6 hours and increased passage of ^{14}C sucrose at 18 hours after carotid artery occlusion (sensitive gerbils).

Significance to Biomedical Research and the Program of the Institute: The basic comprehension of the blood-brain barrier (BBB) functions concerned with the transport of nutritional substances from blood to brain following cerebral ischemia is of major importance (1) for the understanding of the pathophysiological process occurring in cerebrovascular disease and many other neurological disorders, and (2) for selecting the best therapeutic approach to a given disease.

Proposed Course of Project: This model system will be useful in studying the effect of ischemia on the transport of amino acids and biogenic amines.

Keyword Descriptors: Brain uptake, 3-O-methyl-D-glucose, sucrose, ischemic gerbils

Honors and Awards: None

Publications: None

Project No. Z01 NS 02000-03 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Brain edema in cerebral ischemia of gerbils

Previous Serial Number: Same

Principal Investigator: K. Gwan Go, M.D.

Other Investigators: Maria Spatz, M.D.
Igor Klatzo, M.D.
Hanna Pappius, Ph.D.

Cooperating Units: Montreal Neurological Institute
Department of Neurosurgery, University of Groningen

Man Years:

Total:	.87
Professional:	.75
Other:	.12

Project Description:

Objectives: In human cerebral ischemia, brain edema is considered 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. and Payan, H., Exp. Neurol. 16: 252-255, 1966; Kahn, K., Arch. Path. 69: 544-553, 1972; and Ito, U., Go, K. G., Klatzo, I., and Spatz, M., American Association of Neuropathologists Meeting, 1973). Therefore, the gerbils were chosen as an animal model system for the investigation of brain edema in ischemia.

In our recent studies of ischemic brain edema in gerbils, a decrease in the per cent of dry weight (i.e., an increased water content) was found in the cerebral hemisphere ipsilateral to the carotid artery occlusion as compared to the controls. The present investigation has been a continuation of this study to determine the electrolyte concentration in the brain in order to correlate the changes of the electrolyte to the water content of the brain in cerebral ischemia.

Methods Employed: Several groups of adult gerbils were subjected to unilateral clipping of the left common carotid artery for various periods of

time (1, 3, 6, and 18 hours), and release for 5 or 60 minutes. For this study, only the gerbils with definite ischemic cerebral symptoms (established clinically or by EEG) were selected for the determination of the brain water and electrolyte content. The brains from the decapitated animals were removed quickly and the separated hemispheres weighed immediately (fresh weight). Following drying at 98°C for six days, the samples were weighed again (dry weight) and used for the analyses of Na and K content by flame photometry.

Major Findings: The effect of the unilateral common carotid artery occlusion on the cerebral Na and K content was seen already after one hour of arterial clipping. There was a net loss of potassium (34 meg/kg dry weight) and a net gain of sodium (46 meg/kg dry weight) in the affected, as compared to the unaffected and the control cerebral hemispheres. These changes progressed (as was reported in the brain water content) with the length of the carotid artery occlusion. There were no significant differences observed in the Na and K concentration of the affected hemispheres whether the release period lasted 5 or 60 minutes after carotid artery occlusion.

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 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 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 ischemic brain in gerbils.

Keyword Descriptors: Brain edema, cerebral ischemia, gerbils

Honors and Awards: None

Publications: None

Project No. Z01 NS 02001-03 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The effect of oxygen saturation and pCO_2 tension on amino acids transport in the rabbit brain

Previous Serial Number: Same

Principal Investigator: Maria Spatz, M.D.

Other Investigators: Frank Berson, M.D.
Tsukasa Fujimoto, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	1.50
Professional:	1.25
Other:	.25

Project Description:

Objectives: Previous studies have shown a decreased brain uptake of 2-deoxy-D-glucose and 3-O-methyl-D-glucose in severe hypoxia and hypercapnia while an increased uptake of these substances was seen in hypocapnia. The purpose of this investigation has been to evaluate the effect of oxygen saturation and carbon dioxide tension on the transport of amino acids from blood to brain in rabbits.

Methods Employed: Young adult rabbits were anesthetized and ventilation controlled with a small animal respirator. By varying the rate of ventilation and concentrations of inspired gases, it was possible to regulate the pO_2, pCO_2 and pH of arterial blood. Blood pressure was monitored throughout the experiments and blood gas levels measured in a pH/blood gas analyzer. A radioactive mixture of tritiated H_2O and a carbon-14 labeled glucose analogue was injected into the cerebral circulation via the right common carotid artery 15 seconds prior to decapitation. The brain was removed and a portion of cortex processed by standard techniques for radioactive counting. Uptake of the test substance was expressed in terms of a brain uptake index (BUI).

Major Findings: In hypoxia (pO_2< 18%) the brain uptake index (BUI) of essential (L-Alanine, L-Serine and D-Leucine) was decreased as compared to the control animals. In hypercapnia a significantly decreased brain uptake index (BUI) was found with the non-essential amino acids while the passage of essential amino acids was not different or increased when compared to the controls. The brain uptake index (BUI) of the essential amino acid was also increased in hypocapnia. These results indicate that the brain uptake of the tested radiolabeled essential amino acids was different from the uptake of labeled glucose analogues and non-essential amino acids in hypercapnia, but not in hypoxia.

Significance to Biomedical Research and the Program of the Institute: Oxygen saturation, pCO_2 and pH represent factors, which individually or combined, may play a role in altering the blood-brain barrier and contribute to the tissue damage resulting from cerebral ischemia. Since many of the amino acids are essential cerebral nutrients elucidation of the factors affecting their uptake of the brain should help in understanding the pathophysiologic process involved in ischemia and may suggest means of preventing irreversible damage. Part of this work was presented at the Erwin-Riesch Symposium on the Cerebral Vessel Wall in West Berlin March 14-16, 1975. The paper will be published in the Symposium Proceedings in a book for 1975.

Proposed Course of the Project: This model will be used to study the transport of several other essential and non-essential amino acids from blood to brain. Also, blood flow studies using xenon clearance method are planned in order to quantitate any effect the cerebral blood flow may have on uptake of glucose analogues under these particular experimental conditions.

Keyword Descriptors: Oxygen saturation, carbon dioxide tension, transport of amino acids from blood to brain

Honors and Awards: None

Publications:

Spatz, M., Berson, F., Fujimoto, T., and Klatzo, I.: Transport of nutrients and non-nutrients across the blood-brain barrier in pathological conditions. Symposium Proceedings, 1975 (in press).

Project No. Z01 NS 02083-02 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Uptake of radiolabeled glucose analogues in cerebellar explants

Previous Serial Number: Same

Principal Investigator: Krystyna Renkawek, M.D.

Other Investigators: Maria Spatz, M.D.
Margaret R. Murray, Ph.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	1.50
Professional:	1.50
Other:	0

Project Description:

Objectives: Glucose transport across the blood brain barrier was found to be increased in gerbils following cerebral ischemia, but decreased in acute hypoxic and hypercapnic rabbits (Spatz, M., Go, K. G., and Klatzo, I.: The effect of ischemia on the brain uptake of ^{14}C glucose analogues and ^{14}C sucrose. In Cervos-Navarro, J. (Ed.): Pathology of Cerebral Microcirculation. Berlin, Walter de Gruyter & Co., 1974, pp. 361-366, and Go, K. G., Berscn, F., Klatzo, I. and Spatz, M., American Association of Neuropathologists Meeting, 1973). The changes in brain uptake of various substances in a disease process may depend on the integrity of: (1) the cerebral vascular bed and/or (2) some of the cellular elements, for example, astrocytes. Thus, these experiments were designed to utilize tissue culture for the study of transport phenomena across the cellular membrane elements in the brain under normal and pathological conditions. The brain uptake of nutrient and non-nutrient substances in a diseased process may depend on the integrity of: (1) the cerebral vascular bed and/or (2) other CNS cellular elements. Therefore, it was thought that CNS organotypic cultures may be suitable for selective investigation of transport phenomena across the living cellular membranes in the brain under normal and pathologic conditions. This report describes the investigation of ^{3}H glucose analogue uptake in the cerebellar explants.

Methods Employed: The Maximow technique was used for cultivation of explants from newborn rat cerebellum. The cultures were fed twice a week with equal parts of Eagle basal media containing glutamine, fetal calf serum, bovine serum ultrafiltrate, Simm's balanced salt solution (BSS) supplemented with 10% dextrose. Fourteen day old explants, washed with dextrose-free BSS, were used for ^{3}H methyl-D-glucose or ^{3}H 2-deoxy-D-glucose (10 µc/1 ul) transport studies. The culture incubated for various periods of time contained .5 µc of the labeled substance in .5 ml dextrose-free media or BSS. For the inhibition studies glucose, methyl-D-glucose, 2-deoxy-D-glucose, xylose and phlorizin in various concentrations were added to the labeled solution. In addition, the extracellular space was determined with ^{14}C sucrose or ^{14}C inulin. After the incubation, the medium was removed and the cultures were washed three times with BSS. Pooled samples (2-7 cultures) were either weighed or assayed for protein content (Lowry, O. H.), solubilized and processed for liquid scintillation counting.

Major Findings: The passage of ^{3}H glucose analogues from the incubation media into the cerebellar explants occurred by both pH dependent facilitated carrier transport and diffusion. The greatest saturable uptake was found at pH 7-7.2. At this pH, 60% of the labeled glucose analogues uptake can be reduced by self-inhibition (addition of unlabeled 3-O-methyl-D-glucose or 2-deoxy-D-glucose to the respective labeled solution) or competitive inhibition (addition of labeled glucose or xylose or phlorizin). The non-saturable uptake of the labeled glucose analogues was lower at pH 7-7.2 than at pH 7.4-7.6. The amount of diffusion of glucose analogues determined from the cerebellar explant uptake of double isotopes consisting of ^{3}H labeled glucose analogue and ^{14}C labeled sucrose or inulin, was found to be 30-40% at pH 7-7.2, but higher at pH 7.4-7.6. The highest accumulation of the labeled glucose analogues in the cerebellar explants was found after 30 minutes of incubation. The activity of cerebellar explants was higher for the 2-deoxy-D-glucose than 3-O-methyl-D-glucose (100.000 DPM/100 µ P, and 14.000 DPM/100 µ P, respectively). (Presented at the Neuroscience Meeting, 1974.)

Significance to Biomedical Research and the Program of the Institute: The cerebellar tissue culture is an excellent model for the study of transport across the cellular membrane elements without the interference of cerebral vessels. These investigations have been focused on creating the environmental and cytogenic conditions in tissue cultures similar to the one existing in normal and diseased brain in order to determine the cellular functions in many neurological disorders associated with ischemia, edema, neurotoxicity and others.

Proposed Course of the Project: The continued investigations will be concerned with defining and eliciting the factors responsible for the glucose transport under normal conditions. Therefore, the relationship of the glucose uptake to the duration of the incubation, the kinetic and the influence of electrolytes, metabolites and drugs on the glucose transport

will be determined in these cultures. Thereafter, this model will be used to study the glucose transport under pathological conditions. Furthermore, amino acid transport will be investigated, too.

Keyword Descriptors: Transport phenomena, CNS organotypic cultures, cellular membranes, ^{3}H glucose analogue uptake, facilitated carrier transport and diffusion.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02084-02 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Morphologic and enzymatic properties of endothelial cells in cerebellar and lepto-meningeal culture

Previous Serial Number: Same

Principal Investigator: Krystyna Renkawek, M.D.

Other Investigators: Margaret R. Murray, Ph.D.
Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	1.8
Professional:	1.2
Other:	0.6

Project Description:

Objectives: The concept of blood-brain barrier (BBB) is essentially functional. Nevertheless, realization of the function to admit or exclude certain types of substances to the cerebral tissue must be based, at least in part, on morphological and histochemical factors of the capillaries. It occurred to us that the organotypic cerebellar and pia arachnoid cultures might be suitable for the isolation, identification and histochemical studies of the BBB capillaries in order to define and differentiate their function from the capillaries of tissue unrelated to the BBB system.

Methods Employed: The Maximow technique was used for cultivation of capillaries from cerebellar or lepto-meningeal explants. The cerebellum was stripped from meninges and each one sectioned into small fragments. The cerebellar tissue was placed while the meninges were spread in collagen-coated coverslips. The cultures were fed and washed twice weekly. The nutrient medium was composed of equal parts of balanced salt solution (BSS), fetal calf serum, some serum ultrafiltrate supplemented with 300 mg% of glucose. The outgrowth of cells and the formation of capillaries have been observed by light and phase microscopy for over two months. Representative cultures were photographed living, with phase and subsequently fixed and

stained with Jenner-Giemsa, Azan, Trichrome, Toluidine blue and Foot-Bielschowski methods. The major part of the cultured material was used for histoenzymatic studies of butyryl-cholinesterase, alkaline phosphatase and gamma-glutamyltranspeptidase activity. Capillaries in organotypic cultures of somatic tissues not involved in the BBB, e.g., embryonic skin and rib, were tested similarly.

Major Findings: In about 70% of cerebellar culture formed capillaries are distinguishable in the explants after 24-48 hours. After a week, capillaries can be seen in the outgrowth zone, and in the thinner areas direct connection can be traced between these and their sources in the explants. In pia arachnoid cultures sprouts can be identified on the third day in vitro. The endothelial cells of both explants appear in parallel and/or crossed chains of cells forming small loops easily distinguished by form and pattern from other cells. The capillaries of rat cerebellum are characterized by the activity of three enzymes: BuChE, Alk. Phosphatase, and GGTP, which have been implicated in the function of the BBB system in situ. The activity of BuChE is found only in cerebellar capillaries, not in pia arachnoid vessels, though in situ, these are continuous with the brain vessels. Therefore, BuChE rates as a marker for the brain vascular system itself. Vessels of somatic tissues (rib, skin) do not show activity in any of these three enzymes.

Significance to Biomedical Research and the Program of the Institute: The cerebellar and/or lepto-meningeal organotypic cultures are a suitable model for studying the function of the endothelial cells and their role in transport and/or metabolism of the brain in health and disease. Such information is of great importance for the understanding of many neurological disease processes, especially the cerebrovascular disorders.

Proposed Course of the Project: This model will be used for histochemical, biochemical and isotope studies, glucose, amino acids and biogenic amine transport and/or metabolism.

Keyword Descriptors: Organotypic cerebellar, pia arachnoid cultures, BBB capillaries, BBB system, rat cerebellum, BuChE, Alk. Phosphatase, GGTP

Honors and Awards: None

Publications: None

Project No. Z01 NS 02085-02 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Ultrastructural changes in cerebral ischemia of gerbils

Previous Serial Number: Same

Principal Investigator: Erik Westergaard, M.D.

Other Investigators: Maria Spatz, M.D.
Igor Klatzo, M.D.
K. G. Go, M.D.

Cooperating Units: Anatomy Department, University of Copenhagen
Department of Neurosurgery, University of Groningen

Man Years:

Total:	1.75
Professional:	1.00
Other:	.75

Project Description:

Objectives: Cerebral infarctions occur in 30-50% of gerbils subjected to the occlusion of a single common carotid artery (Ito, U., Spatz, M., Walker, Jr., J. T., and Klatzo, I.: Experimental cerebral ischemia in Mongolian gerbils: I. Light microscopic observations. Acta Neuropath. 1975, (in press).

In the animals with cerebral injury, an augmented facilitated transport of glucose analogues was observed after six hours while an increase passive diffusion of glucose occurred after eighteen hours of carotid artery clipping. In gerbils without apparent cerebral damage, only the facilitated transport of glucose analogues was found to be increased irrespective of the duration of carotid artery occlusion. These findings suggested that the observed change and the type of glucose transport may depend on the integrity of the cerebral capillaries (Spatz, M., Go, K. G., and Klatzo, I.: The effect of ischemia on the brain uptake of ^{14}C glucose analogues and ^{14}C sucrose. In Cervos-Navarro, J. (Ed.): Pathology of Cerebral Microcirculation. Berlin, West Germany, Walter de Gruyter & Co., 1974, pp. 361-366). Thus, the present investigation was designed to evaluate the status of the cerebral capillaries by electron microscopy in ischemic gerbils.

Methods Employed: Several groups of gerbils submitted to various periods of left common carotid artery occlusion and one hour release were injected with horseradish peroxidase (10 mg/100 g body weight) as the blood-brain barrier (BBB) tracer. The horseradish peroxidase was allowed to circulate for five minutes and thereafter the brains were perfused with paraformaldehyde. Selective tissue sections of the cerebral hemisphere were processed for light and electron microscopy from animals with and without cerebral symptoms.

Major Findings: An increased capillary vesicular transport was observed in cerebral hemisphere ipsilateral to carotid artery occlusion in symptoms positive animal. In gerbils subjected to 3, 6, and 18 hours carotid artery clipping and one hour clip release, the reaction product of the peroxidase tracer was seen in the increased number of endothelial vesicles and in the capillary endothelial membrane except for the endothelial junctions in all groups. The peroxidase reaction product was also observed in the cerebral parenchyma around the cellular membrane in animals with 6 and 18 hours of cerebral ischemia none of the examined tissues showed damage of the capillary endothelium.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the nature and sequence of vascular and parenchymatous ischemic brain injury is of major importance in the understanding of the cerebrovascular disease process. The exploration of the established animal model system for cerebral ischemia should be helpful and useful in the comprehension and treatment of this disease process in man.

Proposed Course of Project: This investigation has been and will be concerned with the study of ultrastructural changes in the brain during and following different periods of cerebral ischemia in order to delineate the sequential pathological processes occurring in the cerebral vessels and parenchyma.

Keyword Descriptors: Ultrastructural changes, cerebral ischemia, gerbils, horseradish peroxidase, increased capillary vesicular transport

Honors and Awards: None

Publications: None

Project No. Z01 NS 02165-01 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Uptake of ^{3}H glucose analogues in pia arachnoid explants

Previous Serial Number: None

Principal Investigator: Maria Spatz, M.D.

Other Investigators: Krystyna Renkawek, M.D.
Margaret R. Murray, Ph.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	1.8
Professional:	1.2
Other:	0.6

Project Description:

Objectives: Little information has been available about pia arachnoid function, although it has been described to possess "blood-brain barrier properties." It was thought that the isolated pia arachnoid explants could provide a suitable model system to study the uptake of various substances by its cellular elements under normal and pathologic conditions. This investigation was concerned with the uptake of glucose analogues in pia arachnoid explants.

Methods Employed: The pia arachnoid explants were cultivated in the Maximow double coverslip depression-slide assembly (according to the method of Allerand and Murray, 1968). Fourteen-day old explants washed with dextrose-free BSS were used for this study. They were incubated with .05 ml medium composed of either fetal serum with glucose-free BSS or with later solution only containing .5 µc of ^{3}H labeled 3-O-D-glucose or 2-deoxy-D-glucose. For the inhibition studies various concentrations of nonlabeled 3-O-methyl-D-glucose, 2-deoxy-D-glucose, glucose and phlorizin were added to the labeled solution. In addition, in some experiments the labeled glucose analogues were used together with ^{14}C labeled sucrose or inulin for the determination of the extracellular substrate uptake. After the incubation period, the cultures were washed three times with BSS. then they were either weighed or assayed for the protein content (O. H. Lowry Method) and processed for liquid scintillation counting.

Major Findings: The maximum uptake of the labeled 3-O-methyl-D-glucose or 2-deoxy-D-glucose was observed at pH 7.4 - 7.6 of the medium after 60 minutes of incubation. Up to 80% reduction of the labeled substrate uptake occurred when the labeled substances were incubated with unlabeled glucose, glucose analogues and phlorizin. This self and competative inhibition was demonstrated at every pH of medium tested, indicating a saturable uptake of glucose analogues by the pia arachnoid explants. The nonsaturable uptake of glucose analogues (10-20%) was found to be equivalent to the uptake of the labeled sucrose or inulin which are extracellular space markers. All the data suggest that the glucose uptake in pia arachnoid explants take place by facilitated carrier mediated transport.

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 relation to blood and central spinal fluid in the normal and diseased state.

Proposed Course of Project: The continued investigations will be concerned with defining and eliciting the factors responsible for the glucose transport under normal conditions. Therefore, the relationship of the glucose uptake to the duration of the incubation, the kinetic and the influence of electrolytes, metabolites and drugs on the glucose transport will be determined in these cultures. Thereafter, this model will be used to study the glucose transport under pathological conditions. Furthermore, amino acid transport will be investigated too.

Keyword Descriptors: Uptake, glucose analogues, pia arachnoid explants

Honors and Awards: None

Publications: None

Project No. Z01 NS 02166-01 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: ^{3}H 2-deoxy-D-glucose transport in synaptosomes during cerebral ischemia in gerbils

Previous Serial Number: None

Principal Investigator: Maria Spatz, M.D.

Other Investigators: Bogomir B. Mrsulja, M.D.
Branislava J. Mrsulja, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	1.0
Other:	0.2

Project Description:

Objectives: In previous studies we have demonstrated an increased brain uptake of labeled glucose analogues from blood to brain 5 minutes after cerebral recirculation in gerbils subjected to unilateral common artery occlusion for 6 and 18 hours. The augmented brain uptake of glucose analogues was saturable in the former but not in the latter group (Spatz, M., Go, K. G., and Klatzo, I.: The effect of ischemia on the brain uptake of ^{14}C glucose analogues and ^{14}C sucrose. In Cervos-Navarro, J. (Ed.): Pathology of Cerebral Microcirculation, Berlin, Walter de Gruyter & Co., 1974, pp. 361-366). The cerebral transport phenomena may not only depend on the integrity of the capillary endothelial cells, but also on the glia cells and neurons. Since neurons exhibit greater oxidative metabolism than glia (Rose, J. P. R., Applied Neurochemistry, pp. 332, 1968), and nerve terminals may account for a high proportion of the oxidative metabolism in gray matter (Lowry, O. H. et al., J. Biol. Chem. 270: 39, 1954), it was thought to investigate the glucose transport in isolated synaptic nerve endings in ischemic gerbils.

Methods Employed: Several groups of gerbils were subjected to left common carotid artery clipping for various periods of time. The gerbils were killed by decapitation and the synaptosomes of left and right hemispheres were prepared separately by the method of Diamond, I. and Molfay, D.

(J. Neurochem. 19: 1899, 1972). Aliquots of synaptosomal suspensions (.1 ml) were incubated in duplicate for 15 minutes at 37°C in 0.5 ml of pH 7.4 solution containing 264 mM sucrose, 26 mM potassium phosphate, and 140 mM of ^{3}H 2-deoxy-D-glucose. The nonspecific entry of radioactive sugar was measured in synaptosomes in which non-radioactive glucose analogue replaced an equal concentration of sucrose in the incubation solution. The uptake was stopped by ice-cold sucrose and the contents were immediately filtered and rinsed with cold BSS (Diamond, I. and Fishman, R. A., J. Neurochem. 20: 1533, 1973).

Major Findings: The radioactive synaptosomal uptake of 2-deoxy-D-glucose by carrier-mediated transport was affected in both the ischemic and control hemispheres as compared to the synaptosomal uptakes from control animals. However, the ^{3}H 2-deoxy-D-glucose synaptosomal uptake was lower in the ischemic than in control hemisphere. The inhibition of glucose uptake was 45% and 70% in the ischemic, while 30% and 45% in the control hemisphere after 30 and 60 minutes of ischemia, respectively. The 2-deoxy-D-glucose uptake in the synaptosomes from the ischemic hemisphere was 32% lower at 30 minutes and 55% lower at 60 minutes than in the contralateral control hemisphere. The saturable glucose uptake of synaptosomes was almost completely abolished in the ischemic hemisphere of gerbils subjected to 3 hours of common carotid artery occlusion. The synaptosomal transport activity was not recovered by the addition of various metabolites to the synaptosomal suspension.

Significance to Biomedical Research and the Program of the Institute: The basic comprehension of neuronal function in cerebral ischemia is of major importance for: (1) understanding of the pathophysiological process occurring in the cerebrovascular disease, and (2) for selecting the best therapeutic approach to this disease.

Proposed Course of Project: This model system will be used to study the glucose transport during the recovery period after ischemia. In addition, future investigations include the transport of amino acids and biogenic amines in the ischemic synaptosomes.

Keyword Descriptors: 2-deoxy-D-glucose transport, synaptosomes, cerebral ischemia of gerbils

Honors and Awards: None

Publications: None

Project No. Z01 NS 02173-01 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Histochemical distribution of peptidase activity in the central nervous system of different animal species

Previous Serial Number: None

Principal Investigator: Maria Spatz, M.D.

Other Investigators: Branislava J. Mrsulja, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	.75
Professional:	.75
Other:	0.

Project Description:

Objectives: The action of tissue proteolytic enzymes have been implicated to play a role in both normal and abnormal growth processes (Pope, A., J. Neurochem. 4: 31-41, 1959; Glenner, G. G. et al., J. Nat. Cancer Inst. 23: 857-873, 1959). The substrate specificity of whole brain peptidase activity described earlier for a large variety of peptidase substrates indicate that more than one type of peptidase may be present in the brain. During the investigation of the activity of several enzymes in cerebral ischemia of gerbils we have noticed the presence of leucyl-aminopeptidase in normal neurons which to the best of our knowledge, has not been described previously in any animal species. In order to clarify whether this observation represents a unique occurrence in Mongolian gerbils, we decided to investigate this enzyme in brain tissue of several animal species.

Methods Employed: The localization of GGTP (gamma-glutamyltranspeptidase) and leucyl-aminopeptidase activity in the central nervous system were studied in the following, two months old animal species: mouse, hamster, rat, guinea pig and the gerbil. Freshly removed brains were frozen on an aluminum plate placed on dry ice and fixed on the holders. Sections of 20μ in thickness were cut in a cryostat, mounted on non-albuminized cover glass, dried at room temperature for several minutes, and then stored

temporarily in the refrigerator at 4°C. The period between cutting and incubation was no longer than 5 hours for aminopeptidase and 24 hours for cold absolute or cold acetone-fixed sections for gamma-glutamyl-transpeptidase. The following incubation methods were used for GGTP: (1) the N(Y-L-glutamyl)β-naphtylamide method of Glenner et al., (1961); (2) Albert's method using Y-L-glutamyl-2 naphtylamide as substrate (Albert, Z. et al., Nature 191: 767-768, 1961). Incubation was done at room temperature for 2-3 hours. L-leucyl-β-naphtylamide was used as a substrate according to the technique described by Burstone, M. S. et al., J. Histochem. Cytochem. 4: 217-226, 1956. The sections were incubated 120 minutes at 37°C. The pH of incubation medium was 7.2 for all the methods.

Major Findings: No species differences were found in enzymatic activities of both enzymes GGTP and leucyl-aminopeptidase. The only and the most prominent GGTP activity localization was seen in the vessels of both the gray and white matter of the cerebrum, cerebellum and medulla. The endothelium of the capillaries and larger vessels, as well as stroma of the choroid plexus, showed diffuse and granular, dark reddish-brown reaction product of glutamyltranspeptidase activity. The enzymatic activity of GGTP was especially visible in dilated capillaries. The glial cells and neurons did not show any transpeptidase activity. In all investigated animals, histochemically demonstrable activity of the proteolytic enzyme leucine-aminopeptidase was not only confined to vascular walls, leptomeninges and stroma of the choroid plexus (as previously described), but was also found to be present in the neuronal cytoplasm of Ammon's horn and Purkinje cells of cerebellum. In addition, some nuclei (N. vestibularis, N. cochlearis ventralis, N. cochlearis dorsalis, N. N. vagi, N. N. hypoglossi, nucl. n. trigenimi, N. N. Facialis) showed an intense activity of leucine-aminopeptidase within the perikaryon of the nerve cells without any activity in the intervening tissue.

Significance to Biomedical Research and the Program of the Institute: This investigation was concerned with the histochemical localization of leucyl-aminopeptidase in the cell bodies of the brain. The presence of this enzyme in the neuronal cytoplasm was not demonstrated previously.

Proposed Course of the Project: This project is completed.

Keyword Descriptors: Proteolytic enzymes, gamma-glutamyltranspeptidase, leucyl-aminopeptidase, neuronal cytoplasm, Purkinje cells

Honors and Awards: None

Publications: None

Project No. Z01 NS 01443-09 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The intracerebral movement of proteins injected into the blood and cerebrospinal fluid of rodents

During FY 1975 this project was incorporated with Project No. Z01 NS 02144-01 LNNS.

Project No. Z01 NS 01587-08 LNNS

1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June June 30, 1975

Project Title: A blood-brain barrier to peroxidase in the normal and injured brain of elasmobranchs

During FY 1975 this project was incorporated with Project No. Z01 NS 02144-01 LNNS.

Project No. Z01 NS 01678-07 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Distribution of exogenous proteins in brain tumors

During FY 1975 this project was incorporated with Project No. Z01 NS 02144-01 LNNS.

Project No. Z01 NS 01805-07 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Structural changes with the membranes of smooth muscle cells at rest and under tension

Previous Serial Number: Same

Principal Investigator: Lise Prescott, M.D.

Other Investigators: Milton W. Brightman, Ph.D.
Harold Gainer, Ph.D.
Jeffery L. Barker, M.D.

Cooperating Units: Behavioral Biology Branch, NICHD

Man Years:

Total:	0.9
Professional:	0.6
Other:	0.3

Project Description:

Objectives: To examine changes in structure of sarcolemmal caveolae which have hitherto been considered as pinocytotic.

Methods Employed: Ganglia in the mollusk Aplysia, are surrounded by a sheath in which is embedded isolated, separate, smooth muscle cells. The sheaths were either quenched in liquid nitrogen or fixed first before freezing. Bits of sheath were then fractured in vacuo. Other sheaths, stretched three times their normal length were also fixed, frozen, and cleaved. Portions of all sheaths were embedded in plastic and sectioned for electron microscopy including several that had been soaked in HRP for 30 minutes to 6 hours and one specimen in 90 μM $CaCl_2$.

Major Findings: The sarcolemma, like that of vertebrate smooth muscle bears hemi-desmosomes and is indented to form many pits or caveolae. Unlike most other species, the pits have a natural marker in the form of "bumps", about 65-130Å wide, within their membranes. In the stretched connectives, the fractured sarcolemmas display many more clusters of these "bumps" indicating that the caveolar membrane had become flattened and pulled into the fracture plane. The muscle cells were thinner in the stretched preparations and the sarcolemma of some were broken. HRP coated the pits but did not appear to have been pinocytosed.

Significance to Biomedical Research and the Program of the Institute: The numerous pits can be unfolded and thus increase the extensibility of the sarcolemma. The pits do not pinch off and migrate but, instead, might be involved in calcium binding. The intrinsic marker could be useful in studying enzyme content of pit membrane vs. the rest of the sarcolemma in isolated membrane fractions. The hemi-desmosomes on the sarcolemma not only look like attachment plaques but are here shown to be candidates for acting like anchors since the separate muscle cells can be passively stretched when the entire sheath is pulled.

Proposed Course of Project: These observations and conclusions are being written and the calcium-soak experiments are to be repeated.

Keyword Descriptors: smooth muscle cells, caveolae of smooth muscle, freeze-fracture of smooth muscle, relaxed and stretched smooth muscle

Honors and Awards: None

Publications: None

Project No. Z01 NS 01965-04 LNNS

1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The effect of biogenic amines on blood-brain barrier to peroxidase

During FY 1975 this project was incorporated with Project No. Z01 NS 02144-01 LNNS.

Project No. Z01 NS 02086-02 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Regeneration in vertebrate and invertebrate nerves

Previous Serial Number: Same

Principal Investigator: Richard Shivers, Ph.D.

Other Investigators: Milton W. Brightman, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.2
Other:	0.3

Project Description:

Objectives: To examine structural changes inside the membranes of nerves and surrounding glial cells during degeneration and regeneration.

Methods Employed: The nerve roots of the 6th abdominal ganglion in crayfish are so superficial that only a little dissection is required for their exposure. Scarring is, therefore, minimal. Exposed roots were soaked in peroxidase for 1 hour. In other crayfish intact roots and those that has been cut 1 to 5 weeks prior to fixation were frozen and fractured.

Major Findings: In the normal and regenerating nerve roots of crayfish, a hitherto unsuspected system of transglial channels accessible to peroxidase, has been recognized in both thin sections and freeze-fracture replicas. The channels, about 240Å wide, are straight and short, being as long as the thin glial sheets are wide. Then frequency is about 16 per μ^2m normal roots and 13 per μ^2m in regenerating area. In replicas, the channel openings appear as circular depressions or pits and protuberances or bosses.

In regenerating roots, the glial cells become disorganized but still lie close to axons. The gap junctions between glial cells increase in number but diminish in size.

Significance to Biomedical Research and the program of the Institute: The diffusion path from periglial fluid spaces to axonal membrane is shortened by trans-glial channels which could act as shortcuts for the flow of metabolites and ions.

Proposed Course of Project: The work on trans-glial channels is being prepared for publication. The observations on glial junctions are to be extended to longer time periods of regeneration.

Keyword Descriptors: glial channels, regeneration and glia, crayfish glia, freeze-fracture of regenerating nerve roots

Honors and Awards: None

Publications: None

Project No. Z01 NS 02144-01 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effects of hypertension on the permeability of cerebral endothelium to proteins

Previous Serial Number: None and incorporating Project No. Z01 NS 01443-09 LNNS, Project No. Z01 NS 01587-08 LNNS, Project No. Z01 NS 01678-07 LNNS and Project No. Z01 NS 01965-04 LNNS

Principal Investigator: J. S. Robinson, M.D.

Other Investigators: Milton W. Brightman, Ph.D.
Stanley R. Rapoport, M.D.

Cooperating Units: Laboratory of Neurophysiology, NIMH

Man Years:

Total:	1.2
Professional:	1.1
Other:	0.1

Objectives: To see if and how a rise in the intraluminal pressure of cerebral blood vessels affects their permeability to blood-borne protein.

Methods Employed: The baseline arterial pressure is recorded continuously from the femoral artery of adult rats. A solution of Evans blue, then peroxidase (HRP) is injected into a femoral vein followed by 0.5mg/kilo body weight of the vasopressor, Aramine. When the blood pressure (bp) exceeds 60mg Hg above resting level, the brain is fixed and processed for HRP activity. The HRP exudates appear as brown spots which are counted for each of five regions of the grain. In a second group, the blue dye is given first and from 2 minutes to 20 hours after Aramine, HRP is given to test for reversibility of barrier opening. In a third group, a bolus of saline is rapidly infused into one carotid artery.

Major Findings: In 30 rats, the rise in bp during Aramine injections was accompanied by the appearance of randomly scattered blue spots in the cortex and in the parenchyma and many more brown, HRP spot-exudates. The number of spots is greatest in the cerebrum and least in the medulla. The opening of the barrier is reversed within 1 to 2 hours. In 12 rats given the fluid bolus, the number of HRP exudates were far more numerous but their distribution was similar.

Significance to Biomedical Research and the Program of the Institute: A moderate, transient hypertension is accompanied by an escape from cerebral blood vessels of blood-borne substances as large as albumin, HRP and, presumably, smaller substances. Thus, therapeutic or toxic agents, normally excluded from the brain, can enter during such a brief episode.

Proposed Course of Project: To complete the tabulation of the number of exudates in terms of pressure rise and to see whether the protein leaks through opened junctions or, improbably, by increased vesicular transport.

Keyword Descriptors: hypertension and blood-brain barrier, capillary permeability and hypertension, vascular leaks in hypertension

Honors and Awards: None

Publications: None

Project No. Z01 NS 02145-01 LNNS
1. Laboratory of Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Identification of neurons having terminals in the median eminence and area postrema

Previous Serial Number: None

Principal Investigator: Richard D. Broadwell, Ph.D.

Other Investigators: Milton W. Brightman, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: To identify the cells projecting to median eminence and area postrema and to ascertain the manner in which protein-laden vesicles travel within axons.

Methods Employed: Large concentrations of peroxidase (HRP), from 30 to 150 mg (type VI), are given intravenously in divided doses over a 12 to 24 hour period to mice. In some, the area postrema is destroyed electrolytically. Other mice are to be hypophysectomized. In still others, hypoglossal and facial nerves are ligated unilaterally before protein injection. The fixed brains are examined for HRP distribution.

Major Findings: The supraoptic, paraventricular, periventricular and infundibular nuclei as well as a discrete noradrenalin-containing neuron population in the pons, are labeled by retrograde transport of HRP that had leaked around their terminals from fenestrated vessels of the median eminence and neurohypophysis. In the medulla and pons, cranial nerve nuclei such as III, IV, V, VI, VII, X and XII and in the spinal cord ventral horn motorneurons were labeled. Since the nucleus of ns. VII and XII were not labeled on the ligated side, the HRP must have reached their terminals from muscle capillaries known to be permeable to protein. However, the nucleus solitarius may have been labeled from the area postrema if such labeling is absent in the mice whose area postrema had been destroyed.

Significance to Biomedical Research and the Program of the Institute: This is the first demonstration that cranial nerve axons imbibe protein indirectly from the blood. The ensuing retrograde transport thus circumvents the blood-brain barrier to bring protein into cells within the central nervous system. The cells involved in the emetic reflex may be demonstrated by this method.

Proposed Course of Project: To complete these experiments and to determine, electronmicroscopically, the intra-axonal fate of protein taken up by hemo-neural (neurosecretory) endings and myo-neural terminals.

Keyword Descriptors: retrograde transport, neuroendocrine organs, cranial nerve labeling, circumventricular organs

Honors and Awards: None

Publications: Brightman, M.W., Prescott, L. and Reese, T.S.: Intercellular junctions of special ependyma. In Scott, D.E. and Kobayashi, M. (Eds.): Proceedings of the Second International Symposium on Brain-Endocrine Interaction, Switzerland, S. Karger, 1975. In press.

LAB. OF BIOPHYSICS

LAB. OF NEURAL CONTROL

LAB. OF NEUROPHYSIOLOGY

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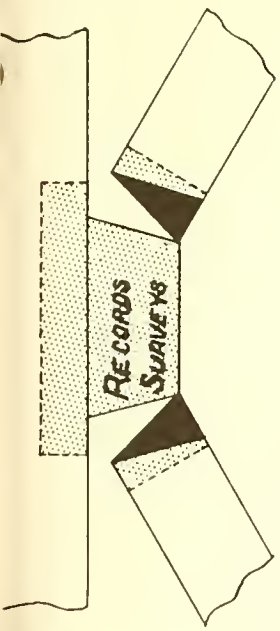

TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1974 through June 30, 1975
Laboratory of Neural Control, Intramural Research
National Institute of Neurological and Communicative Disorders and Stroke

Karl Frank, Ph.D., Chief (Retiring)
Robert E. Burke, M.D., Chief (Acting)

The Laboratory of Neural Control began in 1967. Its objectives have been five-fold:

1. The development of techniques for leading information from the central nervous system outside the body for the purpose of controlling external devices, such as limb prostheses.

2. Development of techniques for delivering patterns in time and space of electrical or other stimuli to the nervous system for controlling it in some desired fashion, such as a visual or auditory prothesis.

3. Basic neurophysiological research to study neuronal mechanisms operating within the nervous system by which it controls input-output relations, e.g., how neural patterns from the brain are interpreted and translated into patterns of excitation and inhibition leading to coordinated movement.

4. The clinical application of basic research findings for aid to the neurologically handicapped.

5. A concern for the social consequences arising from the application of the principles of neural control to human societies.

All the members of the Laboratory of Neural Control have had multidisciplinary training in two or more of the fields of neurophysiology, physics, bioengineering, mathematics and computer techniques.

The total staff of the Laboratory numbers 17. Eleven of these have either permanent Civil Service or permanent Commissioned Corps appointments. Two have limited tenure appointments. There are in addition one Visiting Fellow and three Guest Workers.

This year marks a change in leadership of the Laboratory. Three members are transferring to the Extramural Fundamental Neurosciences Program. The Neural Prosthesis Program will also be formally transferred out of the Laboratory of Neural Control to the new Fundamental Neurosciences Program. However, it is anticipated that close collaboration and communication between present and former staff members of the Laboratory of Neural Control will continue.

Neural Prosthesis Program

This overall program is designed to utilize contracts with outside Institutions in collaboration with members of the intramural staff of NINCDS and is intended to explore the feasibility of combining recent advances in basic neurosciences, natural sciences and microelectronics for the development of prostheses to aid the neurologically handicapped. Contracts with ten outside institutions have yielded progress on a number of fronts.

1. Functional Neuromuscular Stimulation

One of the most impressive successes of the application of implanted microelectrode techniques has been the development of functional neuromuscular stimulation, primarily through the work of a team of scientists at Case-Western Reserve University. Electrical stimulation under voluntary control through implanted electrodes has been able to produce smooth functional contractions of both forearm flexors and extensors in a C-5 quadriplegic patient. Using this system, the patient can grasp and pronate with his otherwise paralyzed hand. In related animal studies further evidence has been provided that muscle fibers can be converted from rapidly fatiguing to fatigue-resistant type by a systematic program of muscle activation using electrical stimulation through implanted electrodes.

2. Bladder Control in Paraplegics

One of the greatest hazards for the paraplegic is urinary tract infection due to incomplete bladder evacuation. Implantation of stimulators in the spinal cord has shown initial successes in a number of such patients for improved evacuation of the bladder. Ideally, the detrusor muscle of the bladder wall should be stimulated to contract at the same time the external sphincter is caused to relax. Recent studies through two contracts indicate, however, that the neurons controlling these two muscles are mixed in the same population within the spinal cord. This greatly reduces the possibility of developing a practical bladder evacuation prosthesis based on electrical stimulation of the spinal cord.

3. Neural Prosthesis for the Blind

One of the most crucial factors in determining the feasibility of a sensory prosthesis for the blind through direct electrical stimulation of the visual cortex is the rate of information transfer into the brain. This rate is determined not only by the spatial resolution of electrodes whose stimulation can be discriminated by the patient but also the rapidity with which a given electrode can be stimulated again. In a recent human striate cortex stimulation case, the relationship between stimulation pulse train duration and threshold for phosphene production was found to be independent of train length for durations greater than 200 msec.

In other experiments on cats it has been shown that direct electrical stimulation will produce after-discharges in normal cortical cells. The

use of Dilantin increases the electrical current thresholds for producing such after-discharges.

Electrodes can be roughened to increase their effective surface area without increasing their size. An in vitro technique for measuring this real surface area of stimulating electrodes has been extended and successfully tested in vivo.

4. General Neural Prosthesis

One of the most serious problems facing the practical development of a neural prosthesis is the establishment of parameters for stimulation which maximize the safety factor between stimulation and the production of neural damage. An extensive histopathological study of brain tissue damage after essentially continuous electrical stimulation of the cerebral cortex for 36 hours, has just been completed. The results indicate significant neural damage was found for stimulation levels above 0.45 microcoulombs per pulse, a level close to that required for stimulation of cells near the electrode.

Low temperature isotropic carbon, once suggested as a possible electrode material, has been shown to be an unsuitable candidate for this purpose. Severe corrosion appeared in in vitro stimulation testing and the corrosion products were found to be toxic to the nervous system in in vivo tests. However, other electrode materials research has shown that antimony doped stannous oxide has extremely good corrosion resistance.

Platinum, one of the most practical electrode materials in use so far, has been tested for "gassing limits" in simulated cerebral spinal fluid solution and these are found to be greater than 300 microcoulombs per real square centimeter. This indicates that gas evolution on electrode tissue interfaces will not be a problem at least with the types of electrodes currently under test for stimulation of the cortical surface.

Connections with the Nervous System

All of the research activities of the Laboratory of Neural Control involve either inward or outward information transfer through electrodes in contact with nervous tissue. A number of these activities, some of them in collaboration with the contractors of the Neural Prosthesis Program, are reported together under one Annual Report project "Techniques for making Connections with the Nervous System." Studies continue in the Laboratory of new materials with regard to their physical properties and biocompatibility. Both insulators and conductors are candidates for study and include tantalum, Parylene-C, iridium, tungsten, Teflon and silastic rubbers.

New techniques have been devised for removing the insulation from the tips of the highly successful new "map pin" electrode arrays implanted in monkeys and cats. These studies continue to supply data about the feasibility of chronic single cell recording for time periods of months or even years.

A new "hair brush" electrode array with tip separations under 100 microns is being developed and tested in animals. Histological evaluation of neural damage resulting from long-term implantation of such arrays is proceeding.

The new microelectrode introducer for tracking single cells in the pulsing human brain has been evaluated in three humans undergoing craniotomies for removal of epileptogenic foci. Single unit activity in such a moving brain can be followed indefinitely with the air-bearing device. The device is now being tested in cats and monkeys to investigate the possibility of intracellular recordings.

A split tube array for recording from single axons in dorsal or ventral root filaments and from small peripheral nerves continues to show promise as a technique permitting recording from single peripheral neural elements during normal motor behavior.

Another type of microelectrode has been developed for recording from chronic single units in the dorsal root ganglion.

Further progress has been made on the development of an implantable, remotely-controlled, fixed-dose injector which will release a 5-50 microliter dose of any drug into a distant tissue space.

A 16-channel telemetry system for human EEG has been custom designed and fabricated under contract. The system is being tested and will be used to study EEG patterns before, during and after epileptic seizures in ambulatory patients.

A single-unit amplifier and remote-controlled electrode impedance detector has been designed in a small probe arrangement that can be gas sterilized. This system is particularly useful in evaluating electrodes during human neurosurgical procedures.

Voluntary Mechanisms of Motor Control

This project attempts to determine whether it is possible to extract enough information from the spike trains of small sets of cortical neurons to be able to predict the time course and amplitude of various motor response measurements. Numbers of different types of cell responses have been described for different neurons in the motor cortex. Since the animal has under voluntary control the movements associated with firing patterns of cortical neurons, he has also voluntary control over the firings of these cells directly. The degree of control possible over the firing rate of specific neurons is of paramount importance in predicting the success of a centrally controlled prosthesis.

Monkeys were tested for their ability to modify the firing rate of single cortical cells to obtain a juice reward. It was found that of the cells whose firing patterns could be modified (80%), those most easily affected were usually associated with a specific arm movement. Only

rarely could firing rates be modified, if they were not associated with limb movements.

Tests with the "map pin" electrodes show that a particular cortical cell can be followed for up to 25 days; however, relative movement occurs between the cell and the electrode that limits the time the cell can be observed. The source of this relative motion is still not understood. Emphasis on this project will continue the search for better methods for long-term chronic recording from single cells and will explore the degree to which two cells can simultaneously control two degrees of freedom of an external device. A related project, just beginning, will study the ability of humans to control the firing pattern of single motor units in order to obtain a better understanding of the organization of motor neuron pools.

Dorsal Root Ganglion Studies

Methods have been developed for chronic recording of single cell activity in the dorsal root ganglia of cat spinal cord. By using this technique, it has been possible to explore the frequency with which atypical sensory and motor fibers can be found in the ventral and dorsal roots respectively. Four out of 186 cells studied were found to send processes into the spinal cord via ventral rather than dorsal roots and one sent processes in via both roots. Whether this small percentage of abnormal afferents represents developmental accidents or whether they subserve some special role, remains to be determined.

Neuron Activity during Locomotion

One of the broad objectives of all parts of the Laboratory of Neural Control has been the study of neuronal activity during movement, particularly that associated with locomotion. Many of the techniques described bear directly on progress with this objective. It is important to determine the roles of spinal neurons in controlling locomotion, to develop preparations which utilize electrode arrays for monitoring and stimulating neurons of the unrestrained animal and to develop techniques and concepts for dealing with patterns of simultaneous neuron activity. It has been shown possible to monitor a moderate population (1 to 10 units) in a dorsal rootlet (1-150 μm) or small peripheral nerve (250 μm) for a period of several weeks. Lead wire breakage has limited recording from single dorsal root fibers to less than 30 days. In peripheral nerve preparations, there has been a steady loss of population after implantation, the larger spikes dropping out first within six days to three weeks -- probably the result of continual stressing of the nerve during movement; however, it has been possible to record a fluctuating spontaneous activity in muscle afferents in unanesthetized animals whichis influenced by arousal as well as in phase with the stepping cycle.

Models of Neural Interaction

In parallel with the experimental and developmental research of

the Laboratory, theoretical studies are being conducted to explore the properties of a neural model capable of detecting special features of the information it receives. One reason for seeking such a model is the observation that the cells of the mammalian visual cortex appear to detect independently varying features of the patterns of activity coming from the retina. It is suggested that the neural connections responsible for accomplishing independent feature recognition are not entirely determined genetically, but that during development the synaptic strengths are influenced by the afferent activity in such a way that correlated neuron firing is selected against. Initial computer analysis of the model looks promising.

Motor Systems in the Spinal Cord

Over the past several years, this project has concentrated on examination of interrelations between the characteristics of alpha motoneurons and the muscle fibers which they innervate. Systematic study of the entire motor unit (i.e., both motoneuron and muscle unit portions simultaneously) has led to a clearer picture of motor unit organization in large limb muscles than was previously available, especially in that it has also included analysis of the organization of synaptic input from a variety of input systems to defined types of motor units. The test system chosen has been, and remains, the motor unit population of the gastrocnemius muscle in the cat hindlimb, which for many reasons seems a good model of large limb muscles generally.

Using information developed to date about the unit population in normal animals, it is now possible to study the effects of various conditioning treatments on particular types of units in adult animals. For example, the effects of compensatory hypertrophy and of immobilization atrophy have been examined as they affect synaptic organization, intrinsic motoneuron properties and the mechanical, histochemical and morphological characteristics of muscle units. It seems likely that this experimental approach may well prove fruitful in the further elucidation of unresolved questions about the "trophic" interaction between motoneurons and their muscle units. This aspect of the ongoing work will continue as a new project, directed mainly at further elucidation of the range and malleability of motor unit properties with variations of "usage" and functional demand.

In our attempts to study central nervous system mechanisms in control of movement, we have focused recently on the organization of synaptic input from both primary afferent and supraspinal systems onto motor units of defined type. There is considerable evidence that the range of unit properties observed in mixed unit populations such as cat gastrocnemius reflects functional specialization of particular unit types for particular physiological roles. The available quantitative information on synaptic organization in relatively direct projection systems to motoneurons appears to make good sense in this regard.

We are now faced with the problem of analyzing input systems which

project to motoneurons through series of segmental interneurons, which represents an area of major concern to further progress in elucidation of CNS control of movement. We intend to pursue this inquiry by combining techniques of neuroanatomy, using marker materials for pathway tracing and marking of individual neurons, with conventional and novel methods for electrophysiological analysis of single neuron connectivity and functional behavior. Although technically difficult, this research strategy appears to have great promise.

General Statement Concerning Future Goals of the Laboratory

It is anticipated that this year's change in the Chief of the Laboratory of Neural Control will mean a change in emphasis among the various goals outlined at the start of this Summary. LNLC will no longer formally encompass those projects designed to facilitate immediate application of prosthetic devices to human subjects. The formal emphasis in the future will be on a broad-based attack on the problem of CNS control of movement. However, close communication and interchange between the present LNLC staff and its former members concerned with application of research advances will and should continue, as such interchange is obviously beneficial to all concerned.

It seems important to note here that a very large gap exists in our present knowledge of how components of the CNS motor system operate during movement in intact animals. Satisfactory methods for systematic analysis of this problem are in rather early stages of development and the staff of LNLC have been leaders in this field. The presence of facilities and particularly of scientists with the vision, ability and interest to develop novel and sophisticated new techniques and to apply (and evolve) them in application to important biological questions, all within one small group, is quite rare and represents a tribute to long-range vision and planning by the Laboratory's original Chief. This strength should be preserved and every effort will be made to do so. The Laboratory will remain committed to the study of neural control in mammals using control of movement as the basic process of interest, and it will continue to employ a broad range of techniques, mainly but not exclusively based on electrophysiology and neuroanatomy.

Project No. Z01 NS 01686 07 LNLC
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Motor control systems in the spinal cord

Previous Project Number: Same

Principal Investigator: Robert E. Burke, M.D.

Other Investigators: William Z. Rymer, M.D., Ph.D.
John V. Walsh, M.D.
Kenro Kanda, M.D.
*Peter Strick, Ph.D.

Cooperating Unit: *Laboratory of Neurophysiology, NIMH

Man Years:

Total:	1.2
Professional:	0.8
Other:	0.4

Project Description:

Objective: This project is designed to provide information on the organization of those neuronal systems in the spinal cord of mammals which ultimately control the discharge of alpha motoneurons, and on the interaction of primary afferent and supraspinal descending systems in the control of information flow in the segmental motor mechanisms.

Methods Employed: In the past year, this project has utilized two experimental approaches: (a) acute experiments on anesthetized cats with intracellular microelectrode recording and stimulation techniques; and (b) anatomical identification of neuronal pathways using a recently developed technique using axonal transport of protein labels, specifically, horseradish peroxidase. In the first type of experiment, intracellular potentials or the discharge patterns of alpha motoneurons are recorded while stimulating various peripheral afferent receptors either electrically or with natural stimuli. Supraspinal structures or their descending axonal projections are stimulated with electrical pulses delivered through carefully placed electrodes. In the anatomical experiments, purified horseradish peroxidase protein is deposited in precisely localized motoneuron nuclei using antidromic field potentials recorded through the injection pipette for orientation. Enzyme

deposition is done in anesthetized cats under sterile conditions and after approximately 24 hours' survival, the animals are perfused with fixative under deep anesthesia and the spinal cord removed for frozen section histology.

Major Findings:

A. Organization of synaptic input to motor units of defined type.

One of the several input systems which excite alpha motoneurons of the ankle extensor gastrocnemius in the cat is a polysynaptic pathway activated by cutaneous afferents of low electrical threshold, originating particularly from receptors in the skin of the ankle and foot. Of particular interest is the fact that this system produces stronger excitation of fast twitch motor units (both FF and FR units with previously described properties) than is observed in most slow twitch (type S) unit motoneurons. In the past year, preliminary experiments have been performed to assess the possibility that the above interneuronal pathway receives convergent input from supraspinal descending systems. Contrary to initial expectation, there appears to be no convergence from rubrospinal or corticospinal descending axons, at least in anesthetized animals. Convergence from vestibulospinal axons onto the excitatory cutaneous pathway appears possible but has not yet been established with assurance. Further progress with this aspect of the work requires planned acquisition of a signal averaging instrument.

Another approach to the study of the interaction of input systems in spinal segmental interneurons is the use of the tonic vibration reflex (TVR) to assess the excitability of the gastrocnemius motor unit pool under conditions such as the decerebrate state in which intracellular recording is technically difficult. Marked excitation of gastrocnemius motor units by large cutaneous afferents is found when the TVR is conditioned by brief tetani of low intensity to skin nerves from the ipsilateral foot and ankle. Complex effects are produced by further conditioning with input from supraspinal structures, but these are difficult to interpret and the TVR results are viewed at present as preliminary to investigation using intracellular recording techniques.

B. Anatomical identification of last-order interneurons.

Over the past several years, a quantitative analysis of synaptic input to identified gastrocnemius motor units has been accomplished in this laboratory for those input systems projecting directly, or via a single interposed interneuron, to the motoneurons in question. A similar analysis of input systems projecting through longer chains of interneurons is not currently possible because of lack of information about the interneuronal chains themselves. As a first attempt to deal with this difficult question, we have begun to study the size, shape and anatomical position of interneurons presumed to project directly to gastrocnemius motoneurons. This

can be accomplished in first approximation by injecting a protein tracer, purified type VI horseradish peroxidase, into the gastrocnemius motoneuron nucleus and later examining those spinal cord neurons which exhibit uptake of the label in suitable histological preparations. While this does not definitively identify the last-order interneurons, the results so obtained do provide maps of neuron concentrations which are sufficiently well localized so that later experiments with multiple microelectrodes can be done on those spinal cord regions known to contain a high density of candidate last-order cells.

This work is being done in collaboration with Dr. Peter Strick of the Laboratory of Neurophysiology, NIMH. Tissue processing is done in that laboratory and analysis of results is done collaboratively.

C. The central effects of secondary muscle spindle afferents.

This year saw the conclusion of a study of the mechanism by which secondary spindle afferents produce excitation in extensor alpha motoneurons of decerebrate animals. Although still regarded as controversial by some workers, such excitation appears clear in results from this laboratory. It now appears likely that at least one mechanism contributing to the effect is a presynaptic disinhibition of excitatory synaptic transmission from spindle primary afferents to gastrocnemius motoneurons, produced by impulses arriving at the spinal cord over spindle secondary afferents.

Significance to Biomedical Research and the Program of the Institute:

With few exceptions, active movement of mammals in space is accomplished by motor units with motoneurons located in the spinal cord. Analysis of the central nervous system control of movement requires detailed understanding of the organization and interaction of input systems to the spinal cord segments, both from peripheral afferent sources and from supraspinal structures. There is now considerable evidence for the existence of functional specializations among the muscle fibers of different motor unit types, indicating rather precise patterns of motor unit "usage" during movements of various sorts. The long-range goal of the present project is to analyze the patterns of neuronal organization present in the spinal cord as they relate to motor unit type in order to further our understanding of how motor units, and therefore movements, are controlled. Such studies are of clear relevance to analyses of both normal and abnormal movement patterns in man and bear importantly on the interpretation of results of clinical neurophysiological investigations in human subjects.

Proposed Course of Project:

Analysis of interactions among segmental interneuron systems will continue, primarily using intracellular recording techniques to assess synaptic events in alpha motoneurons belonging to type-identified motor units. Emphasis will be placed in two areas: 1.) examination of the

effect of conditioning transmission in selected polysynaptic pathways (primarily cutaneous) by inputs arriving over descending axons originating in medullary and mesencephalic structures; and 2.) recording the behavior of interneurons demonstrated to be last-order to motoneurons using two microelectrodes, one intracellular in identified motoneurons. Anatomical tracing of interneuronal connections will continue as a necessary adjunct to (2.).

Keyword Descriptors:

Motoneurons
Motor units
Interneurons
Spinal cord

Honors, Awards and other Scientific Recognition:

In July 1974, Dr. Burke joined the Editorial Board of the Journal of Neurophysiology at the invitation of the Publications Committee of the American Physiological Society.

Dr. Burke is currently serving as President of the Potomac Chapter of the Society for Neuroscience and as a member of the Council and President-elect of the Assembly of Scientists, NINCDS, NIMH, NEI.

Publications:

Bergmans, J., Burke, R.E., Fedina, L. and Lundberg, A.: The effect of DOPA on the spinal cord. 8. Presynaptic and "remote" inhibition of transmission from Ia afferents to alpha motoneurones. Acta Physiol. Scand. 90: 618-639, 1974.

Rudomin, P., Nunez, R., Madrid, J. and Burke, R.E.: Primary afferent hyperpolarization and presynaptic facilitation of Ia afferent terminals induced by large cutaneous fibers. J. Neurophysiol. 37: 413-429, 1974.

Rudomin, P., Burke, R.E., Nunez, R., Madrid, J. and Dutton, H.: Control by presynaptic correlation: A mechanism affecting information transmission from Ia fibers to motoneurons. J. Neurophysiol. 38: 267-284, 1975.

Project No. Z01 NS 01687 07 LNLC
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Techniques for Making Connections with the Nervous System

Previous Project Number: Same

Principal Investigator: F. T. Hambrecht, M.D.

Other Investigators: Martin Bak
Karl Frank, Ph.D.
*Seth Goldstein, Ph.D.
**J. A. Hoffer
George Laird
Gerald Loeb, M.D.
William Marks, Ph.D.
W. Zev Rymer, M.D., Ph.D.
*** Michael Salcman, M.D.
Edward Schmidt, Ph.D.
**** John Van Buren, M.D.

Cooperating Units: *Division of Research Services - Biomedical Engineering and Instrumentation Branch - Project No. Z01 RS 00012-04 BEI

**Johns Hopkins University
Baltimore, Maryland

***Columbia Presbyterian Medical Center
New York, New York

****Surgical Neurology Branch, NINCDS
Project No. Z01 NS 00100 21 SN

Man Years:

Total:	5.0
Professional:	3.4
Other:	1.6

Project Description:

Objectives: Successful applications of neural control require the development of techniques for making connections to the nervous system.

These include both acute and chronic techniques for recording, stimulating and inhibiting individual nerve cells or small groups of cells of similar function.

Methods Employed:

A. Materials suited for biological implantation.

1. The corrosion resistance during electrical current passage into saline of Hercules graphite fiber and Technical Wire Products carbon-filled silicone rubber was evaluated.

2. Pyra-ML insulated platinum was substituted for gold as a flexible lead-out material.

3. The evaluation of the physical properties and biocompatibility of tantalum, Parylene-C, iridium, tungsten, Teflon and certain medical-grade silastic rubbers has continued.

B. Designs for a chronic recording intracortical microelectrode.

1. The tip insulation of the "map pin" electrode is now removed by a d.c. voltage arc and the gluing of the electrode to the pia-arachnoid with cyanoacrylate has been eliminated. This type of electrode has been implanted in three monkeys and four cats during the past year.

2. A "hair brush" electrode array consisting of three or more microelectrodes with tip separations within 100 microns has been designed, fabricated and implanted in animals.

3. Histological evaluation of neural tissue after implantation of these electrodes is being performed.

C. Microelectrode introducer capable of tracking the moving brain.

Development of this device for use in obtaining single unit recordings from the pulsating brain during neurosurgical procedures has continued. The device has been evaluated in three humans before removal of epileptogenic foci. A cortical surface electrode was added so that correlations between the ECoG and the single unit activity in the region of the focus can be made. Modifications to permit intracellular recordings from neurons are being tested in cats and monkeys.

D. Capacitor stimulating electrode.

Chronic testing in vitro and in vivo has continued by contractors in the Neural Prosthesis Program.

E. Electrode arrays for chronic recording from nerve fibers.

Separate split tubes have been developed for recording activity from dorsal root filaments and the tenuissimus nerve in the rabbit. Each tube has one or two recording contacts at mid-tube and indifferent electrodes at the ends. A larger insulated cuff of silastic containing gold foil has been placed around the recording cuff as a Faraday cage to minimize EMG artifacts. The lead out wires have been changed from 1 mil gold to 1 mil coiled stainless steel to reduce breakage.

F. Chronic single unit recording from dorsal root ganglia.

The current microelectrode design consists of individual 50-75 micron platinum-iridium alloy Teflon-insulated wires which are electrolytically sharpened and coated with 3 microns of Parylene-C insulation. The insulation in the tips is removed by a d.c. voltage arc. The electrodes are anchored to the connective tissue of the ganglion with a cyanoacrylate adhesive. Animal testing has been initiated.

G. Forced aliquot release technique.

An implantable device for chronic intermittent administration of precise amounts of drugs or other chemicals is being developed. Work has been concentrated on the valve for releasing and blocking flow from the pressurized reservoir, on the leak barrier, and on the flow detector. Present designs for the valve include an ultra-thin walled Parylene tube which rests in a drop of magnetic fluid between the poles of a special electromagnet. When this electromagnet is energized with an initial current pulse, it becomes magnetized and holds the magnetic fluid under pressure between its poles, occluding the collapsible Parylene tube. A current pulse in the opposite direction demagnetizes the electromagnet which releases the magnetic fluid pressure and permits flow through the tube. The leak barrier consists of a pair of short parallel segments of exposed platinum wire along the inside walls of a Teflon delivery catheter a few centimeters proximal to the catheter outlet. When a d.c. current is passed through the electrodes, a gas bubble (whose size is regulated by the current and its application time) is generated. This bubble occludes the catheter. The flow detector consists of a pair of toroidal-shaped platinum electrodes within the catheter about a millimeter from the outlet. The increase in electrical impedance between the electrodes when the bubble moves by is sensed. In normal operation the valve is opened and the bubble is carried by the column of drug solution to the detector electrodes. The increased impedance that is detected is used to initiate valve closure. The bubble then remains at this point near the catheter outlet acting as a barrier to diffusion leakage.

H. 16-channel EEG telemetry system.

A custom-designed 16-channel telemetry system for human EEG was fabricated on contract. This system has a frequency response of 0.5 Hz to 100 Hz, a dynamic range of 5-500 microvolts and a range of 300 feet. The radio transmitter, differential preamplifiers, multiplexer and batteries are contained in a small package that is attached to the patient's head by a special head holder. The system will be used to study epileptic patients, including patients receiving cerebellar stimulation.

I. Single unit amplifier and remote-controlled electrode impedance detector.

This instrument consists of a high input impedance amplifier and an electrode impedance testing circuit in a small probe that can be gas sterilized. The output of this amplifier is optically isolated from other instrumentation and the impedance testing circuit is isolated and remotely controlled by relays. This system permits safe recording and electrode evaluation in humans during neurosurgical procedures.

Major Findings:

Use of a d.c. voltage arc to remove Parylene insulation from microelectrode tips permits a clean, reproducible recording surface. The degradation and poor long-term stability of Parylene associated with previous heat removal techniques appear to have been eliminated.

Both the Hercules graphite fibers and the carbon-filled silicone rubber corroded excessively during current passage, making these materials unsuitable candidates for chronic stimulating electrodes.

Using the microelectrode introducer, cortical single unit recordings have been obtained in the region of epileptic foci in two patients undergoing surgery for removal of these foci. The recordings were stable despite brain pulsations exceeding 1 millimeter.

Studies by contractors in the Neural Prosthesis Program have confirmed that the capacitor electrode passes current without oxidation-reduction reactions.

Single unit recordings from dorsal root fibers and peripheral nerve fibers have been monitored up to 30 days using tube electrodes in unrestrained awake animals.

Bench testing of the forced aliquot release technique with a tritiated amino acid solution has demonstrated a dose regularity within 1% and negligible leakage rates.

Significance to Biomedical Research and the Program of the Institute:

The successful development of techniques for recording signals from the nervous system and means of selectively controlling the activity of small populations of neurons is mandatory for the success of the Laboratory's objectives. Such developments are also of value to the neurophysiological research of other laboratories, including those working on prostheses for the neurologically handicapped.

Proposed Course of Project:

An animal kinesiology facility is being established in the Laboratory and will include a treadmill and video tape recording equipment. The recording techniques in this project will be used in awake animals in this facility to study the relationships between neural activity and movement. The microelectrode introducer will be used to obtain a better understanding of human epileptic foci on a neuronal basis. The telemetry system will provide EEG recordings from unrestrained epileptic patients 24 hours a day. Some of the projects discussed need further development and work will continue on them. Further cooperation with contractors in the Neural Prosthesis Program is anticipated.

Keyword Descriptors:

Chronic single unit recording
Microelectrode
Capacitor electrode
EEG telemetry

Honors and Awards:

Patent No. 3,826,244, "Thumbtack Microelectrode and Method of Making Same," awarded to M. Salcman and M. Bak.

Publications:

Guyton, D.L. and Hambrecht, F.T.: Theory and design of capacitor electrodes for chronic stimulation. Med. Biol. Eng. 12: 613-620, 1974.

Salcman, M. and Bak. M.J.: Evaluation of a new chronic recording intracortical microelectrode. Fed. Proc. 33: 331, 1974.

Salcman, M. and Bak, M.J.: A new chronic recording intracortical microelectrode. Med. Biol. Eng. In press.

Goldstein, S.R., Schmidt, E.M., Bierley, F.L. and Bak, M.: Atraumatic electrical recording from the exposed pulsating human cerebral cortex - a new mechanism. In Brighton, S., Goldstein, S. (Ed.): Advances in Bioengineering. New York, The American Society of Mechanical Engineering, 1974, pp. 52-54.

Goldstein, S.R., Schmidt, E.M., Bierley, F.L. and Bak, M.J.: A gas bearing mechanism for stable electrical recording from individual neurons in pulsating human cerebral cortex. Trans. ASME J. Dynamic Systems, Measurement and Control. In press. 1975, 97, Series G, #3.

Project No. Z01 NS 01688 07 LNLC
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cortical Mechanisms of Voluntary Motor Control

Previous Project Number: Same

Principal Investigator: Edward M. Schmidt, Ph.D.

Other Investigators: Martin Bak
George Dold
Karl Frank, Ph.D.
Joan McIntosh
J. Stevenson Thomas, Ph.D.
N. Mutsuga, M.D.

Cooperating Units: Surgical Neurology, NINCDS

Man Years:

Total:	3.3
Professional:	1.4
Other:	1.9

Project Description:

Objectives: The major goal of this project is to study the feasibility of using cortical neurons for the control of external devices such as a prosthesis. This involves the study of relationships between the firing patterns of cortical neurons and the measurable parameters associated with simple movements. Our aim is to determine whether it is possible to extract enough information from the spike trains of small sets of cortical neurons to be able to predict the time course and amplitude of various motor response measurements. This requires a careful study of the types of cells to be found in the motor cortex, their frequency of occurrence, location and specific function. At the same time, we are anxious to determine how much control an individual has over the firing patterns of these cortical cells. If an individual can influence the firing rate of specific neurons, the chances for building a centrally controlled prosthesis are markedly improved.

Methods Employed: Monkeys were initially trained to move a handle

to obtain a liquid reward. After implantation of up to a dozen "map pin" chronic microelectrodes in the motor cortex, the animals were allowed to make free arm movements and were given liquid rewards dependent on the firing patterns of individual cells.

Major Findings:

1. Monkeys were required to modify the firing rate of single cortical cells to obtain a juice reward. During approximately one half of the recording sessions, the animal could significantly alter the firing rate of the cell. The cells whose rate could easily be modified were usually associated with a specific arm movement. Only rarely could firing rates be modified if they were not associated with limb movement.

2. Recordings from the same cortical cell for up to 25 days have been obtained with the chronic "map pin" electrodes. However, relative movement occurs between the cell and electrode that limits the time the same cell can be observed. This relative movement was still occurring four months after electrode implantation. The source of this motion as yet is unknown and remains a topic for future investigations.

3. Previously we had found that our insulating material for the microelectrode, Parylene-C, was degrading after several weeks of implantation. By exposing the electrode tip through an arcing procedure electrode impedances have stayed relatively constant for four months.

4. Chronic "map pin" microelectrodes have also been implanted in the vicinity of an area in precentral motor cortex that had previously been rendered epileptogenic with alumina cream. With these electrodes it has been possible to record extracellularly from the same cortical neuron prior to, during, and after a clinical seizure. The ability to record long periods from a single neuron may help to elucidate the behavior of epileptic neurons.

5. An instrument for separating the activity of multi-unit neuroelectric activity into the activity of a single cell has been developed. The instrument is much simpler and easier to use than ones we previously employed. In addition, the instrument incorporates a semiconductor delay line that makes it possible to display only spikes that meet the criteria of a single cell.

6. Computer software has been developed for the PDP-12 computer to allow BASIC to utilize the "real-time" capabilities of system. The use of a high level language such as BASIC for computer control of experiments and data analysis minimizes computer programming time and provides for more flexible experiments.

Significance to Biomedical Research and the Program of the Institute: Through our studies we are obtaining a better understanding of the function of the motor cortex and the types of control signals we can obtain. We are quite optimistic that long-term chronic recordings can be obtained from the motor cortex and these signals used to control the stimulation of paralyzed muscles or control a prosthetic device.

Proposed Course of Project: Work will continue in the area of obtaining long-term chronic recordings from cells of the motor cortex. The degree to which the firing patterns of cells can be controlled will be examined in refined tasks to determine if proportional control of an external system is possible with single cortical cells. The degree to which two cells can simultaneously control two degrees of freedom of an external device will be of major concern in these investigations.

Further studies will be conducted on the cause of what appears to be movement between the cell and electrode trip. The duration that recordings may be obtained from a single cell will greatly influence the type data processing that will be required to use the signals for control of a prosthetic device.

Keyword Descriptors:

Chronic recording
Conditioning
Single Cell
Motor cortex
Computer programs
Epilepsy
Spike discriminator
Spike delay

Honors and Awards: None

Publications:

Schmidt, E.M., Jost, R.G. and Davis, K.K.: Cortical cell discharge patterns in anticipation of a trained movement. Brain Res. 75: 309-311, 1974.

Schmidt, E.M., Jost, R.G. and Davis, K.K.: Reexamination of the force relationship of cortical cell discharge patterns with conditioned wrist movements. Brain Res. 83: 213-223, 1975.

Schmidt, E.M.: PDP-12 Functions for OS/8 BASIC, DECUS Program Library, March 1975.

Project No. Z01 NS 02015 03 LNLC
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Neural Prosthesis Program

Previous Project Number: Same

Project Officers: Frederick T. Hambrecht, M.D.
Karl Frank, Ph.D.

Other Investigator: Herbert C. Lansdell, Ph.D.

Cooperating Unit: C&FR, NINCDS

Man Years:

Total:	2.6
Professional:	1.8
Other:	0.8

Project Description:

Objectives: An attempt is being made to combine recent advances in basic neurosciences, natural sciences and microelectronics for the development of prostheses for the neurologically handicapped. In particular, the feasibility of a sensory prosthesis for the blind utilizing direct electrical stimulation of the visual cortex, a bladder prosthesis for patients with neurogenic bladders utilizing electrical stimulation of the sacral spinal cord, functional neuromuscular stimulation with intramuscular electrodes in patients with upper motor neuron lesions and evaluation of the effects of chronic cerebellar stimulation for relief of medically intractable epilepsy are being investigated.

Methods Employed: The feasibility studies are being carried out primarily through contracts with 10 institutions. These contracts are budgeted and administered mainly through C&FR. Project officers and intramural collaboration are within LNLC-IR.

Functional Neuromuscular Stimulation: Case Western Reserve University has implanted intramuscular electrodes in paralyzed patients and is studying the degree of proportional control that can be achieved. Sequential stimulation through multiple electrodes is utilized to provide smooth muscle contractions and to reduce fatigue. They are also studying the effects of

electrical stimulation on the physiological, histological, and histochemical properties of skeletal muscles in both normal and spinal animals. Preliminary studies in animals have been initiated to determine the feasibility of stimulating the paraspinal muscles to correct idiopathic scoliosis.

Bladder Evacuation: Both Duke University and the University of California at San Francisco are stimulating the spinal cords of both normal and spinal animals to determine if cell populations exist in the cord that can be selectively stimulated to cause bladder detrusor contraction with simultaneous sphincter relaxation.

Sensory Prosthesis: The University of Rochester, using sighted and blinded rhesus monkeys, is studying methods of optimizing information input to the visual cortex, survival of electrical excitability during long-term stimulation, and the effects of blindness on these factors.

The Massachusetts General Hospital is studying the mechanism and extent of neuronal activation by electrical stimulation of the striate cortex. They are also studying the effects of changes in electrode parameters and electrode size by stimulating visual cortex of human subjects who require occipital craniotomies.

Cerebellar Studies: The University of California, Los Angeles is producing epileptic foci in the hippocampus of monkeys with alumina cream. They are studying the effects of cerebellar surface stimulation on the seizure behavior of the animals and the neuronal firing patterns of the abnormally discharging neurons.

The University of Minnesota is trying to determine the neurophysiological mechanism of seizure alteration by cerebellar surface stimulation. During stimulation in epileptic monkeys, they record neural discharges from the deep cerebellar nuclei and brain stem nuclei.

Basic Studies: Environmental Impact Center is determining the electrochemical reactions at the electrode-electrolyte junction during the passage of current and methods of identifying and minimizing possible toxic by-products.

Major Findings: Functional contractions of both forearm flexors and extensors in a C-5 quadriplegic patient have been demonstrated. Using a 6-electrode sequential electrical stimulation system under voluntary control, the patient is now using the system to provide grasp and pronation on a continuous basis. Also, in animal studies, further evidence has been provided that muscle fiber conversion to fatigue resistant types is possible with a program of muscle exercise using electrical stimulation.

Results from neurophysiological studies indicate that the neurons controlling detrusor contraction are mixed in the same population with the cell bodies of pudendal neurons that control the external sphincter. This

greatly reduces the possibility of developing a practical bladder evacuation prosthesis based on electrical stimulation of the spinal cord.

Dilantin increases the electrical current thresholds for producing afterdischarges in cats. In a human striate cortex stimulation case, the relationship between pulse train duration and threshold for phosphene production was determined and was found to be independent of train length for durations greater than 200 msec. In this patient, a phosphene could be produced with a single 3 ma, 0.5 msec, capacity coupled monophasic pulse.

An in vitro technique for measuring the real surface area of stimulating electrodes has been extended and successfully tested in vivo.

A histopathological study of brain tissue after essentially continuous electrical stimulation of the cerebral cortex for 36 hours has been completed. Significant neural damage was found for stimulation levels above 0.45 microcoulombs per pulse.

Low temperature isotropic carbon is not a suitable candidate as a potential electrode material. It corroded severely in in vitro stimulation testing and was found to be neural toxic in in vivo tests. Antimony doped stannous oxide has extremely good corrosion resistance.

The gassing limits for platinum electrodes in simulated cerebrospinal fluid solution were found to be greater than 300 microcoulombs per real square centimeter. This indicates that gas evolution at electrode-tissue interfaces will not be a problem in the neural prostheses that are presently being studied and are utilized in stimulation of the cortical surface.

Significance to Biomedical Research and the Program of the Institute: As these feasibility studies continue, the possibility of developing truly useful neural prostheses for the neurologically handicapped becomes more promising. The results of these studies are already proving useful to current neurosurgical techniques involving electrical stimulation and manufacturers of equipment for such stimulation have utilized results from these studies for the design of their equipment.

Proposed Course of Project: The studies on bladder evacuation by spinal cord stimulation are being terminated because separate centers in the spinal cord for control of detrusor and sphincter activity were not found.

Development of functional neuromuscular stimulation will be extended to totally implanted systems. Also, if the animal studies on scoliosis show promise, the technique will be extended to clinical trials.

As the major problem facing neural prostheses development is safe, effective connections with the nervous system, emphasis will continue on the development and evaluation of new biomaterials, electrodes, and stimulation techniques.

Keyword Descriptors:

Neural stimulation
Neural prosthesis
Visual prosthesis
Bladder prosthesis
Functional neuromuscular stimulation

Honors and Awards: None

Publications: None by Project Officers

Project No. Z01 NS 02078 02 LNLC
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Input-Output Pathways of Dorsal Root Ganglion Cells

Previous Project Number: Same

Principal Investigator: Gerald E. Loeb, M.D.

Other Investigators: None

Cooperating Unit: None

Man Years:

Total	0.7
Professional:	0.6
Other:	0.1

Project Description:

Objectives: Evidence from a number of investigators is mounting to indicate that the classical interpretation of dorsal root ganglion cells with a peripheral branch coursing distally via a single spinal nerve and a central branch coursing proximally via associated dorsal root is incomplete. There are known to be ganglion cells scattered in the ventral roots; spinofugal fibers carrying primary afferent signals have been identified in dorsal roots; and a large proportion of unmyelinated fibres in the ventral roots have been found to have cell bodies in the dorsal root ganglia. This project is designed to identify and quantify these and possibly other unusual pathways of processes from somata in the dorsal root ganglia.

Methods Employed: Cat L7 dorsal root ganglion cells are identified by extra-cellular single unit recording from their somata by dual tungsten-Parylene insulated microelectrodes. Receptor type and conduction velocity in sciatic nerve are obtained and the units tested for antidromic stimulation via the proximally cut dorsal and ventral roots.

Major Findings: A total of 186 units have been isolated in the L7 dorsal root ganglia of 11 cats. Of these, 130 have been characterized by conduction velocities in both (sciatic) and proximal (dorsal and/or

ventral roots). Of these, four units were found to send processes into the cord via ventral rather than dorsal roots and one sent processes in via both roots. Both the typical dorsal root pathway and the ventral root pathway cells showed the typical slowing of conduction velocity in the proximal arm to about 43% of the sciatic velocity. The units were divided into 77 proprioceptors, 65 cutaneous receptors and 37 unknown receptor types. Of cells with ventral root proximal branches, 3 were proprioceptors (71-84 m/sec sciatic conduction velocity), 1 was a cutaneous hair cell receptor (69 m/sec) and one of unknown receptor type (104 m/sec). The previously described search for recurrent collaterals (done with cord connections left intact) was terminated after 47 cells were characterized without encountering any such drivable pathways. The extracellular electrode methods employed appeared highly selective for units faster than 10-20 m/sec and it was not possible to study unmyelinated afferents.

Significance to Biomedical Research and the Program of the Institute:

The presence of a small percentage of myelinated afferents in ventral roots with cell bodies in the ganglion has been demonstrated. This small percentage is probably insufficient to provide useful amounts of sensory information to a spinal cord acutely deprived of afferents by dorsal rhizotomy but might, given recent evidence of sprouting, eventually play some crude role in the return of function seen in deafferented animals. Whether this small percentage of afferents represents developmental accidents or whether it subserves some special role remains to be determined.

Proposed Course of Project:

The above described data is being prepared for publication as part of a paper reviewing the question of this exception to the Bell-Magendie hypothesis and the several other exceptions hypothesized or explored by other investigators. Previously proposed histochemical experiments remain to be done. The electrophysiological and surgical techniques developed for the work to date are being converted to the problem of chronically recording single unit afferents in unrestrained animals to study the role of proprioceptive receptors in initiation and control of movements.

Keyword Descriptors:

Dorsal Root Ganglion
Neural Pathways
Ventral Root Afferents

Honors and Awards: None

Publications: None

Project No. Z01 NS 02079 02 LNLC
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Models of Neural Interactions

Previous Project Number: Same

Principal Investigator: William B. Marks, Ph.D.

Other Investigators: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0

Project Description:

Objectives: The primary objective of this project is the generation of a model of a part of the nervous system capable of detecting special features of the information it receives. One commonly observes in the nervous system a group of nerve cell bodies, each receiving a different mixture of excitatory and inhibitory connections from a shared group of afferent nerve fibers. It appears useful to hypothesize that each neuron of such a group is indicating by its firing rate the amount of some independently varying property, or "feature," of the patterns of activity in the afferent fibers. It is proposed that the connections for accomplishing this are not determined entirely genetically, but that during development the synaptic strengths are influenced by the afferent activity. If the activity of these neurons is correlated then they are not detecting independently occurring features of the afferent patterns. Therefore, the synaptic strengths should change in a direction which tends to reduce the correlations in the activities of the cells.

A justification for seeking such a model is the observation that the cells of the mammalian visual cortex appear to detect independently varying features of the patterns of activity coming from the retina. A further goal of this project is to present a series of visual patterns to the modeled system and to compare the features it discovers to the naturally occurring feature detectors of the visual cortex. Features of the patterns of activity from muscle and joint afferents during limb movements might also be studied using such a model for comparison with

naturally occurring afferent connections on the cells of the spinal cord.

Methods Employed:

A. It was assumed that there existed an array of signal sources X(1), I = 1, 2, 3 which varied independently and had a probability distribution that was skewed about the mean. These sources were linearly mixed to form 5 variables Y(J), J = 1, 2, 3, 4, 5. These were taken as data, and 5 linear mixtures of these, Z(K), K = 1, 2, 3, 4, 5, were formed. That is,

$$Z(K) = \sum_{J} L\ (K,J)\ Y(J).$$

The mixing coefficients L(K,J) were varied under the influence of the correlations among the Z(K) in an effort to reduce the correlations among the Z(K). The "growth rule" for the L(K,J) was

$$\frac{d\ L\ (K,J)}{dt} = -\sum_{I \neq K} \frac{\overline{Z(K)\ Z(I)}\ L(I,J)}{[Z^2(K)\ Z^2(I)]^{1/2}}$$

B. After the correlations in the Z(K) were eliminated by this rule, and the number of the Z(K) had been reduced to the number of sources X(I), a second growth rule was tested to eliminate the mixing that was still present between the X(I) and the Z(K). The new L, called $\hat{L}$, was given by

$$\hat{L}\ (K,J) = \sum_{I} \frac{\overline{Z^2\ (K)\ Z\ (I)}}{Z^3\ (K)}\ L(I,J)$$

Major Findings:

A. Growth rule "A" above worked. Not only did it produce uncorrelated Z(K), but it automatically eliminated excess Z(K) so that only the number remained that could be related 1 to 1 to the X(I). ((A Z(K) was dropped when its variance became small compared to that of the other Z(K).) The time course of the reduction in correlation among the Z(K), when plotted, showed sudden drops when a Z(K) was eliminated. 20,000 samples of the Z(K) were required to reach a final stable variance.

B. The second growth rule also worked. To measure the degree to which each Z(K) was influenced by only one X(I), we used the fraction of the variance of a Z(K) that was accountable to all of the X(I) other than its dominant one. Before application of the second growth rule this contamination was as much as 30%. The growth rule was then applied. 3000 Samples were used to compute the $\frac{}{Z^2(K)\ Z(I)}$.

With this many samples, the sample error was such that application of this growth rule did not eliminate all mixing, but reduced the maximum contamination in any Z(K) from 30% to 7%. A larger sample would have allowed less contamination.

Significance to Biomedical Research and the Program of the Institute:

This begins to be a method for detecting signals, lost through mixing, by a procedure simple enough to be implemented by neuron-like devices. It utilizes the principle of independent sources, and may have applicability to the general problem of estimating the number and strength of connections within a system of interacting variables.

Proposed Course of Project:

Large numbers of samples are required to provide reliable measures of correlation from which to calculate accurate mixtures of the data. Thus it may be necessary to reprogram these models into our PDP-12, and thereby avoid expensive lengthy iterations on the NIH PDP-10. The latter computer would continue to be used to develop the programs, using the language APL, which is convenient for manipulation of arrays.

Then it would be possible to apply these feature defining programs to larger, more interesting, arrays, to test my formulas which predict the number of samples required for a given accuracy, and to find the limits of the method. In particular, much computer time will be required to compute the features of visual patterns, for comparison with the receptive fields of the visual system.

Keyword Descriptors:

An adaptive model of neuronal interactions for feature detection.

Honors and Awards: None

Publications: None

Project No. Z01 NS-02080-02 LNLC
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Neuron Activity during Locomotion

Previous Project Number: Same

Principal Investigator: William B. Marks, Ph.D.

Other Investigators: *Joaquin-Andres Hoffer
George Dold

Cooperating Unit: *Johns Hopkins University
Biophysics Department
Baltimore, Maryland

Man Years:

Total:	0.6
Professional:	0.4
Other:	0.2

Project Description:

Objectives: This project has several related objectives: a) to obtain information about the roles of spinal neurons in controlling locomotion; b) to develop preparations which utilize our electrodes for monitoring and stimulating neurons of the unrestrained animal; and, c) to develop techniques and concepts for dealing with patterns of simultaneous neuron activity. We are particularly interested in the role of muscle afferent fibers in controlling the movement of the limb, and in the degree to which motoneuron activity reflects the "expected" movement of the limb.

Methods Employed:

Progress in chronic recording from nerve fibers resulted largely from improvements in the design of the tube electrodes. In Fiscal Year 1974 these were made by covering an array of grooves in a plastic chip after loading the grooves with dissected spinal rootlets. Three problems had to be solved with this design: a) Nerve filaments became ischaemic when captured within the grooves and ceased to conduct within about 30 minutes. This was caused by the thickness of the array, which stretched the filaments and cut off circulation at the bends. To solve this, we separated the grooves into individual, split silastic tubes (4 mm long,

300 μm diameter, 4 in number) which fit between the dorsal roots, rather than resting on them. For the first time, with this undeviated path, filaments survived 30 days and more. b) At this point, the breaking of our leadouts became limiting. Flexible .001" gold leads had been required to prevent stressing the nerve filaments. For these, we have substituted .001" stainless steel leads, which should not break, coiled to provide easy flexion. c) Our percutaneous button connectors have each been rejected after about 30 days. We have recently begun to pass our 1 mm cable directly through a small stab wound in the skin. This should eliminate infection and rejection.

For our peripheral nerve preparation, J. A. Hoffer chose the tenuissimus nerve for its small size (25 μm) and the rabbit was chosen because the tenuissimus is more accessible there. The cat tenuissimus remains a possibility. This peripheral nerve, lodged between the lateral muscles of the thigh, branches from the sciatic nerve about 3 cm after its exit from the spinal column. It consists entirely of efferents and afferents to the tenuissimus muscle. It is captured in two electrically independent cuffs with indifferent contacts at mid-tube. Thus, efferents and afferents can be distinguished by propagation direction, and some idea of conduction velocity obtained. EMG interference has been controlled after much effort by enclosing the recording cuffs within a larger insulated Faraday cage of silastic and platinum shorting bars.

The spinal root filaments are 100-150 μm in diameter and the tenuissimus nerve has a diameter of 250 μm. Both produce about 10 large spikes and, in each, preparations have occurred in which a single large spike could be distinguished even during bursts.

Major Findings:

It is possible to monitor a moderate population (1-10) of units in a dorsal rootlet (100-150 μm) or a small peripheral nerve (250 μm) at least for several weeks. For the dorsal root preparation, wire breakage has become limiting at 30 days. In the peripheral nerve there is a steady loss of population, the larger spikes dropping first within 6 days to 3 weeks. This is probably caused by the continual stressing of the nerve by movement. Muscle afferents have a fluctuating spontaneous activity in unanesthetized animals, which is influenced by arousal as well as by the phase in stepping.

Significance to Biomedical Research and the Program of the Institute:

Other laboratories have expressed interest in applying our cuff recording technique to human quadriplegics. Grasping is elicited in these patients by muscle stimulation. Chronic multi-unit records from the median nerve using a cuff might provide finger pressure and slip information for controlling the grip.

The clinical usefulness of capturing spinal roots is less immediate, because a laminectomy is required. However, eventually the flexibility of root recording may overcome this factor. Roots can be split more easily, all nerves are equally accessible, sensory and motor fibers are segregated, and there is little movement within the spinal column.

These techniques may be used to generate basic information, by recording the behavior of single units along with the estimated time course of muscle length and tension during various movements, followed by an acute experiment to characterize the unit. This may augment data from acute experiments by showing how control strategy varies with the task. By recording several units simultaneously, we may be able to detect differences between their activities that are not known now, for example, in the recruitment order of motor neurons, or among apparently similar muscle afferents.

Proposed Course of Project:

We now have a system for video recording movements on a treadmill and simultaneously tape recording nerve activity, and have begun to analyse their relations in slow and step motion. During the next year we will be producing graphs of rate of activity of groups of afferents and efferents and of single identified fibers, with graphs of simultaneously measured muscle length, joint angle, phase in stepping, and muscle tension (EMG). We will also be improving unit isolation by paring down filaments in the spinal root preparation, and by distinguishing unit events by their simultaneous effect on two or more contacts in a common tube in both preparations.

Earlier dissections in the rabbit suggest that nerves to the periphery of the limb in the rabbit are fasciculated as though each muscle may have its own dissectible small fascicle. This should be explored in the rabbit, cat, and monkey.

In theory the nerve root preparation should be able to tap fibers to any muscle of interest. During the coming year we will attempt to record from fibers to the ankle extensor muscles. We will also attempt to capture ventral root fibers to those muscles.

We will also collaborate with Dr. Gerald Loeb, whose microelectrode approach to the dorsal root ganglion is potentially good for chronic use. As compared with the cuff approach, this method should give better unit isolation, but would not allow such free control of the population (muscle) that is to be sampled.

Keyword Discriptors:

Recording chronic neuronal activity during movement by capturing nerve fiber bundles, in relation to the control of muscles and prostheses.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02160 01 LNLC
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Intrinsic Properties of Motor Units

Previous Project Number: None

Principal Investigator: Robert E. Burke, M.D.

Other Investigators: William Z. Rymer, M.D., Ph.D.
John V. Walsh, M.D.
*Richard F. Mayer, M.D.
**Peter Tsairis, M.D.
***V. Reggie Edgerton, Ph.D.
Kenro Kanda, M.D., Ph.D.

Cooperating Units: *University of Maryland School of Medicine,
Department of Neurology
Baltimore, Md.

**Cornell University Medical School
Department of Neurology
New York, N.Y.

***University of California
Department of Kinesiology
Los Angeles, Ca.

Man Years:

Total:	2.1
Professional:	2.1
Other:	0

Project Description:

Objective: This project is designed to provide information on the ranges and distributions of electrophysiological, mechanical, histochemical and morphological characteristics of individual motor units in mammals, principally in the cat.

Methods Employed: For the most part, acute experiments are carried out in anesthetized cats, using intracellular recording and stimulation of alpha motoneurons to ensure functional isolation of single motor units. The

electrophysiological properties of the innervating motoneurons can be examined using conventional techniques and then the mechanical properties of the innervated muscle unit is assessed by recording tension production at the appropriate tendon during stimulation of the motoneuron through the intracellular pipette. For specific experimental series, various conditioning situations have been used to alter the activity of the motor unit pool under study, such as surgical removal of synergist muscles or immobilization of the appropriate limb by joint pinning.

Major Findings:

A. The normal situation: Previous work in this laboratory, described in earlier reports, has provided data enabling classification of the motor unit population in the cat medial gastrocnemius muscle into two major categories, fast twitch (F) and slow twitch (S), on the basis of the mechanical responses of the muscle unit. The F category can be subdivided into two major subclasses, one with relatively little resistance to fatigue (type FF) and the other with considerably greater fatigue resistance (type FR), plus a small group of units with fatigue resistance intermediate between these extremes (called type F(int)). This body of data from normal animals now serves as a reference base against which comparisons can be made of altered motor unit properties produced by altered usage patterns.

B. Compensatory hypertrophy.

During the past year, a study of the effect of removal of all ankle extensor synergists of the medial gastrocnemius muscle was completed. Surgical preparation and eventual motor unit studies (after 3 to 7 months of survival) were carried out in this laboratory, the relevant muscles were then frozen and sent for histochemical preparation to Dr. Peter Tsairis, of Cornell University Medical School, and the histochemical and morphological results were largely assessed here. After an initial recovery period, cats adapt very well to having only the medial gastrocnemius as an ankle extensor, and the animals walk, run and jump effectively. Compensatory hypertrophy of the remaining gastrocnemius muscle is evident in a modest increase in weight (X up to +13%) compared to the contralateral control. However, the motor units in hypertrophic gastrocnemii showed striking increases in mean tetanic tension output (in FF: +49%; in F(int): +157%; in FR: +140%; and in S: +114%) as compared to a very large sample of units from normal animals matched for body weight and muscle weight. There were no significant changes in mean twitch contraction time for any motor unit group. Also, no change was evident in the percentage composition of the gastrocnemius motor unit population in hypertrophic muscles and the histochemical mosaic was unchanged. Likewise, no change in fatigue resistance was found in careful comparison with units from normal animals. The marked increase in tetanic tension output was not matched by a corresponding increase in twitch tension, so that the mean twitch/tetanus ratio actually decreased with compensatory hypertrophy. Measurement of the muscle fiber cross-sectional areas on the operated and control sides in one animal (data limited by requirement for excellent technical preservation of equivalent regions of muscle on the two

sides) showed only a modest increase in fiber area in those fibers belonging to FR and S motor units. Compensatory hypertrophy of the type studied appears to result in alteration only of tetanic tension output per motor unit without any other clear change, and this increase appears to require either a proliferation of muscle fibers belonging to individual units (more in FR and S units than in FF) and/or a change in the specific tension output per unit cross-sectional area of fiber, implying a possible alteration in the sliding filament mechanism generating tension.

C. Motor units in muscle atrophy.

This year saw initiation of a study of the effect of limb immobilization by joint pinning on the motor unit population of the cat medial gastrocnemius. Initial surgical pinning was done in the Department of Kinesiology, UCLA, Los Angeles, and the animals were sent to NIH for motor unit analysis after 6 - 8 months' survival. Assessment of the motor unit population was done here and the appropriate muscles frozen and processed histochemically both at UCLA and in the Department of Neurology, University of Maryland School of Medicine by Dr. Richard Mayer, who participated in the experiments here as a Guest Worker.

Data analysis is incomplete at this time, but preliminary review indicates a very striking degree of reduction in tetanic tension output from both type FR and type S motor units, with comparatively less reduction in output from FF units, in relation to data from normal animals matched for body and muscle weight. Also striking was the finding that the average twitch/tetanus ratio in atrophic units was higher than in normal motor units, and very much higher than observed in compensatory hypertrophy (see above). This physiological evidence for muscle unit atrophy was borne out in histochemical analysis, at least in part, for fibers which could be identified as type FR on the basis of histochemical profiles were very much more atrophic than FF fibers. Surprisingly, fibers belonging to type S muscle units showed little evidence of morphological atrophy even though S unit tensions were much below normal. The proportion of F to S units in the overall population remained the same after atrophy but there was a decreased number of FR units in the atrophic sample. The apparently selective mechanical and morphological atrophy of fibers belonging to type FR units, and the dissociation between mechanical and morphological atrophy in S units, were both quite unexpected and require further work.

Significance to Biomedical Research and the Program of the Institute:

Analysis of the control of movement by the central nervous system requires consideration of the properties and functional specialization of motor units, as these are the quantal elements from which all skeletal movements are composed. Study of the interrelation between the intrinsic properties of motor units, including both the motoneuron and muscle unit portions, and the organization of synaptic input to the same units has aided our understanding of the control problem and has suggested new

avenues for research. In addition, elucidation of the detailed interrelation between the physiological, morphological and histochemical characteristics of muscle units in animal muscle has immediate relevance to investigations of human neuromuscular disease, in which electromyography and muscle histochemistry play important diagnostic and research roles, as there is growing evidence that the basic pattern of motor unit organization in animals and man is similar in principle.

Proposed Course of Project:

The present work on the effects of limb immobilization on motor units in the cat hindlimb will continue, with particular reference to the time course of development of the changes and their degree of reversibility. In addition, contact with our group has been initiated by another outside laboratory who propose a pilot study of motor unit properties during regeneration of muscle fibers as occurs in muscle transplantation. Preliminary results with this system appear promising and initial acute experiments are planned for the Fall of 1975.

Keyword Descriptors:

Muscle fibers
Contractile properties of muscle
Motoneurons
"Trophic" effects

Honors, Awards and other Scientific Recognition:

Invited lecture (Dr. Burke) to the III International Congress on Muscle Diseases, Newcastle-upon-Tyne, England, September 1974.

Invited tutorial lecture (Dr. Burke) to the 21st Annual Meeting of the American Association for Electromyography and Electrodiagnosis, San Francisco, California, November 1974.

Publications:

Burke, R. E., Levine, D.N., Salcman, M. and Tsairis, P.: Motor units in cat soleus muscle: Physiological, histochemical and morphological characteristics. J. Physiol. (Lond.) 238: 503-514, 1974.

Burke, R.E. and Edgerton, V.R.: Motor unit properties and selective involvement in movement. In Wilmore, J.H. and Keogh, J. (Eds.): Exercise and Sport Sciences Reviews, Vol. 3, New York, Academic Press, 1975, pp. 31-81.

Project No. Z01 NS 02161 LNLC
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Control of Single Motor Unit Firing Patterns in Humans

Previous Project Number: None

Principal Investigator: Edward M. Schmidt, Ph.D.

Other Investigators: F. Terry Hambrecht, M.D.
J. Stevenson Thomas, Ph.D.
Joan S. McIntosh

Cooperating Unit: None

Man Years:

Total:	0.4
Professional:	0.3
Other:	0.1

Project Description:

Objectives: The objectives of this study are twofold: the first being to obtain a better understanding of the organization of motoneuron pools, and the second to determine the extent of control a human has over the firing frequency of a single motor unit.

Methods Employed: Platinum wires (1 mil) will be introduced into either the abductor pollicis brevis or abductor digiti minimi muscles of the hand with a 27 gauge hypodermic needle. Normally several motor units are recorded simultaneously with intramuscular microelectrodes. Each motor unit has a distinctive action potential shape allowing multiple unit records to be separated into single unit activity. The multi-unit data will be analysed by a waveshape discriminator to provide "on-line" classification of the potentials. Each time an action potential occurs corresponding to the activation of a specific motor unit a tone burst will be generated and presented to the subject. Each separable unit, in the multi-unit record, will be associated with a different frequency tone burst. Using a different frequency tone burst to represent each motor unit will provide the subject with sufficient information to attempt conscious control of motor unit activity.

Recruitment order of motor units will be established at different muscle lengths and forces with a torque motor loading system. The maximum output of the torque motor is below the level where physical damage to the subject could occur. One task for the subject will be to determine the extent to which motor unit recruitment order can be modified by conscious control.

Currently, we are studying the ability of monkeys to control the firing patterns of individual cells in the motor cortex to determine if these signals could be used to control paralyzed muscles, or artificial limbs. The ability of humans to control the firing patterns of single neurons is of great interest to our laboratory and needs to be correlated with our animal studies. Some data exists from humans indicating that control of single motor unit firing patterns is feasible but the information transfer rate of such a signal source is quite low. Further studies in both animal and man are required to determine the range of control of single cell firing patterns and if different signal processing techniques will provide a higher rate of information transfer.

Significance to Biomedical Research and Program of the Institute:

The ability of a person to control the firing patterns of single motor units and the ability to record these signals may provide a source of signals that could be used to control stimulation of paralyzed muscles or control a prosthetic device in a more refined method than is possible with gross EMG electrodes.

Keyword Descriptors:

Single motor units
Recruitment order of muscle units
Voluntary control of motor units

Proposed Course of Project: This project has been approved by the Clinical Research Committee of NINCDS and is currently being reviewed by the Medical Board of NIH because human volunteers will be used in the study.

Honors and Awards: None

Publications: None

LAB. OF
BIOPHYSICS

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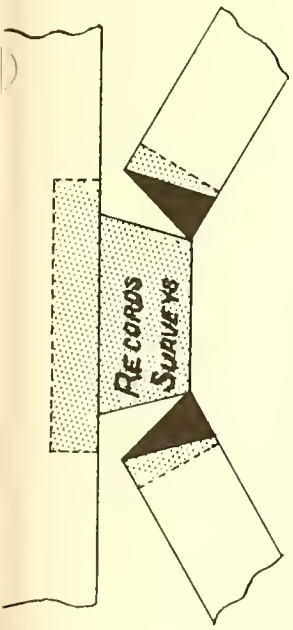

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LAB. OF
NEUROPHYSIOLOGY

ANNUAL REPORT
July 1, 1974 through June 30, 1975
Laboratory of Neurophysiology, Intramural Research
National Institute of Neurological and Communicative Disorders and Stroke

M.G.F. Fuortes, M.D., Chief

During FY 74 the Laboratory of Neurophysiology lost ten scientists; four were foreign scientists who returned to their home countries; three were Staff Fellows who obtained positions elsewhere in the United States; two went to work abroad and one completed the thesis work required for her Ph.D. degree. Twelve scientists joined the Laboratory during the year; four Guest Workers supported by Fellowships; five Visiting Scientists or Visiting Fellows from Czechoslovakia, Italy, Japan and Switzerland; and three Staff Fellows. With twelve new scientists, the staff of LNP now includes nineteen professionals. Two more young scientists are expected to join the Laboratory within the next three or four months. The professional staff is supported by one secretary and three technicians.

The major results of the work performed in the Laboratory are described in sixteen articles published during FY 75.

Experiments on muscle cells of chick embryo in tissue culture have revealed that at early stages of development these muscles produce a novel type of slow spike which is controlled by membrane permeability to chloride. In addition to this slow spike, the cell can develop a conventional fast spike controlled by sodium and a slower spike controlled by calcium. Studies in invertebrate neurons have been continued. It has been found that the rhythmical activity of the so-called "bursting" neurons is influenced by divalent cations and by amino-acids including the anti-diuretic hormone vasopressin.

Continuing previous work, two types of receptors have been studied in the mollusk Hermissenda: photoreceptors and statocyst hair cells. In the photoreceptors it has been found that the electrical response is brought about by an increase of sodium permeability, followed by increase and latter reduction of permeability to potassium. It seems probable that the changes in potassium permeability are brought about by corresponding changes in the intracellular concentration of calcium ions.

Qualitative analysis of the generator potentials evoked in hair cells by mechanical stimulation has led to the conclusion that hair cell responses are not a direct consequence of the deformation of the membrane but arise from intermediary processes which are triggered by the stimulus. These processes are

temperature-dependent with a Q_{10} of approximately 2. Noise and steady-state responses of hair cells are now under investigation.

As in previous years a major part of the work at LNP has been dedicated to the study of the vertebrate retina. Using light- and electron-microscopy it has been possible to describe the organization of rod and cone pathways in the retinas of the cat. Rods are connected to "rod bipolars," and through them to amacrine cells which in turn form synapses on ganglion cells. Cones instead are connected to ganglion cells directly through "cone bipolars" without making use of amacrine cells. Important anatomical information has been derived also from microspectrophotometry. It has been seen that a correlation exists between shape and size of a cone and the type of visual pigment which the cone contains. In addition, it has been established that double cones in the goldfish consist of a long member with red pigment and a short member with green pigment. With regard to horizontal cells it has been seen that two types of luminosity units which differ both in their structure and in their functional properties are in fact two parts of the same cell, connected one to the other by a thin axon; what were once called type I cells are terminal processes while the cells classified as type II include somata and dendrites. In addition somata of different cells are connected by electrical junctions and it is possible that chemical synaptic connections exist between the terminals of one cell and the somata of others. The activities of LNP were reviewed during the year by the Board of Scientific Counselors. It was a pleasure for the staff to learn that the Counselors stated that "this laboratory has become one of the world's real centers of excellence in terms of research and as a place for training of young investigators."

Project No. Z01 NS 01239-11 LNP
1. Laboratory of Neurophysiology
2. Section on Sensory Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Photoreceptors in the Limulus Eyes

Previous Serial Number: Same

Principal Investigator: Thomas G. Smith, Jr., M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0
Professional:	0
Others:	0

Project Description:

This project has been terminated.

Project No. Z01 NS 01690-07 LNP
1. Laboratory of Neurophysiology
2. Section on Sensory Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Rapid Scanning Microspectrophotometry in Visual Cells

Previous Serial Number: Same

Principal Investigator: Thomas G. Smith, Jr., M.D.

Other Investigators: Judith Oldak[1]
Franco Malerba, Ph.D.[3]
Theodore Colburn, Ph.D.[2]
Ferenc Harosi, Ph.D.

Cooperating Units: [1]Department of Biomedical Engineering
University of Maryland
College Park, Maryland
[2]Section on Technical Development
NIMH,NINCDS
[3]C.N.R., Laboratorio Di Cibernetica E
Biofisica, Camogli, Italy

Man Years:

Total:	1.0
Professional:	1.0
Others:	0

Project Description:

Objectives: The objective of this research is the development of rapid scanning microspectrophotometric techniques for investigation of the chemical kinetics within excitable cells. Although these techniques are being developed to study the molecular steps coupling photoexcitation of visual pigments to the excitation of the electrical response of the cells, their more general application to cellular physiology will also be considered.

Methods Employed: The microspectrophotometer samples transmittance at spectral wave bands between 350 and 650 nm, either sequentially or in random order, at rates of 600 microseconds per sample point. The system uses a rapid scan monochromator in which the entrance slit has been replaced by the image of a cathode ray tube face.

Major Findings: Progress on the development and use of the microspectrophotometer was slowed this past year because Dr. Malerba was unexpectedly recalled to active military service in January 1974. A replacement for Dr. Malerba, Ms. Judith Oldak, a graduate student at the University of Maryland, was able to join this project in July 1974, and has assumed day-to-day responsibility for the instrument. Several months of full-time effort was required for Ms. Oldak to become familiar with the technical aspects of the microspectrophotometer and educated in the scientific nature of the biological proglems to be investigated.

Subsequently, several months of redesign and reconstruction efforts were required to improve the stability and control of the light source, a cathode ray tube. Extensive tests have been run on the improved instrument. The results indicate that sufficient stability may have been achieved to allow the execution of the proposed biological experiments. Currently, such experiments are in progress.

Furthermore, a new logic control system has been designed and constructed. This system has increased the ease of use and flexibility of the instrument.

In addition to work on the instrument itself, further computer programs have been written, tested out and used successfully to convert and analyze the data on the PDP-12 computer.

Significance to Bio-medical Research and the Program of the Institute: This project should provide the specific instrumentation and techniques for the measurement and analyses of the transduction steps which couple the stimulus to the electrical changes in the excitable membrane of photoreceptors. It will also provide a useful tool for similar study of other molecular systems functioning within living cells, for which an increasing need has developed.

Proposed Course of the Project: With the aforementioned technical improvements in the instrument, the proposed study of the kinetics of visual pigments in the frog retina are in progress.

Keyword Descriptors:

rapid scanning microspectrophotometer
chemical kinetics
visual pigments
photoreceptors

Honors and Awards: None

Publications:

Harosi, F.I. and Malerba, F.E.: Plane-polarized light in microspectrophotometry. Vision Res. 15: 379-388, 1975.

Project No. Z01 NS 02019-03 LNP
1. Laboratory of Neurophysiology
2. Section on Sensory Physiology
3. Bethesda, Maryland

PHD-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Electrophysiology of Simple Cellular Systems

Previous Serial Number: Same

Principal Investigator: Thomas G. Smith, Jr., M.D.

Other Investigators: Jun Fukuda, Ph.D.[3]
Tadashi Akaike, M.D.[3]
Harold Gainer, Ph.D.[1]
Jeffery Barker, M.D.[1]
Gerald Fishbach, M.D.[2]
Maryanna Henkart, Ph.D.[1]

Cooperating Units: [1]Behavioral Biology Branch, NICHD
[2]Department of Pharmacology,
Harvard Medical School
[3]Department of Physiology
University of Tokyo
Tokyo, Japan

Man Years:

Total:	3.5
Professional:	3.4
Others:	0.1

Project Description:

Objectives: The objective of this research is to gain insight into the membrane mechanisms which are responsible for the activity of single cells.

Methods Employed: The electrical properties of single cells are recorded with intracellular microelectrodes and analyzed under a variety of experimental conditions. The technique of voltage-clamping the membrane potential is the main method employed in these experiments. This technique requires the penetration of single cells with two microelectrodes, one for monitoring the membrane potential, the other for supplying the current necessary to clamp the membrane potential at the desired level. Using this technique the role of the various ionic conductances responsible for the electrical responses of the membrane can be studied.

Because of the necessity of taking many measurements from each experiment, the data is often recorded on magnetic tape and analyzed with a PDP-12 computer.

The anatomical properties of the cells are studied with conventional light and electronmicroscope techniques.

Major Findings: This project involves the investigation of the electrical characteristics of nerve and muscle tissue, with particular emphasis on slow membrane processes. This past year most investigations have been on chick muscle cells grown in tissue culture and on neurosecretory cells of molluscs.

A number of electrical properties of the chick muscle cells have been identified and studied during the course of development of these cells from their immature, myoblastic stage to an apparently "mature" stage. One particularly interesting electrical response in these cells is a slow "spike" which associated with a slow contraction of the muscle. This spike appears quite early in development and changes with maturation. The results of electrophysiological investigations indicate that it is generated by a voltage-and time-dependent change in the muscle membrane's chloride permeability. Thus, the chick muscle has three types of spikes: 1) a conventional, fast sodium spike, 2) a slower calcium spike and 3) a very slow chloride spike.

During this past year experiments were begun to characterize in detail the properties of the voltage-dependent chloride conductance which underlies the slow chloride spike. While these investigations are not yet completed, they concern the refractory periods of the spike, the activation and inactivation properties of chloride conductance, the dependence of the chloride currents and conductance to the transmembrane chloride concentration gradient and the effect of drugs on the chloride spike. A particularly interesting finding was that caffeine blocks the chloride spike (see section on Significance).

In order to have a technically manageable preparation for the voltage clamp experiments, the muscle cells have been grown in 10-20 nanomolar colchicine, which results in a spherical, isopotential cell (a myosac) with apparently normal membrane electrophysiological properties. The electronmicrographic studies indicate that colchicine prevents the formation of microtubules and hence the elongation of the cell into a tubular fiber. These myosacs have all the spikes (sodium, calcium, and chloride) of the normal muscle, as well as sensitivity to acetylcholine. Moreover, they can contract spontaneously or when stimulated.

The aim of the study of the snail neurosecretory cells is an understanding of the membrane mechanism underlying the bursting, pacemaker activity (BPA). Voltage-clamp analysis has shown that bursting cells have an N-shaped steady-state current-voltage (I-V) curve. The cell has no "resting" potential but oscillates between the two positive slope regions of the I-V curve.

As previously reported, ionic studies have shown that the two positive slopes of the I-V curve result from high potassium conductances which are voltage and time dependent. The positive slope at large negative potentials is due to anomalous rectification while that at small negative and positive potentials is due to delayed rectification. The negative slope region of the N-shaped curve is due to a voltage dependent but apparently time independent sodium conductance.

During this past year a qualitative model of the membrane mechanisms underlying pacemaker activity was devised and published. Central to this model are the differences between the voltage and time dependencies of the sodium conductance and the delayed potassium rectification. Quantitative measurements of these characteristics are in progress in an attempt to develop a quantitative model. Moreover, since the divalent cations (Ca, Mg, Sr) are known to affect bursting, the voltage clamp I-V curves of cells bathed in different divalent cations are being studied. The results support the qualitative model, but have not been tested quantitatively.

A particularly interesting finding has been that lysine vasopression (Antidiuretic Hormone:ADH) and other nine membered amino acid peptides have an effect on BPA. The BPA of the neurosecretory cell in active snails is absent in hibernating snails, but can be elicited within a few minutes after the addition of nanomolar amounts of ADH to the extracellular bathing medium. ADH does not have any demonstratable effect on other neurons tested. Consistent with our model, the quiescent neurosecretory cell has no negative slope in its I-V curve but the ADH-activated cell does have a region of negative slope. As yet, we have been unable to demonstrate whether the ADH acts on the sodium or the potassium conductance or both.

Significance to Bio-medical Research and the Program of the Institute: Since this project is relatively new and the experiment results are incomplete, it is impossible to be precise about the significance of this research. Our results however, do suggest a number of areas of potential significance.

In the first place, the study of the development of the chick muscle cell in tissue culture may provide insight into the

manner in which growth and maturation of membrane excitability occurs and the mechanisms which control these processes. Such insights would be of scientific interest in their own right, but are of potential medical interest for a rational understanding of muscular disorders which may have a developmental basis.

The finding that caffeine blocks the chloride spike, together with others' observation that a caffeine contracture can be evolved only in the presence of chloride (Stephanson and Podolsky, 1973), raises the possibility that the chloride spike may play a role in muscle excitation-contraction coupling. For example, a change in intracellular chloride concentration, following a change in chloride conductance, may play a role in intracellular calcium release, which initiates contraction.

Second, rhythmic or pacemaker activity is a property found in a variety of excitable tissues (normal and epileptic nerve cells, heart muscle, and smooth muscle) and an improved understanding of the membrane mechanism underlying this activity would be of interest to a number of scientific and medical disciplines. Now that we have proposed a model of a membrane mechanism to account for pacemaker activity in one tissue, it will be possible to examine other pacemaking tissues to see if a similar mechanism are involved there.

Furthermore, the finding that a hormone, ADH, affects a cell membrane in the manner observed has several potentially significant implications. For example, the speed of action of the drug suggests that hormonal action triggers or activates a membrane component that is "in place" rather than controlling the production of the requisite membrane component. In addition, the observation that ADH acts on, voltage dependent, i.e., non linear conductances is probably unique. Previously, hormones and neurotransmitters has been shown or were thought to act on voltage independent conductances. Regulation of a cell's activity via a non linear conductance has special advantages and potential hazards, especially when this regulation is not transient but long lasting, as is the case with hormones. The advantages include an increased dynamic range of action, since non linear mechanisms can act as amplifiers. Coupled with this advantage, however, is the hazard of possible loss of control since such systems are potentially unstable and can either turn on or off completely, unless regulation is very tightly controlled by fine tuning or by some additional feedback mechanisms. In physiological processes, involving hormones, such loss of control of regulation could produce catastrophic effects.

The third and perhaps most interesting area of potential significance of this research stems from the apparent similarity of the membrane mechanisms with different ionic bases underlying

two quite distinct physiological processes in completely different tissues, viz. 1) slow contractions in immature chick muscles and 2) periodic oscillations in the membrane potential of a snail cell that controls the release of hormones. Electrophysiologists have heretofore concentrated mainly on the "fast" processes of excitable tissues and have found a considerable ubiquity in nature in the membrane mechanism which underlies these "fast" processes. Many excitable tissues, have, however, a variety of "slow" processes which appear to be important in the regulation of physiological activity. It would, therefore, be of considerable interest to know if there were also an ubiquity in nature in the membrane mechanisms underlying these "slow" processes.

Proposed Course of the Project: It is proposed to continue the research on both chick muscle in tissue culture and on the snail cells by means of the voltage-clamp technique. One aim will be a more detailed documentation on the electrical characteristics and the time course of development of the excitable membrane of the chick muscle. In addition, a more complete analysis of the regulation of the time- and voltage-dependent ionic conductances underlying the "slow" processes in both the chick muscles and the snail neurons will be attempted. Particular emphasis will be given to the effects of divalent cations and drugs on these conductances.

Keyword Descriptors:

voltage-clamp
tissue culture
chloride spikes
pacemaker activity
hormones

Honors and Awards: None

Publications:

Smith, T.G., Barker, J.L. and Gainer, H.: Requirements for bursting pacemaker potential activity in molluscan neurons. Nature 253: 442, 1975.

Fukuda, J., Henkart, M.P., Fischbach, G.D. and Smith, T.G., Jr.: Physiological and structural properties of colchicine treated chick skeletal muscle cells grown in tissue culture. Dev. Biol. In press.

Fukuda, I., Fischbach, G.D. and Smith, T.G., Jr.: A voltage clamp study of the sodium, calcium and chloride spikes of chick skeletal muscle cells grown in tissue culture. Dev. Biol. In press.

Project No. Z01 NS 01659-07 LNP
1. Laboratory of Neurophysiology
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Synaptic Contacts of Visual Cells

Previous Serial Number: Same

Principal Investigator: Arnaldo Lasansky, M.D.

Other Investigators: Silvana Vallerga, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.5
Professional:	2
Others:	0.5

Project Description:

Objectives: To investigate the fine structure and function of the synapses between receptors and other retinal neurons.

Methods Employed: Electron microscopy combined with silver impregnations by the method of Golgi. Electrical recordings with intracellular microelectrodes.

Major Findings: The responses to light of horizontal cells were intracellularly recorded in the retina of the larval tiger salamander. All the units studied had a large summation area and were hyperpolarized by circles of light of any wave-length centered on the recording electrode, but two types could be distinguished according to the properties of their receptive fields. Type A units were hyperpolarized following illumination of any portion of their receptive field, while type B units were not hyperpolarized by illumination of their surround unless the centre was simultaneously illuminated, stimulation of the surround alone resulting in either a small depolarization or virtually no response.

Procion yellow injections showed that type A responses are recorded from thick and long processes not directly continuous with an identifiable cell body, while type B responses originate from the cell body of cells that send very fine and tortuous processes towards the receptors. The histological observations

also suggested that type A and B units are just different parts of a single kind of horizontal cell.

The large summation area of type A units can be explained, just as for horizontal cells in other retinae, by supposing that they are electrically coupled to other units of the same type. The receptive field properties of type B units, however, can only be partly explained by electrical coupling, and then only if the existence of voltage-dependent junctions is postulated. Instead, the reversal of the polarity of responses to an annulus of light during steady illumination of the centre, plus the available electron microscopic evidence, suggest that the effect of the surround on the type B units is due to a chemical synaptic impingement from the type A units.

Significance to Bio-medical Research and the Program of the Institute: It is hoped that these observations will help in identifying the mechanisms of synaptic transmission between photoreceptor cells and second order neurons, and provide a better knowledge of the neuronal networks involved in the processing of visual information within the retina.

Proposed Course of the Project: The properties of the synapses between photoreceptors and bipolar cells will be investigated by analyzing the characteristics of the bipolar cell responses and the organization of their receptive fields.

Honors and Awards: None

Publications:

Lasansky, A. and Vallerga, S.: Horizontal cell responses in the retina of the larval tiger salamander. J. Physiol. (London). In press.

Project No. Z01 NS 01889-05 LNP
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Agents Affecting Cell Proliferation and Differentiation in the Crystalline Lens

Previous Serial Number: Same

Principal Investigator: Ludwig von Sallmann, M.D.

Other Investigators: Patricia A. Grimes, B.A.

Cooperating Units: None

Man Years:

Total:	1.6
Professional:	0.8
Others:	0.8

Project Description:

Objectives: To investigate the possible hormonal regulation of cell proliferation in the rat lens epithelium and to determine the role of cyclic AMP in mediating such action.

Methods Employed: Lenses, carefully isolated from young rats were maintained in vitro in small Petri dishes containing 4 ml of medium 199 supplemented with additional bicarbonate buffer and calcium chloride. The lenses were incubated at 37 degrees C in equilibrium with a gas phase of 95% air and 5% carbon dioxide. After appropriate intervals the lenses were examined for clarity and fixed. Mitotic activity was determined by methods previously described.

Major Findings: The results of previous studies demonstrated that stimulation of beta receptors in the isolated lens by isoproterenol (IPR) and elevation of cyclic AMP levels can directly affect cell division in the epithelial layer. In an effort to further define the responsiveness of this cell system to hormonal regulation, we have examined the effects of prostaglandin E_1 (PGE_1) which is known to influence cyclic AMP levels and proliferation in certain cultured cell populations.

Mitotic activity is severely inhibited when lenses are cultured in the presence of PGE_1 at a concentration of 5×10^{-5}M. No significant differences between mitotic counts of treated and control lenses were detected after 2 and 4 hours of incubation. By 6 hours the number of mitoses in lenses exposed to PGE_1 fell to 75% of control values and after 16 and 24 hours to 45% of the normal count. There was no morphological evidence of toxicity at this drug level.

The time course of mitotic suppression induced by PGE_1 does not correspond to that observed in the presence of IPR or cyclic AMP derivatives. Response to the latter agents is characterized by a rapid and transient fall of mitoses presumably due to a block in the G_2 period of the cell cycle, whereas the delayed mitotic suppression evoked by PGE_1 suggests that inhibition occurs prior to DNA synthesis. However, when the concentration of calcium in the incubating medium is reduced from 1.5 mM to 0.5 mM, PGE_1 appears to induce an early fall in mitosis, corresponding to that of IPR, together with the suppression manifest at 24 hours

Significance to Bio-medical Research and the Program of the Institute: The clarification of the effects of these agents on the non-vascularized, non-innervated lens epithelium may contribute to the understanding of the complex mechanisms governing the control of proliferation and differentiation in cell population of different functional properties.

Proposed Course of the Project: Studies will be continued to determine the effect of PGE_1 on DNA synthesis, to clarify the role of calcium, and to test the possible mediation of cyclic AMP in this cell system.

Honors and Awards: None

Publications: None

Project No. Z01 NS 01943-04 LNP
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Spontaneous Retinal Degeneration in Osborne-Mendel Rats

Previous Serial Number: Same

Principal Investigator: Ludwig von Sallmann, M.D.

Other Investigators: Patricia A. Grimes, B.A.

Cooperating Units: None

Man Years:

Total:	0.4
Professional:	0.2
Others:	0.2

Project Description:

Objectives: To study the development of the retinal degeneration of mature Osborne-Mendel rats, and to compare this progressive retinal destruction with a possibly similar degeneration reported to occur in a strain of spontaneously hypertensive Wistar rats.

Methods Employed: The presence and extent of retinal degeneration was determined by histological examination of rats of various ages from four colonies maintained by the NIH Animal Production Unit: inbred Osborne-Mendel (OM/N), non-inbred Osborne-Mendel (OM), spontaneously hypertensive Wistar (SHR/N), and non-hypertensive Wistar (WsKy/N). The spontaneously hypertensive animals bred at NIH originated directly from the line of hypertensive Wistar rats developed in Japan. The non-hypertensive Wistar rats descend from the original Wistar colony at Kyoto University from which the SHR were isolated.

Major Findings: We have previously reported that retinal degeneration affects approximately 70% of mature Osborne-Mendel rats and spontaneously hypertensive Wistar rats maintained at NIH. The degeneration which appears after the age of six months is characterized by progressive loss of photoreceptors and appears to be similar in both strains of rats.

Study of the disease in Osborne-Mendel rats has been hampered by the lack of suitable control animals. However, further examination of the WsKy/N colony, a non-hypertensive control for the SHR/N strain, indicates a very much lower incidence of retinal degeneration. Ten of sixteen SHR/N killed between the ages of 8 and 15 months demonstrated retinal degeneration, whereas only one of 15 WsKy/N of comparable ages was affected.

In view of the emphasis in recent years on the damaging effects of light on the rat retina, and particularly of continuous exposure to relatively low levels of illumination, we have begun to investigate the susceptibility of these three strains to light damage. Results of preliminary experiments in which rats were kept under normal animal room illumination 24 hours per day for 5 to 7 days demonstrated that WsKy/N were relatively resistant to light-induced photoreceptor destruction when compared with SHR/N and OM/N.

Significance to Biomedical Research and the Program of the Institute: The retinal degeneration observed in these rats resembles certain human retinal degenerations more closely than do other types of animal retinal dystrophies. It may provide a valuable model for identification of the cause of photoreceptor degeneration in such conditions.

Proposed Course of the Project: We will continue to study the rat colonies affected with retinal degeneration to evaluate the influences of genetic and environmental factors in development of the disease with particular attention to the contribution of light.

Honors and Awards: None

Publications:

von Sallmann, L. and Grimes, P.: Retinal degeneration in mature rats. Comparison of the disease in an Osborne Mendel and a spontaneously hypertensive Wistar strain. Invest. Ophthal. 13: 1010-1015, 1974.

Project No. Z01 NS 01945-04 LNP
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of Neural Organization of the Vertebrate Retina

Previous Serial Number: Same

Principal Investigator: Elliott J. Simon, M.D.
Amrei Richter, Ph.D.

Other Investigators: M.G.F. Fuortes, M.D.
Paul O'Bryan, Ph.D.

Cooperating Units: None

Man Years:

Total:	0
Professional:	0
Others:	0

Project Description:

This project has been terminated.

Project No. Z01 NS 02017-03 LNP
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechano-Transduction in Invertebrate Hair Cells

Previous Serial Number: Same

Principal Investigator: Peter Detwiler, Ph.D.

Other Investigators: M.G.F. Fuortes, M.D.

Cooperating Units: None

Man Years:

Total:	0
Professional:	0
Others:	0

Project Description:

This project has been terminated.

Project No. Z01 NS 02018-03 LNP
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Ionic and Pharmacologic Basis of the Inhibitory Interaction Between Photoreceptors of a Simple Visual System

Previous Serial Number: Same

Principal Investigator: Leona M. Masukawa

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0
Professional:	0
Others:	0

Project Description:

This project has been terminated.

Project No. Z01 NS 02153-01 LNP
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Description of an Oscillatory State in the Vertebrate Retina

Previous Serial Number: None

Principal Investigator: Richard A. Normann, Ph.D.

Other Investigators: Jiri Pochobradsky, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.9
Professional:	1.9
Others:	0

Project Description:

Objectives: The principal objectives are 1) to describe the conditions which elicit the oscillatory state and 2) to determine whether the oscillatory state is a single cell or a network phenomenon.

Methods Employed: Intracellular recordings were performed in the eyecup of Bufo Marinus using high resistance microelectrodes. Retinal temperature was controlled with a Peltier device. The retina was kept in an oxygenated or anaerobic state by the use of externally applied gasses. Retinal stimulation was accomplished with a two-channel photostimulator. The color, size, shape, and intensity of the light stimulation could be controlled independently in the two channels.

Major Findings: The retinal neurons of Bufo Marinus can exist in at least two states. In the classical state, the responses recorded from the rod photoreceptors, the horizontal cells, the bipolar cells and the ganglion cells are similar to those described in other vertebrate retinae. The responses are most similar to those recorded in the mudpuppy, Necturus Maculosus but exhibit somewhat slower kinetics than those recorded

in the two species of turtle, Chelydra Serpentina and Pseudemys Scripta Elegans. The other state is characterized by oscillatory responses. In this state, the retinal neurons of Bufo and Necturus exhibit either transient oscillations following test flash stimulation or sustained oscillations which can persist for up to fifteen minutes. The rod oscillations are quite sinusoidal in character in either case. The horizontal cell oscillations usually exhibit rectification. Oscillating components which are more depolarizing than the dark-adapted baseline were observed while hyperpolarizing components were absent. This oscillatory state is transient and lasts typically for three to fifteen minutes. Before and after the oscillatory state is observed, the more classical behavior occurs.

The stimulus conditions which are optimal to elicit oscillatory responses when the retina is in its oscillatory state are as follows: 1) very intense 20 msec stimulation (5.5 log units above threshold), 2) moderately bright stimulation superimposed over a very dim background illumination and, 3) dim test flashes preceeded by bright preadapting flashes. All three of these conditions have one common feature; they cause desensitization and hyperpolarization of rod photoreceptors. We have tried to mimic this state by artifically hyperpolarizing the rods with extrinsic current but have failed to elicit an oscillatory response.

The frequency of the oscillations (approximately 2.2 Hz at 20°C) recorded in rods and horizontal cells is quite constant throughout a given experiment but varies somewhat from animal to animal. Frequency does however, have a high dependence on temperature. Changing retinal temperature from 20°C to 15°C prolongs the period of the oscillations by a factor of approximately 2.

The sustained oscillatory state can be inhibited by moderate to bright test flashes or by moderate background illumination. Thus, very dim backgrounds augment the oscillations but further increases in background level by as little as 0.7 log units will completely inhibit them.

Modulation of metabolic activity was accomplished by switching from an oxygenated to an inert environment. While this affected the resting potentials and the response amplitudes of the rods, horizontal cells, bipolar cells and their receptive field properties, it did not elicit the oscillatory state. Changing the retinal temperature in the range from 30°C to 10°C did not elicit the oscillatory state.

Interaction between receptors and second order neurons was suggested as a possible factor in the oscillations on the basis of receptive field measurements. Rod oscillations were not triggered by light in the form of 60 micron spots but were observed for 1200 micron spots even though the amplitudes and kinetics of the rod responses to the big and small spots were quite similar.

Significance to Bio-medical Research and the Program of the Institute: This project has significance at two levels. First, at the basic level, the oscillatory state can be used to obtain information about neural interactions in the retina, i.e., do the oscillations result from excitatory and inhibitory interactions between the rods and horizontal cells or is the oscillatory phenomenon a result of a feedback mechanism working in the single cell? Secondly, at the applied level, the retinal oscillations which occur spontaneously and transiently might serve as a model system for testing drugs used as antiepileptics. Also, if the oscillations result from neural interactions, a detailed understanding of the oscillations and the conditions which generate the oscillatory state might provide basic insight into the mechanisms of epilepsy.

Proposed Course of the Project: The possible correlation between spreading depression and the oscillatory state will be investigated using infrared visualization of the retina during the course of the experiment. If a correlation can be found, agents which inhibit (glutamate) or enhance (potassium) spreading depression will be applied to the retina in a perfusion system. Also, synaptic blockers such as cobalt will be used to help answer the question of whether the oscillations are a single cell or a network phenomenon. Further receptive field measurements of oscillating neurons will be performed to obtain more information about network involvement in the oscillations. Finally, anti-epileptic agents (barbituates, hydantoins, etc.) can be perfused during the oscillatory state and their effect and mechanism of action can be determined.

Honors and Awards: None

Publications: None

Project No. Z01 NS 02154-01 LNP
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Md. 20014

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Organization of Vertebrate Retinal Neurons

Previous Serial Number: None

Principal Investigator: Paul M. O'Bryan, Jr., Ph.D.

Other Investigators: Guiliana Bertrand, Ph.D.
M.G.F. Fuortes, M.D.
Walter Stewart[1]

Cooperating Units: [1]Laboratory of Experimental Pathology, NIAMDD

Man Years:

Total:	2.4
Professional:	2.4
Others:	0

Project Description:

Objectives: The objective of this study is to determine the characteristics of the electrical signals generated by vertebrate retinal neurons and to establish how these signals are affected by changes in the chromatic and spatial composition of the light. Experiments of this type will provide a basis for determining how the responses of various retinal neurons are modified by synaptic interactions, which cells are mediating the interactions and the mechanisms underlying these interactions.

Methods Employed: The electrical responses of neurons in the retina of the turtle Pseduemys scripta elegans are recorded with fine intracellular micropipettes. Light stimuli of varying spatial and chromatic composition are delivered to the retina in order to determine the physiological characteristics of the responses (i.e. receptive field and wavelength dependence). A correlation of the physiological characteristics with the morphology of the various neuronal classes is made by staining the cell recorded from with a dye passed from the recording electrode. The dye used is a flourescent stain developed by Mr. Walter Stewart of LEP/NIAMDD which appears to be far superior to the previously used intracellular stain Procion yellow.

Major Findings: To date experiments have concentrated on staining cells with the dye mentioned above. Because this dye has a higher quantum yield and appears to diffuse throughout the cell more readily than Procion yellow it provides a means of making more precise correlations between the physiological characteristics of the cells and their fine structure.

Previous studies have shown that the response of cones in the turtle retina are modified by two types of synaptic interactions due to activity in surrounding cones. One of these interactions, an enhancement of the response with illumination of neighboring cones appears to result from electrical coupling between cones. The dye used for staining appears in some cases to cross electrical junctions and thus to stain the cells coupled to the one from which recordings are taken. Dye injection into a red cone which showed the enhancement of the response resulted in the appearance of a heavily stained central cone surrounded by a hexagonal array of six lightly stained cone nuclei around this cell. Further experiments on this point are required and if successful should provide a basis for determining the number of cones which are coupled in a given area.

Staining of horizontal cells has also been attempted. Previously it was shown by Simon (J. Physiol. 1973) that, based on receptive field size, there are two distinct classes of luminosity horizontal cells in the turtle retina which could be correlated with two distinct morphological classes of cells. Recently, working in the salamander retina Lasansky and Vallerga found luminosity horizontal cells with similar properties. However, they also found indications that while morphologically two classes of cells could be stained these often appeared to be coupled by a thin axon. This suggests that these two classes may in fact be part of the same cell, one being the cell body and the second being only a local enlargement of the dendritic tree of the cell which is isolated from the cell body. Marks in horizontal cells of the turtle retina have shown similar characteristics in a couple of cases; however, this result is not consistently found and further experiments are required to clarify this point.

Significance to Biomedical Research and the Program of the Institute: The increased resolution of the newly developed dye provides a means of making more precise correlation between the physiological and morphological characteristics of retinal neurons. Thus, it should be possible to more accurately determine the synaptic organization of retinal neurons and therefore to define the mechanisms underlying the processing of information about the spectral and spatial characteristics of light falling on the retina by these cells.

Proposed Course of the Project: Intracellular staining experiments will be continued in order to resolve the questions mentioned above. Secondly, attempts will be made to record from and stain cells which synapse in the inner plexiform layer (i.e. amacrine, bipolar and ganglion cells) in order to develop physiological criteria for the identification of their responses, to determine their response characteristic under varying stimulus conditions and to define their synaptic organization.

Keyword Descriptors:

synaptic interactions
intracellular staining
cones
horizontal cells
bipolar cell
amacrine cell
ganglion cell
morphology
receptive field
wavelength dependence

Honors and Awards: None

Publications: None

Project No. Z01 NS 02155-01 LNP
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Organization of a Molluscan Vestibular System

Previous Serial Number: None

Principal Investigator: Roberto Fioravanti, Ph.D.

Other Investigators: M.G.F. Fuortes, M.D.

Cooperating Units: None

Man Years:

Total:	2
Professional:	1.2
Others:	0.0

Project Description:

Objectives: The principal objectives are: 1) to elucidate the mechanism involved in the development of the generator potentials of hair cells and 2) to investigate the responses to accelleration of central nervous system neurons of Hermissenda crassicornis.

Methods Employed: Intracellular recording from single hair cell and from neurons in the pedal and cerebral ganglion has been used.

Stimulation of the hair cells is accomplished by tilting the preparation, by applying sinusoidal accellerations or by delivering brisk taps.

Major Findings: Tilting of the preparation up to ± 180° caused a marked change in the noise as well as in the firing of the hair cells. The same procedures may evoke firing of central neurons. Transduction of sinusoidal accelleration of the cist involves strong non-linearities causing two sharp responses per cycle whenever threshold is exceeded. These repsonses occur when the force reaches a maximum, independently of the sign. This finding suggests that the transduction process is mediated by a critical bending of the hairs. Taps elicit large generator potentials in the hair cell at the top of the statocyst while the hair cells at the bottom (which are covered by statoconia)

respond weakly to this type of stimulus. One giant neuron in the pedal ganglion has been identified, which develops synaptic potentials and nerve impulses following these same stimuli.

Significance to Biomedical Research and the Program of the Institute: The present studies might contribute to our understanding of the responses of hair cells such as those of cochlea and vestibular receptors. Also they may lead to new insights on the organization of the nervous system.

Proposed Course of the Project: The comparative analysis with different stimulation will be continued in order to confirm this preliminary finding.

Keyword Descriptors:

generator potential
hair cell
noise
neuron
statocyst

Honors and Awards: None

Publications: None

Project No. Z01 NS 02157-01 LNP
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Sensory Receptors in Hermissenda Crassicornis

Previous Serial Number: None

Principal Investigator: Peter B. Detwiler, Ph.D.

Other Investigators: M.G.F. Fuortes, M.D.

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.5
Others:	0

Project Description:

Objectives: To study the processes underlying the transduction of mechanical and photic stimuli by hair cells and photoreceptors, respectively, in the invertebrate Hermissenda crassicornis. Both types of sensory receptors are sufficiently large to be reliably recorded from with intracellular electrodes under a variety of experimental conditions.

Methods Employed: Hair cells in the statocyst (a primitive vestibular organ) of Hermissenda were stimulated with a pizoelectrically driven glass stylus. Photoreceptors were stimulated with varying intensities of white light.

Major Findings: A. Hair cells:

1. The hair cell response to a brief mechanical stimulus is a depolarizing wave that reaches peak in about 25 msec and decays slowly. Hyperpolarization by extrinsic currents increase the amplitude of the response and depolarizing currents decrease and eventually reverse its polarity. It is inferred from these results that the primary outcome of the transduction process is an increase in membrane conductance and that the voltage change follows as a secondary event.

2. The features of the conductance change were reconstructed from the time course of the generator potential and the passive properties of the membrane. It was found that the increase of membrane conductance develops slowly and is roughly proportional to the energy delivered by the stimulus.

3. The time course of the conductance change required to reproduce the generator potential is similar to the output of a model involving a sequence of transformations.

4. The generator potential is sensitive to temperature, becomin faster as temperature is raised. These effects are reproduced by the model if the transition rates are assumed to be temperature-dependent, with a Q_{10} of about 2.

5. It is concluded that the hair cell responses are probably not a direct consequence of the deformation of their membrane. It is proposed instead that intermediary processes are interpose between the deformation and the conductance increase of the membrane.

B. Photoreceptors:

1. The photoreceptor responds to a brief flash of white light with a triphasic potential change. The response involves an initial large depolarization followed by a hyperpolarization which is in turn followed by smaller and slower depolarization. Studies of the effects of extrinsic currents on the response reveal that the initial depolarization and hyperpolarization are the consequence of conductance increases and that the slow depolarization arises from a conductance decrease.

2. Ion substitution experiments show that the initial depolarization involves mainly an increase in Na^+ permeability and that the hyperpolarization and slow depolarization involve an increase and decrease, respectively, of K^+ permeability. Furthermore, the changes in K^+ conductance appear to be a secondary effect of changes in intracellular Ca^{++} concentration; an increase in intracellular Ca^{++} increases K^+ permeability and a decrease in intracellular Ca^{++} decreases K^+ permeability.

3. These results suggest that in Hermissenda photoreceptors light causes a) an increase in membrane permeability to Na^+ and b) an increase in intracellular Ca^{++} concentration via either an increase in Ca^{++} permeability and/or a release of intracellularly bound Ca^{++}. The changes in the intracellular Ca^{++} concentration have a secondary effect on K^+ permeability which tends to restore the membrane potential to its original level.

Significance to Bio-medical Research and the Program of the Institute: One of the most fundamental questions concerning any sensory system is 'How is the energy of the applied stimulus transduced into a change in membrane potential,' or in other word's 'How is the chain of events that is culminated in a sensory perception initiated?' The answer to this important question is at the present time unknown. The reported experiments were undertaken with the hope in mind of furthering our understanding of the steps involved in the transduction of mechanical and photic stimuli.

Proposed Course of the Project: The experiments outlined above will be refined, completed, and written up during next several months.

Honors and Awards: None

Publications:

Detwiler, P.B. and Fuortes, M.G.F.: Responses of hair cells in the statocyst of Hermissenda. J. Physiol. In press.

Project No. Z01 NS 01892-05 LNP
1. Laboratory of Neurophysiology
2. Section on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Comparative Physiology of Invertebrate Photoreceptors

Previous Serial Number: Same

Principal Investigator: John S. McReynolds, M.D.

Other Investigators: Anthony L.F. Gorman, Ph.D.

Cooperating Units: Department of Neurobiology
Marine Biological Laboratory
Woods Hole, Massachusetts

Man Years:

Total:	0
Professional:	0
Others:	0

Project Description:

This project has been terminated.

Project No. Z01 NS 01895-05 LNP
1. Laboratory of Neurophysiology
2. Section on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Microspectrophotometric Studies of Vertebrate Photoreceptors

Previous Serial Number: Same

Principal Investigator: Ferenc I. Harosi, Ph.D.

Other Investigators: W.K. Stell, M.D., Ph.D.
D.O. Lightfoot, B.S.
S.H. Orkin, M.D.
P. Leder, M.D.

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: A. Part of this project is intended to reveal the optical properties of vertebrate photoreceptors as they pertain to visual pigment content and photic excitation. B. Another facet of the project is aimed at establishing the principles of cellular interconnections of a retina. C. The third part of the project is concerned with cellular differentiation.

Methods Employed: The main technique used throughout the project is single-cell microspectrophotometry (MSP). Thin preparations are made by mounting cell suspensions of various composition between two cover glasses. Under microscopic examination cells are selected and measured one by one for their absorption of linearly polarized light. The recorded absorptance spectrum, the measured cellular dimensions and the determined linear dichroism permit the estimation of pigment concentration in each cell.

Major Findings: A. Studies on the spectroscopic properties of visual pigments. MSP measurements of absorption spectra of rod cells from mudpuppies, larval and adult tiger salamanders and tropical toads revealed that the visual pigment concentration in such dark-adapted photoreceptors is approximately 3.5 mM, regard-

less of type. Further evidence was found to support the earlier hypothesis of invariance of oscillator strength (see my Project Report of 1972-1973) to exist within each of the two pigment families. This means that the nomogram principle of Dartnall (1953) should no longer be considered universally valid for the interpretation of spectral sensitivity curves. Instead of two standard shapes of extinction curves, two standard areas (oscillator strengths) need to be considered, which are proportional to the products of molar absorptivity and spectral bandwidth of each visual pigment, throughout the two families of pigments.

B. Studies on Cone Cell Morphology. The goldfish retina was found (in collaboration with W.K. Stell and D.O. Lightfoot) to have five types of cones: double with long and short members, long single, short single, and miniature. Visual pigment determinations by MSP showed that the long member of double cones contain a red-sensitive pigment, the short member of double cones a green-sensitive pigment, and the short single cone a blue-sensitive pigment. Although the pigment identification in long single cones is less certain, their content appear to be either red-, or green-sensitive. So far it has not been possible to measure the miniature type. Technical improvements in instrumentation are, however, being implemented so that the limit of measurement may be extended to this type of cone as well.

C. Studies Concerning Cellular Differentiation. Tissue-cultured cells of mouse leukocytes were used (in collaboration with S.H. Orkin and P. Leder) to study the control of gene expression. Those cells, in response to certain treatments (i.e. viruses, chemicals), can be induced to synthesize hemoglobin. It was found that globin gene expression is an all-or-none phenomenon, and that observable differences between clone lines are due to varying proportions of cells participating in erythroid differentiation rather than to uniform gradations in cell response. This property of a clone was termed its "probability of differentiation." The MSP technique enabled us to estimate the hemoglobin concentration in individual cells and to determine the distribution of erythro-differentiating cells among the population of various clones. Cell hybridization and cloning experiments indicated that this phenotype must be controlled by rather stable genetic factors.

Significance to Biomedical Research and the Program of the Institute: Knowledge of the spectroscopic properties of visual pigments are necessary so that physiological responses (spectral sensitivity curves) may be properly interpreted. The establishment of morphological markers are important in facilitating the description of neuronal interconnections in vertebrate retinas. Whereas the former part of the project deals with a fundamental aspect of visual excitation, the latter one is basic for an

understanding of retinal processing of visual information.

Proposed Course of the Project: 1) To continue studying single vertebrate photoreceptors, such as the rods of Xenopus laevis, for spectral properties of the original visual pigment and some in vitro regenerated pigments. This is aimed at obtaining further evidence to elucidate earlier hypotheses (proposed in previous years). 2) To continue studies of cone morphology in the goldfish retina (in collaboration with W.K. Stell) with improved instrumentation, so that all the cone connections to other cells may be specified. 3) To study the optical properties of retinas, both theoretically and experimentally, to elucidate the important aspects of light propagation within them, such as wave-guiding and anomalous dispersion. 4) To conduct chemical studies on isolated photoreceptors which are aimed at probing the mechanism of photo-excitation.

Keyword Descriptors:

photoreceptors
visual pigments
microspectrophotometry
rod cells
absorption spectra
cone morphology
linear dichroism
spectral bandwidth
oscillator strength
hemoglobin
cellular differentiation

Honors and Awards: None

Publications:

Harosi, F.I. and MacNichol, Jr.: Dichroic microspectrophotometer: A computer assisted, rapid, wavelength-scanning photometer for measuring linear dichroism in single cells. J. Opt. Soc. Am. 64: 903-918, 1974.

Harosi, F.I. and Malerba, F.E.: Plane-polarized light in microspectrophotometry. Vision Res. 15: 379-388, 1975.

Orkin, S.H., Harosi, F.I. and Leder, P.: Differentiation in erythroleukomic cells and their somatic hybrids. Proc. Nat. Acad. Sci. USA 72: 98-102, 1975.

Harosi, F.I.: Microspectrophotometry: the technique and some of its pitfalls. In Ali, M.A. (Ed.): Vision in Fishes: New Approaches in Research. New York, Plenum. In press.

Harosi, F.I.: Linear dichroism of rods and cones. In Ali, M.A. (Ed.): Vision in Fishes: New Approaches in Research. New York, Plenum. In press.

Harosi, F.I.: Absorption spectra and linear dichroism of some amphibian photoreceptors. J. Gen. Physiol. In press.

Project No. Z01 NS 02152-01 LNP
1. Laboratory of Neurophysiology
2. Sec. on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Neural Connections in the Retina

Previous Serial Number: None

Principal Investigator: Helga Kolb, Ph.D.

Other Investigators: None

Cooperating Units: Edward V. Famiglietti, Jr., M.D., Ph.D. (NEI)
Ralph Nelson, Ph.D. (NEI)
Donald Bergsma, M.D. (NEI)
Ralph Rosenthal, M.D. (Stanford University)

Man Years:

Total:	1.0
Professional:	1.0
Others:	0

Project Description:

Objectives: The object of this study is to understand the neural circuitry of the retina.

Methods Employed: Retinae were studied by 1) light microscopy of Golgi-impregnated material, 2) electron microscopy of Golgi-impregnated material and 3) by ultra-thin serial sectioning techniques.

Major Findings: By studying the light microscopic appearance of Golgi-impregnated cells of the cat retina we have been able to understand the morphologies of the cell types contributing to the inner plexiform layer (IPL) of the cat retina. Analysis of cat ganglion cells with the aid of a PDP 12 computer has allowed significant morphological criteria which might otherwise have been obscured because of section tilt, to be established for three classes of ganglion cell. The significance of stratification and dendritic morphology of bipolar axon terminals, amacrine cells and ganglion cells have become apparent.

Serial sectioning techniques for the electron microscope has elucidated some of the micro-circuitry of the relationships

between bipolar and ganglion cells and the importance of amacrine cell pathways. Rod bipolars do not synapse directly on ganglion cells but use instead a small, bistratified amacrine cell to get information to ganglion cells. Cone bipolars are segregated by ending in different strata of the IPL and consequently segregate their input to ganglion cells that branch only in the corresponding strata. Thus small tufted ganglion cells ending in the outer 1/3 of the IPL receive mainly bipolar input and from flat bipolars only, while tufted ganglion cells ending in the lower 2/3 of the IPL receive input from invaginating cone bipolars only. Most radiate ganglion cells have their dendrites narrowly stratified in one of five strata of the IPL and the EM analysis shows that their input is predominantly from amacrine cells.

Another ongoing project is in collaboration with Dr. Rosenthal of Stanford Medical School. This investigation is studying the pathology caused to the retina by chloroquine therapy in rhesus monkey. The initial changes are the appearance of membranous cytoplasmic bodies in the retinal neurons. After 2 years of high dosage chloroquine isolated groups of retinal neurons show advanced pathology but the clinical observations are still within normal limits.

Significance to Bio-medical Research and the Program of the Institute: Studies of the structure of the retina will provide a valuable understanding of the connections of the cells within the retina and will in all probability relate to neural circuitry elsewhere in the CNS. Many programs of this Institute are concerned with the physiology and marking of single retinal neurons and thus knowing the morphology and connectivity of these same neurons is essential for our further understanding of visual events.

Proposed Course of the Project: It is proposed to continue the study of the structure of the retina to obtain further insights into the relevance of structure to function in the CNS. In addition we plan to finish the chloroquine study which will help us to understand the human condition of chloroquine retinopathy.

Honors and Awards: None

Publications:

Kolb, H. and Gouras, P.: Electron microscopic observations of human retinitis pigmentosa; dominantly inherited. Invest. Ophthal. 13: 487, 1974.

Kolb, H. and Famiglietti, E.V.: Rod and cone bipolar connections in the inner plexiform layer of the cat retina. Science 186: 47, 1974.

Famiglietti, E.V. and Kolb, H.: A bistratified amacrine, its gap junctions and synaptic circuitry in the inner plexiform layer of cat retina. Brain Res. 84: 293, 1975.

Nelson, R., Lutzow, A., Kolb, H. and Gouras, P.: Horizontal cells in rat retina with independent dendritic systems. Science In press.

Boycott, B.B., Dowling, J.E., Fisher, S.K., Kolb, H. and Laties, A.M.: Interplexiform cells of the mammalian retina and their comparison with catecholamine-containing retinal cells. Proc. Roy. Soc. In press.

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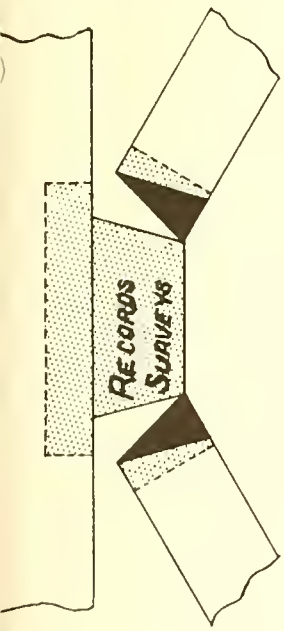

Annual Report
July 1, 1974 through June 30, 1975
Laboratory of Biophysics, IR
National Institute of Neurological and Communicative Diseases and Stroke

William J. Adelman, Jr., Chief

Introduction

The research program of the Laboratory of Biophysics is concerned with uncovering basic molecular and cellular mechanisms associated with the generation of the nerve impulse, sensory transduction in neural receptors, synaptic activity and information processing in simple nervous systems. In order to pursue these goals, physical, chemical, and mathematical methods are employed to solve biological problems. The Laboratory makes use of a number of interesting preparations. These include giant nerve axons from Loligo and Myxicola, isolated synapses in Loligo and Hermissenda, giant muscle fibers from Balanus, tissue cultured muscle fibers from chick embryos, photoreceptors, hair cells and the isolated small nervous system of Hermissenda, as well as artificial lipid bilayer membranes. The techniques used to examine these preparations involve voltage clamp, impedance, electrical noise and electrophysiological measurements which are frequently analysed in terms of physical and mathematical models. In addition to the program in Bethesda, the Laboratory maintains an active summer research program at the Marine Biological Laboratory in Woods Hole, Mass. The Laboratory has continued its collaborative programs with Carnegie-Mellon University, the University of Cincinnati, the University of Rochester, the State Universities of New York at Binghamton and Buffalo, the Eastern Pennsylvania Psychiatric Institute, Juniata College, the University of Pennsylvania, the Technion-Israel Medical School, Cambridge University, England and the University of Chile.

During the fiscal year a major review lecture was given by one member of the Laboratory at the New York Academy of Sciences, two members received invitations to present lectures in England and Germany, one member has been invited to present a plenary session paper at the Society for Neuroscience 1975 meeting in New York, and one member has been invited to prepare a paper for presentation to the New York Academy of Sciences. A comprehensive review on excitable membranes has been published in the 1974 Annual Review of Physical Chemistry, and another review on ion competition, saturation and inhibition in excitable mem-

branes has been completed for publication in Current Topics in Membranes and Transport. One member of the Laboratory will be Chairman of a Gordon conference this coming summer. The following are some highlights of an interesting year of research:

Rapid Analysis of Excitable Membranes by Means of Computer Controlled Voltage Clamp.

In 1971 plans were made to develop modern systems for controlling voltage clamp experiments and acquiring excitable membrane conductance data in a form that could be analyzed by digital computing machines. At present the Laboratory has two operational systems, one rather small system at the Marine Biological Laboratory in Woods Hole, Mass. and a larger more versatile system in Bethesda, Maryland. These systems have been used in experiments on ion competition and inhibition in potassium channels in Loligo giant axons, on ionic selectivity in sodium channels in Myxicola giant axons, and on the voltage dependence of gating currents in Balanus giant muscle fibers.

In accordance with the previous long range planning to modernize the data acquisition and reduction facilities of the Laboratory, a major effort during the past year has been the development of additional systems. This has involved the integration of general purpose data acquisition equipment into dedicated systems, suitable for controlling and, acquiring data from the neurophysiological experiments conducted by the various investigators in the Laboratory. Two new systems have been completely designed and are being implemented. The first system uses a mini-computer as the control unit and magnetic tape for data storage. This system will offer investigators complete facilities for automated pulse train generation while acquiring data at any one of a wide selection of sampling rates. The second system is centered around a digital oscilloscope. This instrument has the capability of acquiring, digitizing and storing data with 12 bit accuracy at various sampling rate up to 1 Megahertz. It has been interfaced with a magnetic tape transport for bulk storage and has read-back capability. A teletypewriter together with custom clocking and control circuitry will provide system control and pulse generation capability. It is expected that this system will be highly utilized since it affords investigators a unique "quick look" at acquired data without the use of any data reduction equipment.

Function and Structure of Ionic Channels: Ion Interactions and Gating Mechanisms

We have made a detailed study using the squid giant axon of the blocking of inward K currents by external cesium ions. The blocking is voltage dependent, causing a region of negative slope in the instantane-

ous current-voltage relation for sufficiently negative transmembrane potentials. The concentration dependence of the blocking, in a range of [Cs] from 5 to 200 mM, is reflected in the reduced current magnitudes and in the appearance of the negative resistance region at voltages closer and closer to zero as the Cs concentration is increased. Although the current-voltage relations have the same qualitative form that is predicted for simple competition between Cs and K, at least two features of the data are inconsistent with a simple, single-site channel model: (a) the blocking is more steeply dependent on [Cs] than predicted, and (b) the voltage dependence of blocking changes with changing [Cs], becoming steeper as [Cs] increases. These observations appear to be qualitatively consistent with the idea that more than one Cs ion can occupy sites in a single K channel at a given time, and that the site of blocking is beyond the first site reached by an ion entering the K channel from the outside. These results are to be reported at the Vth International Biophysics Congress in Copenhagen in August, 1975.

In a collaborative study with the State University of New York at Binghamton and Juniata College, we have found that the lanthanide ions La^{3+}, Eu^{3+} and Yb^{3+} cause major kinetic changes in the sodium and potassium currents for voltage clamped squid giant axon. As the radius of the lanthanide ion species becomes smaller (due to the lanthanide contraction), these changes, as quantitized in the Hodgkin-Huxley parameters. become significantly more pronounced. The kinetic parameters are shifted approximately 10 to 15 mV as 0.1 mM solutions of the lanthanides range from La to Yb. This shift is observed even though the ions have identical charge and very similar chemical properties, and is probably related to asize dependence for ions bound to the surface.

In a project done in collaboration with the Eastern Pennsylvania Psychiatric Institute possible analogies between the kinetics of ion currents across axon membranes and those seen in lipid bilayer preparations treated with channel forming agents have been investigated. The most interesting finding in this study has been the "inertia" in the rate of change of the potassium ion conductance. This finding suggests that an aggregation model used to describe the time and voltage dependent conductances produced by some channel forming agents in lipid bilayer membranes may be applicable to ionic channels in nerve membranes.

In another collaborative study done with the University of Rochester, calcium currents through sodium channels in the squid axon membrane were studied. This study included the effects of external divalent ions on sodium currents through the sodium channel.

Function and Structure of Ionic Channels: Pharmacology and Ionic Selectivity.

In a series of ion selectivity studies on sodium channels in Myxicola giant axons, close agreement was found for ion selectivity sequences between Myxicola channels and frog node channels. Of particular interest has been the selectivity of hydrazine and hydroxylanine. The object of this study has been to probe ion channel dimensions, particularly the diameter. This is based on the theory that only ions which approximate the channel diameter closely show high selectivity.

It has been previously observed in experiments that mercury, which is a known blocker of sulfhydryl groups, decreases the ionic currents in the voltage clamped squid giant axon. Some slight recovery using the sulfhydryl compound, 2-mercaptoethanol, was observed. In our present studies, the effects of silver on the ionic currents of the squid giant axon was investigated. Using μM silver it was observed that similar inhibition of the ionic currents occurred; and that 2-mercaptoethanol produced some slight recovery.

It has been suggested that the asymetrical distribution of lipids across the biological membrane might be attributed to the voltage difference across the membrane. This is based on phospholipid flip-flop in the membrane; i.e., the charged phospholipids would redistribute on the two sides of the membrane according to a change in the electric field across the membrane. This hypothesis was tested by measuring the change in surface charge density caused by long-term membrane depolarization. If charged phospholipids are the source of membrane surface charge, then membrane depolarization to zero membrane potential should cause a flip-flop resulting in a symmetrical distribution of charged phospholipids. The method of determining the change in surface charge density was to measure the shift along the voltage axis of potassium conductance and potassium time constant. Both parameters showed no shift. This demonstrates that the surface charge in the region of axon potassium channels is not composed of phospholipids or else the notions of phospholipid flip-flop inferred from spin label methods are not applicable. (This work was performed in collaboration with the University of Cincinnati.)

In another set of experiments, squid giant axons were subjected to about 150 atmospheres of helium. There was little or no change in the maximum sodium and potassium conductances. The action potentials were prolonged without changing the threshold and the resting potential was not changed. The sodium and potassium currents were prolonged. The effects could be qualitatively duplicated using a computer analysis of the Hodgkin-Huxley equations by decreasing the rate constants of the m and n processes.

The effects of Aconitine on the ionic currents has been tested. This substance has been shown to produce a depolarization of the resting cell membrane due to an increase in sodium permeability and induces repetitive electrical activity. The voltage-clamped sodium and potassium currents were decreased. Thus, this observation lends support to the idea that there are separate resting and active sodium channels. A negative resistance region sometimes occurs for hyperpolarizing pulses. This might be involved in repetitive activity.

Previously it has been shown that lowering the pH decreases the sodium conductance. We wanted to determine the pH of the normal squid blood as well as its temperature dependence. This buffer has a very high temperature dependence and is commonly used as a buffer in voltage-clamp experiments of the squid membrane. We have found that the pH dependence of squid blood upon temperature was -0.0208 pH units/C. At 23 C, the pH was estimated to be 7.16.

The potassium conductance time constant on frog nodes for the same final potential following different conditioning potentials has been determined. Although the Hodgkin-Huxley theory predicts that the time constant should be independent of the conditioning potential, the measurements showed that the time constant was about four times slower following hyperpolarizing potentials than following depolarizing potentials. This work was done in collaboration with a group from the Technion, Israel.

Electrical Fluctuations in Excitable Cells

The study of electrical noise induced by the application of Acetylcholine to tissue cultured muscle cells has been continued using an improved on-line data acquisition system. The initial electrical noise experiments demonstrated that postsynaptic excitation is caused by the activation of ionic channels, having a unit conductance of the order of 30-70 p mho, which open transiently with a lifetime of 1 msec at 35 C. Using computer-controlled iontophoretic application of Ach and on line Fast-Fourier analyses, these experiments were extended to measure the temperature and voltage dependence of the channel activation. Power spectra were measured on 46 preparations. The most striking new finding was the rather sharp temperature dependence of the channel closing rate. By comparing our noise data with the tracer-flux data of W. Caterall we can conclude that the Ach excitation is a two step process in which the actual opening of the ionic channel is preceded by an electrically silent transmitter-receptor complex. Several theoretical models of this process have been worked out which would explain the data. One such model requires two Ach molecules acting cooperatively to open a channel.

Lipid Bilayer Membranes: Simulation and Molecular Description of Excitability

The study of protein additives which induce excitability in synthetic lipid bilayer membranes has progressed to the stage at which the molecular basis of the excitation can be discerned. Our main emphasis has been the study of electric current pulses caused by the activation of individual ionic channels. Such studies have shown that channels induced by the bacterial toxin EIM, are electrolyte pores whose conductance is changed by discrete transitions, when the channel structure is under the stress of an applied potential. Because EIM channels are open in the absence of an applied membrane potential and can be closed by application of either positive or negative potential, a large potential jump can produce a transient burst of conductance analogous to the transient Na conductance observed during nerve excitation. Thus, three discrete states of an EIM channel have been demonstrated - a state of relatively high conductance, whose probability of occurrence is high at or near zero membrane potential, and two different states of relatively low conductance. One low-conductance state has a high probability of occurrence at large positive potentials and the other low-conductance state has a high probability of occurrence at large negative potentials. This three-state system simulates inactivation in nerve membranes. A model for the EIM system consistent with experimental data is that the high-conductance state occurs when the channel is in themiddle of the membrane and a low-conductance state occurs when the channel is on one or the other side of the membrane.

In collaboration with a group of scientists from the University of Chile hemocyanin channels in bilayers have been studied. Experiments on these hemocyanin channels demonstrate two different mechanisms for a voltage-dependent conductance. One mechanism - similar to that observed for EIM and alamethicin - is the voltage-dependent distribution of channels among several discrete single-channel conductance states. The other mechanism-never reported before- is the continuously voltage-dependent conductance of the single-channel states themselves. The relaxation time for the discrete conductance changes is of the order of seconds and the relaxation time of the continuous conductance changes is of the order of 10^{-4} seconds. As salt concentration in the bathing medium is increased, the single-channel conductance first increases linearly and then saturates. The characteristics of the saturation curves suggest that the continuous conductance changes occur at the edges of the channel, and that the mean time an ion spends in the channel is about 4 nanoseconds.

Experiments on bilayers with two different types of carriers or pores have been started - one type for cations and the other for anions. The purpose of these experiments was to determine the interactions between the two ion-transporting systems. Preliminary experiments have shown a megative interaction between valinomycin (a cation carrier) and molecular iodine (an anion carrier). It is planned to look for positive interactions that may be caused by coupling of the two types of ionic flows. Such a mechanism may explain recent flux data on the thin ascending limb of the Loop of Henle in rabbit kidney. Because of the need to

make flux measurements in our bilayer experiments, a new type of bilayer chamber has been designed and fabricated. This work was performed in collaboration with the Laboratory of Physical Biology, NIAMDD.

One of the paradoxes connected with the channels which have been studied in lipid bilayers is why their conductance is as high as it is. Consideration of the electrostatic work needed to bring an ion into a membrane pore suggests a much higher resistance for the channel than is actually observed. Resolution of this paradox requires a consideration of the attractive forces between the polar groups lining the pore and the permeant ion. Calculations have been undertaken for the gramicidin channel.

Mathematical Modeling

A theoretical study of anodal break excitation in the Hodgkin-Huxley equations shows the existence of a critical temperature (17.1 c) at which the threshold to anodal current stimuli of any wave form or duration goes to infinity. In contrast, cathodal thresholds rise continuously as temperature is increased to much higher values, with no indication of a critical temperature. The existence of a critical temperature depends on the saturation of the steady state functions of the conductance variables m, h, n, as functions of membrane potential, and thus would be expected in any nerve membrane model having this property.

The Hodgkin-Huxley equations were modified to include the properties of an external diffusion barrier separated from the axolemma by a thin periaxonal space in which potassium ions accumulate as a result of membrane activity. Computations showed improved agreement with experimental data on action potential shape, threshold, latency, and adaption.

A major emphasis in membrane noise studies has been the analysis of the relation between observed electrical current noise of excitable membranes and the underlying sources of conductance fluctuation. Excitation noise has been observed in the lab for chemically, optically and electrically excitable membranes and analysis of the noise spectrum gives the unit conductance and kinetic information for the ionic channels. However, electrically excitable membranes pose a difficult problem because the spectrum is distorted by the complicated nonlinear response of the membrane. A theoretical study has been made of these effects in the hope of designing more definite noise experiments for electrically excitable membranes.

Electrical noise can also be used as a diagnostic tool when it is the input to an excitable cell. An analysis of the white noise response of the giant axon has been published and shows how the noise response can be used as an alternative to AC impedance measurement. The analysis has been extended to the study of random spike trains in response to white noise inputs.

A corrected derivation was developed for the dependence of spherical aberrations on distance from the axis of symmetry of a lens. In particular, the reason for the characteristic r^3 dependence was shown to be the relations between radial and axial terms in Laplace's Equation.

The object of another study was to develop barrier models of ion permeation through channels. A barrier model for ion competition and saturation effects was developed. For a description of the experimental result of a test of this model on Cs ion blocking of potassium channels see "Function and Structure of Ionic Channel: Ion Interactions and Gating Mechanisms," above.

Subcellular and Extracellular Structures Associated with Nerve and Muscle

Mesosin is the name suggested for the protein or proteins comprising a large portion of the intermediate filaments (10 nm) found attached to dense bodies and in other portions of smooth muscle. Its amino acid composition is unique when compared to other fibrous proteins and the polypeptide chains derived from intermediate filaments are larger than many others. Morphologically similar intermediate filaments with 10 nm diameters have been reported present in axons, tissue cultured baby hamster kidney cells and developing striated muscle cells as well as other types of cells. Mesosin from smooth muscles has a remarkably high content of serine, glutamic acid and glycine and is readily distinguished from most other fibrous proteins by its amino acid composition. Squid axon axoplasm has been examined to see if a protein similar to mesosin exists in neural tissue. This study has been done in collaboration with Carnegie-Mellon University. Polypeptide chains of similar molecular weight to those found in other tissues are present in squid giant axon and these squid axon chains have amino acid compositions very similar to the same molecular weight chains found from smooth muscle. Although the true universality of mesosin awaits further evidence, it is felt that the striking similarity of the same molecular weight chains from such widely different cells as mammalian smooth muscle and squid axon presents a strong inference that mesosin is a universal fibrous portein.

Work on the anatomy of the Schwann sheath of giant axons has progressed toward the stage where convergence is expected with results from biophysical measurements. Values of series resistance across the sheath have been found to be similar with a variety of methods. Axoplasm resistance has been determined and reliable values are now available.

Information Processing in Simple Nervous Systems

The principal objective of this project is to study the mechanisms by which simple neural networks process information with particular emphasis on mechanisms of learning. The nervous system of Hermissenda crassicornis has proven to be an excellent model for information pro-

cessing at several levels: sensory transduction by photoreceptors and hair cells, analysis of synaptic circuitry, changes in synaptic circuitry produced by conditioning paradigns administered to intact animals as well as to isolated nervous systems, membrane properties modified by conditioning, identification of critical developmental stages for the neural networks studied as well as stages critical for learning.

Techniques employed thus far to pursue these questions include: simultaneous intracellular recording from multiple neural elements, paired stimulation of the visual and vestibular pathways using a rotating table, iontophonesis of fluorescent dyes. Other methods planned will allow biochemical and genetic approaches to the above problems. These include mariculture, subcellular fractionation, protein phosphorylation analysis, and uptake of neurotransmitter precursors.

Project No. ZO1-NS-01950-04 LB
1. Laboratory of Biophysics
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Analysis of Excitable Membrane Characteristics by Means of Computer Controlled Voltage Clamp Techniques and Impedance Measurements

Previous Serial Number: NCDS(I) 72 LB/OC 1950

Principal Investigators: W.J. Adelman, Jr., Ph.D., L. Binstock, M.S., K.S. Cole, Ph.D.

Other Investigators: B. Cox, Ph.D., J. Shaw, M.A., S. Takashima, Ph.D., H. Walters, R. FitzHugh, Ph.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Massachusetts
University of Pennsylvania, Philadelphia, Pa.
DCRT, NIH

Man Years:

Total:	3.4
Professional:	2.3
Other:	1.1

Project Description:

Objectives: (a) Rapid analysis of ionic channel conductances by means of computer controlled voltage clamp: to develop modern approaches and systems for controlling voltage clamp experiments and acquiring excitable membrane conductance data in a form that can be analyzed by digital computing machines; to develop programs for carrying out this analysis.

(b) Steady-state analysis by means of impedance measurements: the improvement of bridge impedance techniques and accuracy on axon membranes and investigation of effects of polarizations for comparison with step and ramp techniques and ion conduction models.

Methods Employed: (a) The general method has involved the integration of general purpose data acquisition equipment into dedicated systems, suitable for controlling and acquiring data from biophysical and neurophysiological experiments. The analysis of digitalized data by programs making use of DCRT facilities, such as MLAB running on PDP-10.

(b) An impedance bridge built in 1935 has been reactivated. The development of internal guarded electrodes has been carried forth.

Techniques are used for comparisons with the HH formulations, investigation of apparent anomalies in 1942 and later data and to provide direct comparisons of the advantages and limitations of a simple slow old-fashioned but precise, bridge relative to the new sophisticated, fast and complicated white noise cross-correlation hardware now available.

Major Findings: (a) The software and hardware has been changed and improved for use in the data acquisition system built in 1973 to computer control the voltage clamp for Myxicola axon. This improvement in the software makes the data acquisition easier as well as the analysis. The program and analysis are still essentially the same as that for squid developed earlier but with much greater reliability. Pulse program changes may be necessary for the Myxicola axon. The on-line graphics display unit and input device is now portable and operation of the computer is done at the site of the experiment. The new data and more accurate analysis will produce the Myxicola axon conductances for the various ion selectivity studies. Two new systems have been completely designed and are being implemented. The first system uses a mini-computer as the control unit and magnetic tape for data storage. This system will offer investigators complete facilities for automated pulse train generation while acquiring data at any one of a wide selection of sampling rates. The second system is centered around a digital oscilloscope. This instrument has the capability of acquiring, digitizing and storing data with 12 bit accuracy at various sampling rates up to 1 Megahertz. It has been interfaced with a magnetic tape transport for bulk storage and has read-back capability. A teletypewriter together with custom clocking and control circuitry will provide system control and pulse generation capability. It is expected that this system will be highly utilized since it affords investigators a unique "quick look" at acquired data without the use of any data reduction equipment. Software has been developed to utilize computing facilities at DCRT to analyse data collected with these on-line systems.

(b) In common with measurements on many other cell membranes over a half century, the first impedance data on the squid axon, 1928, could be interpreted in terms of a dielectric loss in the plasma membrane expressed by a mean constant phase angle of 77^{o}. This has been confirmed by similar losses in many subsequent investigations. It was to be expected that a corresponding complication would be found in transient results particularly from voltage and current clamp experiments. However, such calculations have been found difficult in the past with a conventional technique but a preliminary report was made in 1971. A novel rational approximation was found useful for midrange values and the work was published with abbreviated tables, and full results available on request, for a wide range of variables. This, unfortunately, was not included in the Laboratory of Biophysics report for FY74. After publication, attention was called to an article by Kaizumi and Kita in a 1973 Japanese Bulletin on the same problem. Spot checks showed perfect agreement but, with two minor exceptions, the range of their calculations equalled or exceeded the Laboratory of Biophysics 1974 publication.

A "Letter to the Editor" has been published calling attention to the earlier and more extensive article in order that it may be more widely known, appreciated and used.

Significance to Biomedical Research and the Program of the Institute:
(a) The use of the computer program controlled voltage clamp enables one to quickly collect data and do many experiments in a short time. These data acquisition systems are now interfaced to several laboratories performing rather different experiments. Many difficult experiments that have not been possible before can now be done in greater detail. The turnover time from experiment to final analysis by conventional means has been very time consuming. The output rate of information gathering and processing by the new methods has been increased many fold and information on the structure and mechanism of the membrane can thus be obtained more accurately and much sooner than by any other method.

(b) The steady state direct impedance methods compliment the transient non-linear clamp techniques in that there are available the powerful tools of theory and experiment developed since Ohms Law was discovered and are only slowly and with difficulty being extended to non-linear systems. The use of electrical measurements over the decades have given more and more precise data and concepts for nerves, their membranes and their tissues than are yet available from any other. Although the solutions of the basic problems in molecular terms are not yet available, the electrical along with other physical and chemical approaches will be needed for the understanding of normal and abnormal living systems.

Proposed Course of Project: Continual development in this area is planned and expected.

Keyword Descriptors: Excitable membranes, membrane characteristics, voltage clamp, on-line computer control, impedance measurements.

Honors and Awards: Dr. Adelman has been invited to present a symposium address "Modification of Axonal Conductance and Excitability by Periaxonal Potassium: Implications for Information Processing" at the 1975 meeting of the Society for Neuroscience.

Publications: R. FitzHugh and K.S. Cole, 1973. Voltage and Current Clamp Transients with Membrane Dielectric Loss. Biophys. J. 13: 1125-1140.

Project No. Z01-NS 02087-02 LB
1. Laboratory of Biophysics
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Function and Structure of Ionic Channels: Ion Interactions and Gating Mechanisms

Previous Serial Number: NCDS(1)-74 LB/OC 2087

Principal Investigators: W.J. Adelman, Jr., Ph.D., and R. E. Taylor, Ph.D.

Other Investigators: R.J. French, Ph.D., M. Starzak, Ph.D., J.P. Senft, Ph.D., P. Mueller, M.D., F. Sachs, Ph.D., R. Latorre, Ph.D., C.M. Armstrong, Ph.D., M.D., F. Bezanilla, Ph.D., J. Vergarra, Ph.D., Y. Palti, Ph.D., M.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.
Eastern Pennsylvania Psychiatric Institute, Phila.,Pa.
State University of New York at Binghamton, Binghamton, N.Y.
Juniata College, Huntington, Pa.
The University of Rochester, Rochester, N.Y.
Laboratory of Neurophysiology, NIMH
The Technion, Haifa, Israel

Man Years:

Total	2.8
Professional:	2.8
Other:	0.0

Project Description:

Objectives: (1) To obtain information about the position and properties of the sites that limit conductances and determine ionic selectivity by analysis of the interaction between current-carrying and blocking ions. To test the ability of various kinetic models to describe the flow of ions through open channels. (2) To determine the effects of the trivalent lanthanide ions on sodium and potassium conductances in the squid axon. To test whether a "screening" or "binding" hypothesis best describes the lanthanide effect. (3) To investigate possible analogies between the kinetics of ionic currents across the axon membrane and those seen in lipid bilayer preparations treated with channel forming agents, and thus extend our understanding of time and voltage dependent gating of axonal currents. (4) To study the details of the transport of various ions, mainly calcium, magnesium and cesium, through the "sodium" channels of the squid axon. To

study the interaction between calcium (and magnesium) ions and sodium ions moving through the "sodium" channels. (5) To determine the gating currents in ionic channels in single muscle fibers.

Methods Employed: (1) Voltage clamp studies were performed on unperfused axons exposed to a modified artificial seawater containing 240 mM K^+, and Cs^+ at various concentrations from 0 to 200 mM. Ionic strength was maintained constant with Tris-Cl. Under these conditions the outside K^+ concentration and the instantaneous current voltage relation in the absence of Cs is approximately linear. Fitting of the data to mathematical models, and graphical presentation of the results was carried out using the MLAB program on DCRT's PDP-10 computer.

(2) Squid giant axons were voltage clamped using the data acquisition system described in report NDS(1)-72-LB/OC 1950. The membrane current data were analyzed on an IBM 370/158 computer at SUNY - Binghamton using the conjugate gradient method. Values of the Hodgkin-Huxley parameters were obtained as a function of external lanthanide ion concentration.

(3) Ionic currents were triggered conventionally by depolarizing pulses under voltage clamp and the effects of brief (0.1 - 2 msec), superposed, large magnitude (up to 300mV), depolarizing and hyperpolarizing pulses on the current kinetics were observed.

(4) Internal perfusion, voltage clamping and signal averaging techniques are used with the squid giant axon.

(5) Using the laboratory's PDP-11/20, a program was written to measure the currents produced by movement of those charges that are responsible for voltage dependent gating of channels. The characteristics of these gating currents in giant muscle fibers of the barnacle were investigated using the computer based system and a new type electrode system incorporating a perfusion cannula for internal perfusion of the muscle with impermeant ions.

Major Findings: (1) The blocking effect of external Cs was increased by increases in the Cs concentration and by increases in the membrane potential gradient from outside to inside. Comparison of data with predictions of a single site competition model for the interaction between K^+ and Cs^+ exclude such a simple mechanism on at least 3 counts:

(a) the concentration dependence of blocking is too steep,
(b) the voltage dependence of blocking (as indicated by the slope, at 50% blocking, of the ratio of current in the presence of K only) depends on the Cs concentration, and
(c) the voltage dependence at [Cs] greater than about 50 mM is too steep.

(2) Studies with the Ln^{3+} series of ions show that a simple Gouy Chapman model for surface potential is insufficient to describe the effects of these ions on the squid axon. The ions produce extremely large voltage shifts in the K^+ current parameters. The ions shift the curves for both maximum current and time constant τ_n in a parallel fashion implying that their effect can be described as some function of the net voltage across

the membrane. Shifts of this type have been observed frequently and have generally been associated with surface charge effects. The time constant shifts can certainly be explained in this manner since the Hodgkin Huxley time constants are a direct function of voltage.

The weaker voltage shifts induced in the sodium parameters indicates a potential weakness in a simple voltage dependent description of these parameters. In order to resolve the actual changes which take place on the sodium excitation mechanism in the presence of these ions, elimination of the K^+ currents would be most useful. Since subtle changes in the h parameter are easily masked by the n process, it would become easier to resolve the magnitude and form of the voltage shifts in this manner. However, the combined data still provides a strong indicator that size dependent voltage shifts do exist for such systems. The elimination of the Gouy Chapman or "screening" theory as a viable model for voltage shifts on squid giant axon immediately raises two new prospects to explain the data. The Ln^{3+} ions may be binding to the surface. In this case, the degree or energy of binding will increase with the charge to size ratio. Excitation may then be induced by a dissociation process. The second alternative is more direct. The Stern modification of Gouy-Chapman theory postulates two regions of electrostatic interaction. An inner region analogous to a Helmholtz double layer and a diffuse or Gouy Chapman outer layer. The magnitude of the inner potential which is expected to be the dominant potential increases rapidly as the magnitude of the bound ions decreases. This effect is consistent with the results and suggests the system could be modified for the Stern model in a consistent manner.

(3) Preliminary experiments resulted in several interesting observations that deserve further study.

(a) There are indications that the conductance system exhibits an "inertia" by which the rate of change of the conductance immediately prior to a voltage pulse can affect the time course of the conductance change during the pulse. This is not consistent with a first order system such as that on which the Hodgkin Huxley equations are based, but would fit within the framework of an aggregation model used to describe the time and voltage dependent conductances produced by some channel forming agents in lipid bilayer membranes.

(b) Subjecting the membrane to a series of strong depolarizing pulses leads to the appearance of a time and voltage dependent conductance that turns on in response to hyper polarizing pulses.

(4) The general preliminary results are (a) Calcium ions flow through the "sodium" channels about 1/100 as easily as do sodium ions. (b) Magnesium appears not to be permeable at all through the sodium channels. (c) High external calcium interferes with the inward movement of sodium ions but has little or no effect on outward movement of sodium or cesium ions.

(5) A current with some of the properties of a gating current has been observed during the turn-on of the ionic conductances. The response of this presumed gating current to voltages which turn-off the conductance has not yet been clearly observed due to interference of ionic currents. In an effort to eliminate Ca currents, the drug D600 was applied, and clear

inhibition of the Ca currents was observed in the presence of unaltered K currents. D600 appears to block the presumed gating current, suggesting that it may operate by inhibition of the Ca gating mechanism, rather than by plugging the channel in a TTX like manner.

Significance to Biomedical Research and the Program of the Institute: (1) Ion competition provides valuable clues to ion channel structure. These relate to the basic molecular structure underlying all neural activity. (2) Tests of "screening" vs "finding" hypothesis for ion interaction with membrane surfaces provide answers as to the role of membrane surfaces in excitation processes. (3) These experiments are direct tests on real membranes (of uncertain chemical structure) of model descriptions derived from artifically constructed membranes with known chemical structures. Convergence in this area strengthens our understanding of the molecular structure of nerve. (4) A study of this kind is one of the most promising approaches available at this time to attempt to characterize the structure of the "sodium" channel. (5) The barnacle muscle is a unique preparation which has non-inactivating K and Ca currents, and which permits internal perfusion and voltage clamping. An understanding of the gating current properties should provide insight into voluntary muscle function at all phylogenetic levels.

Proposed Course of Project: (1) Cs is an ideal probe of channel properties in studies of this type, since it blocks K currents directed across the membrane from whichever side Cs is present. The results now in hand provide a spring board from which to launch both experimental and theoretical studies.

(a) Using perfused axons we will compare properties of the blocking site occupied by Cs when it is added internally with that for external blocking.

(b) We hope to model the concentration and voltage dependence seen for external blocking and thus be able to suggest a possible physical layout of the binding sites within the channel.

(2) A detailed analysis of both binding models and a Stern model is now in preparation. Resolution of the nature of ion interaction at the surface will then provide a more accurate picture of relevant molecular data, e.g., density of surface binding sites.

(3) Further experiments on the short pulse transients are planned for comparison with the lipid bilayer data. In addition we will perform a detailed study of the "inverted" conductance system which turns on with hyperpolarizing pulses in an attempt to determine its relation, if any, to the channels of normally functioning axons.

(4) In order to be useful the measurements must be done accurately and carefully. We are proceeding to check various sources of possible artifacts and obtain reliable quantitative results.

(5) Further efforts will be made to block the ionic components of the current so that "off responses" can be verified. The assignment of the gating current to a particular channel system will be attempted using a

variety of techniques including the pharmacological properties of D600.

Keyword Descriptors: Loligo axons, Balanus muscle, gating currents, ionic channels, ion interactions, ion competition, ion saturation, ion inhibition, conductance inertia, lanthanide ions, calcium ions.

Honors and Awards: Dr. Adelman has been invited to present the results of his work to the annual convocation of students of the Open University of the United Kingdom at Nottingham University, England on July 24, 1975.

Publications: Y. Palti, W.J. Adelman Jr., J.P. Senft "Modification of Membrane Currents and Potentials by Dynamic Alterations of Ionic Concentration in Periaxonal Space", Act. Neurophysiologiques, 1974.

R. E. Taylor, Excitable Membranes. Ann. Rev. Phys. Chem. 25: 387-405 (1974).

Project No. Z01-NS 02088-02 LB
1. Laboratory of Biophysics
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Function and Structure of Membrane Ionic Channels: Pharmacology and Ionic Selectivity.

Previous Serial Number: NDS(1)-74 LB/OC 2088

Principal Investigators: L. Binstock, B.S.,M.S.; D.L. Gilbert, Ph.D.

Other Investigators: F. Bezanilla, Ph.D.; G. Ehrenstein, Ph.D., J. Henderson, Ph.D., R. Henkin, Ph.D., B. Howell, Ph.D., R.D. Keynes, Ph.D.,F.R.S., H. Lecar, Ph.D., R.J. Lipicky, M.D., E. Rojas, Ph.D., I. Stillman, Ph.D.,M.D., R.E. Taylor, Ph.D.

Cooperating Units: Division of Computer Research and Technology, NIH, Marine Biological Laboratory, Woods Hole, Mass., University of Cincinnati, Cincinnati, Ohio, SUNY at Buffalo, Buffalo, N.Y., Cambridge University, Cambridge, England.

Man Years:

Total:	2.9
Professional:	2.4
Other:	0.5

Project Description:

Objectives: To study the ionic current flow across the nerve membrane without the complication of excitation and propagation in terms of individual ion currents and to observe the effect of changes in normal internal and external environments. These environments are altered by addition of various chemical and pharmacological agents as well as by changing the ionic environment. The long range objectives are the interpretations of the structures and mechanisms by which the permeabilities are controlled.

Method Employed: Standard voltage clamp techniques are employed on the following preparations: a. Myxicola giant axon; b. Squid giant axon. For many experiments on the giant axon of Myxicola, the voltage clamp is under computer control. In addition, the computer provides both on and off line analysis of the data.

Major Findings: (a) Myxicola giant axon: Studies of the selectivity sequence of organic cations substituted for Na in the external

bathing solution were continued. Although there has been some variation in obtaining the selectivity ratio, the sequence is the same as the preliminary results previously reported. Initially the reversal potential was obtained by observation of zero current during a sequence of pulses that were photographed. It is then possible to pick out which potential produced a response in which the current appeared to be neither inward or outward. That potential was taken to be the reversal potential.

In plotting current-voltage curves, correcting for leakage, we easily obtained the reversal potential. In both methods the values obtained were very much the same, or if there was any shift, the net result of the selectivity-ratio varied very little. However, when the selectivity-ratio was small, there was some variability in the reversal potential, but not enough to alter the selectivity sequence of the organic cations.

The ratios of the peaks of the current-voltage plots did not give the same selectivity as the reversal potential. The independence principle did not hold, perhaps due to ion competition.

It should be noted that the selectivity sequence for Myxicola was in close agreement to that obtained by Hille for frog node. The ionic concentration for the species differs by a factor of 4. We are going to test the relation between reversal potential and changes in ionic concentration, and compare the results to the predictions of a simple model. A paper is in preparation.

Hydrazine and some times hydroxylamine hyperpolarizes the axon very rapidly. When these two ions depolarize the axon then we can get the selectivity ratio. This phenomena (hyperpolarization) will occur when the particular ion sea water is in the chamber with only the dual and reference electrodes. As a result one must bear in mind the pharmacological effects that can be produced by these ions. Or possibly some other effect that may not be clearly defined.

(b) Squid giant axon: We have previously observed in experiments that mercury, which is a known blocker of sulfhydryl groups, decreased the ionic currents in the voltage clamped squid giant axon. Some slight recovery using the sulfhydryl compound, 2-mercaptoethanol, was observed. In our present studies, we investigated the effects of silver on the ionic currents of the squid giant axon. We found using 6 μ M silver (Determined by Mr. Becker of the National Bureau of Standards, Gaithersburg, Md. using neutron activation analysis) that similar inhibition of the ionic currents was observed; and that 2-mercaptoethanol produced some slight recovery.

McLaughlin and Harary (Biophys. J. 14:200, 1974) have suggested that the asymmetrical distribution of lipids across the biological membrane could be attributed to the voltage difference across the membrane. This is based on phospholipid flip-flop in the membrane; i.e., the charged phospholipids

would redistribute on the two sides of the membrane according to a change in the electric field across the membrane. We have tested to see if the flip-flop mechanism could be observed in the squid giant axon immersed in high potassium solution, which changes the electric field across the membrane. We measured the surface charge on the outside of the membrane as a function of time after the high potassium solution was introduced to the axon. No change in the surface charge was observed even after a period of one hour at room temperature. Thus, if there is a flip-flop, it is very slow.

Axons were subjected to about 150 atmospheres of helium. There was little or no change in the maximum sodium and potassium conductances. The action potentials were prolonged without changing the threshold; the resting potential was not changed. The sodium and potassium currents were prolonged, which could be qualitatively duplicated using a computer analysis of the Hodgkin-Huxley equations in which the rate constants of the m and n processes were decreased.

We have tested the effects of Aconitine on the ionic currents. This substance has been shown to produce a depolarization of the resting cell membrane due to an increase in sodium permeability and induces repetitive electrical activity (Herzog, J. Gen Physiol. 47:719, 1964). We did observe that the voltage-clamped sodium and potassium currents were decreased. Thus, the observation lends support to the idea that there are separate resting and active sodium channels.

It has been previously shown that lowering the pH decreases the sodium conductance. We wanted to determine the pH of the normal squid blood as well as its temperature dependence. This buffer has a very high temperature dependence and is commonly used as a buffer in voltage-clamp experiments of the squid membrane. We have found that the pH dependence of squid blood upon temperature was -0,0208 pH units/^{o}C, the ph was estimated to be 7.16.

Significance to Biomedical Research and the Program of the Institute: The sizes of the various permeant ions might give the dimension of the conducting pathway. The interaction of the various chemical and pharmacological agents may produce a pharmacological effect. Under these conditions, it appears that the nerve membrane may be altered. Any changes in the axon membrane might possibly give information as to the site of the particular conducting pathway. Therefore, these studies on the giant axon are making it possible to get some kind of insight in the mechanism of the excitable membrane.

Proposed Course of Project: The study of "sodium substitutes" and especially "potassium substitutes" of the quaternary-ammonium ions and their various analogs and others will be continued combined with chemical analysis of the membrane. Mixtures of these ions will also be studied

for ion competition. When internal perfusion is resumed, Naloxone, a morphine antagonist will be studied. Studies on scorpion venom, heavy metals (lead, etc.), Saxitoxin and Tetrodotoxin comparisons leading to dose response curves and a prediction as to the number of Na conducting (data is available for the most part) channels will be resumed. Batrachotoxin, as soon as it is available again, will be rechecked (not enough data for a paper). It is also planned to study other chemicals and pharmacological agents such as anesthetics, tannic acid, pronase and other enzymes. The computer controlled voltage clamp should make these studies much easier.

Keyword Descriptors: Membrane ionic channels, ionic current, nerve membrane, axon, ionic selectivity, Myxicola and squid giant axons, independence principle, reversal potential, organic cations.

Honors and Awards: None

Publications:

Gilbert, D.L.: Potential and time constant effects in the fast ramp voltage clamp. Math. Biosciences 20;67-74, 1974.

Gilbert, D.L. and McCutcheon, F.H.: Respiratory media (Table 207). In Altman, P.L. and Dittmer, D.S. (Ed.); Biology Data Book. Second Edition. Vol III. Bethesda, Md., Federation of the American Societies for Experimental Biology, 1974, pages 1580-1581.

Gilbert, D.L: Physiological uses of the squid with special emphasis on the use of the giant axon. In Arnold, J.M., Summers, W.C., Gilbert, D.L., Manalis, R.S., Daw, N.W., and Lasek, R.J.: A guide to laboratory use of the squid Loligo pealei. Woods Hole, Mass., Marine Biological Laboratory, 1974, pages 45-54.

Henkin, R.I., Stillman, I.S., Gilbert, D.L., and Lipicky, R.J., Ineffectiveness of lysergic acid diethylamide (LSD) on altering Na-K currents in squid giant axon. Experientia 30:916-917, 1974.

Ehrenstein, G., Gilbert, D.L., and Lipicky, R.J.: Does phospholipid flip-flop affect axon potassium channels? Biophys. J. in press.

Keynes, R.D., F.R.S., Bezanilla, F., Rojas, E. and Taylor,R.E.The rate of action of tetrodotoxin on sodium conductance in the squid giant axon. Phil. Trans. Roy. Soc. Lond. B., 1975 (in press).

Project No. ZO1-NS-02089-02 LB
1. Laboratory of Biophysics
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Electrical Fluctuations in Excitable Cells

Previous Serial Number: NDS(1)-74 LB/OC. 2089

Principal Investigators: F. Sachs, Ph.D. and H. Lecar, Ph.D.

Other Investigators: G. Ehrenstein, Ph.D.

Cooperating Units:

Man Years:

Total: 1.4
Professional: 1.4
Other: 0.0.

Project Description:

Objectives: To determine the biophysical properties of the post-synaptic Acetylcholine activated ionic channel. To measure changes in post-synaptic electrical noise produced by pharmacological agents. To develop a method for measuring electrical fluctuations from excitable cells in order to estimate the elementary conductance of the ionic channels responsible for excitation.

Methods Employed: Electrical fluctuations are measured by monitoring the changes in electrical current noise induced when neurotransmitters are iontophoretically released at the postsynaptic membrane. Chick skeletal muscle cells grown in tissue culture are altered by vinblastine and colchicine application and electrical measurements are made using a two-microelectrode voltage clamp. Current noise spectra are recorded by on line computation using a Fast-Fourier Transform program.

Major Findings: Our previously reported experiments (Nature 246: 214, 1973) on the kinetics and conductance of Ach channels were extended to cover a wider range of temperature and membrane potential and to determine the effects of various pharmacological agents.

In order to obtain better spatial uniformity of the membrane potential, chick skeletal muscle cells were modified by treatment with colchicine and vinblastine. This treatment produced spherical cells

30 to 100 μ in diameter, which were more suitable for microelectrode voltage clamp than the original cable-like cells. The transformed cells had weakened contractility and thus were easier to penetrate with microelectrodes.

Current-noise spectra were measured from the voltage-clamped cells by on-line spectral analysis. The noise spectrum is Lorentzian, consistent with a picture of random channel openings and channel closings having no memory of the time of the Ach-induced opening. Analysis of 46 preparations yielded values of 60 pmho for the ion-channel conductances; 5msec for the open-channel lifetime at 25°C and a Q_{10} of 7.1 for the temperature dependence of the channel closing rate.

These results were used as the basis for theoretical analysis of various models for the interactions of Ach with the postsynaptic receptor sites. Comparison of our data with tracer flux data on the same cells suggests that the channel activation exhibits some intermediate state of activation prior to the actual flow of ionic current.

Significance to Biomedical Research and the Program of the Institute: The study of chemical transmission at the neuromuscular synapse by electrical fluctuation methods provides a means of understanding the nature of chemically mediated synaptic transmission, which is the most important membrane process underlying integration in the central nervous system.

Proposed Course of the Project: The fluctuation studies on the Ach channels are being continued to study the effects of pharmacological agents on the electrical fluctuations. The microelectrode voltage clamp and electrical noise measuring methods are being modified to study other excitable cells.

Keyword Descriptors: Acetylcholine,channel, ionic, noise, electrical, post-synaptic membrane, tissue culture, voltage clamp, neurotransmitter.

Honors and Awards: None

Publications: None

Project No. ZO1-NS-02090-02 LB
1. Laboratory of Biophysics
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Lipid Bilayer Membranes

Principal Investigators: G. Ehrenstein, H. Lecar

Other Investigators: R. Latorre, J. Weinstein

Cooperating Units: Dept. of Physiology, Duke University
Laboratory of Theoretical Biology, NCI, NIH

Man Years:

Total:	1.2
Professional:	1.2
Other:	0.0

Project Description:

Objectives: To study the mechanisms for ion transport in lipid bilayer membranes doped with ionic channels or carriers. To develop the lipid bilayer membrane as an assay for ion-conductance-inducing materials isolated from biological membranes. The ultimate goal of the lipid bilayer project is the elucidation of the molecular mechanism of membrane excitation.

Methods Employed: Lipid bilayer membranes are formed from natural membrane extracts, oxidized cholesterol, or purified phospholipids. A small quantity of lipid dissolved in a hydrophobic solvent is placed in a 1mm aperture between two electrolyte solutions, and spontaneously thins to form a bilayer with very low electrical conductance (about 10^{-10} siemens). Ion-conductance-inducing material is added to the solution on one side of the membrane, and spontaneously incorporates into the membrane. The electrical properties of the doped membrane are then studied by voltage clamp techniques. The materials added to the bilayer have been primarily EIM (a protein of bacterial origin which forms ionic channels in the membrane) and hemocyanin.

Major Findings: We have demonstrated that there are three discrete states of an EIM channel - a state of relatively high conductance, whose probability of occurrence is high at or near zero membrane potential, and two different states of relatively low conductance. One low-conductance state has a high probability of occurrence at large positive potentials and the

other low conductance state has a high probability at large negative potentials.

With hemocyanin-doped membranes, we have found two different mechanisms for voltage-dependent conductance. One mechanism-similar to that of EIM and alamethicin - is the voltage-dependent redistribution of channels among several discrete single-channel conductance states. The other mechanism is the continuously voltage-dependent conductance of the single-channel states, themselves. The relaxation time for the discrete conductance changes is of the order of seconds and the relaxation time for the continuous conductance changes is of the order of 10^{-4} seconds. We have also found that as salt concentration in the bathing medium is increased, the single-channel conductance first increases linearly and then saturates. The saturation characteristics can be explained by a model where an ion spends about 4 nanoseconds in the channel, during which time all other ions are excluded.

Significance to Biomedical Research: The EIM results provide a model for inactivation in nerve membranes. According to this model, there is a high-conductance state when the channel is in the middle of the membrane, and low-conductance state when the channel is on one or the other side of the membrane. Membrane potential determines the position of the channel, and the position, in turn, determines the conductance. Membrane depolarization first causes the channel to move to the middle of the membrane thus increasing conductance. Continuing depolarization causes the channel to move all the way to the other side, thus inactivating the channel.

The hemocyanin results show for the first time a continuously voltage-dependent conductance of a single channel. Previously, all voltage-dependent conductance changes had been found to occur in a small number of discrete steps. The continuous conductance change may represent a different type of molecular mechanism. The saturation properties of the hemocyanin channel have been used to model the mechanism for the voltage-dependent conductance. From an analysis of saturation, we found that the continuous conductance changes occur at the edges of the channel and that the interior of the channel is relatively unaffected by membrane potential.

Proposed Course of the Project: We plan to find out more about the molecular mechanism of voltage-dependent conductance in hemocyanin by examining the selectivity of several discrete states. We also plan to test our inactivation model with EIM by determining relaxation rate for various membrane potentials.

Keyword Descriptors: Lipid bilayer, EIM, hemocyanin, ionic channels, voltage-dependent conductance, inactivation, discrete state.

Honors and Awards: None

Publications: H. Lecar, G. Ehrenstein and R. Latorre: "Mechanism for Channel Gating in Excitable Bilayers" Annals of the New York Academy of Science (in press).

O. Alvarez, E. Diaz and R. Latorre: "Voltage-Dependent Conductance Induced by Hemocyanin in Black Lipid Film" Biophys Biochim Acta (in press).

Project No. Z01-NS-02091-02 LB
1. Laboratory of Biophysics
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mathematical Modeling

Previous Serial Number: NDS(I)-74 LB/OC 2091

Principal Investigators: R. FitzHugh, Ph.D., and H. Lecar, Ph.D.

Other Investigators: W. J.Adelman, Jr., Ph.D., K.S. Cole, Ph.D., G. Ehrenstein, Ph.D., R.J. French, Ph.D., R. Guttman, Ph.D.

Cooperating Units: Laboratory of Neurophysiology, NIMH, NIH
Dept. of Biology, Brooklyn College, Brooklyn, N.Y.

Man Years:

Total:	2.9
Professional:	2.9
Other:	0.0

Project Description:

Objectives: (a) Anodal break excitation: To explain theoretically the rapid increase in anodal break threshold with increasing temperature.
(b) Modifications of Hodgkin-Huxley model: To explain the effects of potassium ion accumulation on repetitive firing in axons.
(c) White noise analysis: To calculate the response of nerve membranes to white noise input, as a method of measuring impedance and for predicting the properties of random firing.
(d) New computer programs for nerve models: To increase the convenience of obtaining solutions of the Hodgkin-Huxley and other nerve membrane equations.
(e) Rate theory model of ionic-selective channels: To explain ion selectivity and competition in membrane channels.
(f) Dielectric loss in axon membranes: To investigate the presence and nature of dielectric loss in the nerve membrane.
(g) Optimal control of muscle: to predict theoretically the motor input to a single muscle during voluntary contraction so as to minimize total energy expenditure.

Methods Employed: (a,b,d) Numerical solution of differential equations by computer. (c) Statistical communication theory and nonlinear noise

analysis. (e). Reaction rate theory and curve fitting with the MLAB computer program. (f) Analysis of published data, computation of impedance from nerve models, and solution of partial differential equations. (g) Optimal control theory (Pontryagin's Maximum Principle) nonlinear mechanics, numerical computation.

Major Findings: (a) The earlier conclusion was that the threshold to anodal break excitation in the Hodgkin-Huxley model following an infinitely long anodal current pulse goes to infinity at a finite critical temperature (17.1°C). The same critical temperature has been computed for the threshold to an instantaneous anodal shock. Phase space analysis indicates that as the amplitude of an anodal current of any duration and wave form whatever is increased, the resulting trajectory approaches a limiting trajectory for which, if it is followed backward in time, the membrane potential approaches minus infinity and the variables m, b, and n approach 0, 1, and 0 respectively. This limiting trajectory changes from suprathreshold to subthreshold at the critical temperature, which is therefore the same for all types of anodal stimulus.

(b) The Hodgkin-Huxley equations were modified to include the properties of an external diffusion barrier separated from the axolemma by a thin periaxonal space in which potassium ions accumulate as a function of membrane activity. Further modifications in the equations took into account new values for $\bar{g}_K$ and new functions for α_n, β_n, α_h and β_h derived from voltage clamp experiments on Loligo pealii giant axons. Equations were solved on a PDP-11 computer using the Gear predictor-corrector numerical method. In comparison with the original Hodgkin-Huxley equations, the modified equations gave (1) more accurate representations of the falling and undershoot phases of the membrane action potential, (2) more accurate representation of thresholds and latencies, (3) increases in the periaxonal space potassium ion concentration K_s of about 1 mM/impulse, (4) proper predictions of the time course and magnitude of either undershoot decline or periaxonal potassium ion accumulation during trains of action potentials elicited by repetitive short duration stimuli, and (5) a somewhat more accurate representation of adaptation (finite train and nonrepetitive response) during long duration constant current stimulation.

(c) Electrical fluctuation caused by gated ionic channels have been measured for both natural and synthetic excitable membranes. Theoretical analysis of gating noise relates the observed electrical noise spectrum to the kinetics of the molecular processes underlying excitation (See experimental projects reports). The extraction of useful information from the gating noise in electrically excitable membranes is complicated by the nonlinear membrane impedance. Theoretical analysis of this phenomenon was done in an attempt to separate the true channel noise effects from extraneous membrane noise. A White noise analysis of the subthreshold response of axons has been completed. The analysis demonstrated the applicability of a simplified equivalent circuit to the understanding of subthreshold oscillations. A paper was published comparing the results with experi-

mental results on the squid giant axon. A further analysis was made of the random firing response of axons to white noise stimulation, in which expressions were derived for the frequency of firing and for input-output cross-correllograms.

(d) Programs supporting (a) and (b) above have been developed.

(e) Tests of the model on squid axon have been run (See Project No. ZO1-NS 02087-02 LB).

(f) Transient responses of a constant phase angle impedance to step changes of voltage current were computed, for comparison with experimental records showing dielectric loss in nerve membranes.

(g) Work has commenced on re-deriving the equations for the optimal control of the muscle model without series elastic element in a more compact form for publication, and in computing values for the curves needed for figures of a paper.

Significance to Biomedical Research and the Program of the Institute:
Mathematical modeling of membrane excitation and the ion permeability changes underlying excitation provides a means for using experimental findings on simple nerve preparations to predict physiological phenomena which may not be amenable to controlled experiment. For example, the ion accumulations and depletions, which can be studied experimentally for the giant axon, provide the basis for modeling of accommodation and repetitive bursts of activity in nerve tissue. Thus the kind of modeling reported here provides a factual physiological basis for the more abstract neural modeling needed to explain the more complex phenomena of the nervous system.

Proposed Course of the Project:

(a) Completed.

(b) Methods of computing the change in potassium concentration in the periaxonal space, using the mathematical model and equations of G. Adam, will be studied for the purpose of performing curve fits to experimental data. This would yield values for the width of the periaxonal space and of the channels between the Schwann cells, and for the diffusion constant of potassium inside the channels.

(c) The study of gating noise is being continued with the emphasis on the design of new membrane noise experiments. The study of random firing will be pursued as part of a study of simplified models of random excitation and repetitive firing.

(d) It is planned to provide copies of these programs to the National Technical Information Service (part of the Department of Commerce) for distribution to interested users.

(e) A multi-barrier, multi-site model will be investigated.

(f) No new work is contemplated.

(g) Approximate solutions for the problem of deriving the optimal control of a muscle model including a series elastic element, using singular perturbation theory, appear to be possible, and will help to refine the predictions of this model.

Keyword Descriptors: nerve membrane, computation, potassium space, muscle, optimal control, anodal break, Hodgkin-Huxley model, white noise, dielectric loss, membrane noise, rate theory, ionic channels.

Honors and Awards: None

Publications: R. FitzHugh and K.S. Cole. Letter to the Editor. Dielectric loss transients. Biophys. J. 14: 625-626. 1974.

W.J. Adelman, Jr. and R. FitzHugh. Solutions of the Hodgkin-Huxley Equations Modified for Potassium Accumulation in a Periaxonal Space. Fed. Proc. 34: 1322-1329. 1975.

R. Guttman, L. Feldman, H. Lecar. Squid Axon Membrane Response to White Noise Stimulation Biophys. J. 14: 941-955. 1974.

Project No. ZO1-NS-02092-02 LB
1. Laboratory of Biophysics
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Subcellular and Extracellular Structures Associated with Nerve and Muscle

Previous Serial Number: NCDS(I)-74 LB/OC 2092

Principal Investigators: W. J. Adelman, Jr., Ph.D., L. Binstock, B.S., M.S. and K.S. Cole, Ph.D.

Other Investigators: D. O. Carpenter, M.D., H. Lecar, Ph.D., M. Henkart, Ph.D., R.V. Rice, Ph.D. and J.P. Senft, Ph.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.
Carnegie-Mellon University, Pittsburgh, Pa.
DCRT, NIH
Juniata College, Huntington, Pa.
Behavioral Biology Branch, NICHD, NIH
AFRI, Bethesda, Md.

Man Years:
Total: 1.1
Professional: 1.1
Other: 0.0

Project Description:

Objectives: (1) To examine certain proteins in the axoplasm of nerve fibers.
(2) To investigate the anatomical dimensions of the Schwann cell layer external to the axon membrane and predict some of its physiological properties.
(3) To determine the resistance in series with the excitable membranes of giant axons.
(4) To determine the resistivity of neuronal cytoplasm.

Methods Enployed: (1) Single giant axons were prepared from the hindmost steller nerves of the squid, Loligo pealei. These were carefully cleaned of all surrounding small nerve fibers and any loose connective tissue. The axons were then blotted thoroughly and the axoplasm was squeezed from the axons using a roller in such a manner that no external material contaminated the extruded axoplasm. Extruded axoplasm was collected over a period of several weeks at the Marine Biological Laboratory, Woods Hole,

and stored at -20°C or lower until used for gel electrophoresis in Pittsburgh.

The methods of Laemmli (Nature, 277:680, 1970), Weber and Osborn (J. Biol. Chem. 244:4406, 1969), and Fairbanks, et al. (Biochem 10: 2606, 1971) were used with either 5.7% gels or 10% gels. The polypeptide chains were stained with 0.1% Amido Black or 1% Coomassie Blue. The use of stained gels eliminates ambiguities of band positions even with gels of the entire axoplasm but because fixed polypeptide chains cannot be eluted free of acrylamide, analysis of the basic amino acids is hampered. Loadings of protein ranged from 20 µg to 80 µg per tube. Standards of paramyosin, albumin, ovalbumin, chymotrypsin A and myoglobin were included in each electrophoresis experiment. Slices of bands of axoplasm were hydrolyzed and amino acid compositions determined according to the method of Houston (Anal. Biochem. 44: 81, 1971). Eight slices were combined in a single tube for hydrolysis. As many as 3 groups of hydroxylates of eight slices each were combined for analysis. The amino acid analyzer was a Spinco Model C upgraded with a moderately high pressure resin system.

As controls both the slices of the tubulin band of axoplasm and slices of purified bovine serum albumin (Schwatz-Mann, Orangburg, N.Y., Lot X1422) run in 5.5% gels were analyzed. In our hands the Houston method gave higher contents for glycine and serine and slightly higher for isoleucine and tyrosine. Corrections based on the comparison of amino acid contents of these residues in pure albumin and slices of the same albumin in the gel were used to correct these residues of the smooth muscle bands and the bands found in axoplasm gels including that of tubulin. (The corrections were: glycine x 0.65, serine x 0.78, isoleucine x 0.86, and tyrosine x 0.73.) No corrections were made for losses during the hydrolysis period of 24 hrs. at 120°C.

(2) Squid (Loligo pealei) giant axons were prepared and cleaned of loose connective tissue. These axons were fixed with a variety of fixatives (gluteraldehyde, osmium tetroxide, or a mixture of paraformaldehyde, acrolein, and gluteraldehyde) in sea water and in 10% hyperosmotic sea water. Several concentrations of these fixatives were tried. When osmium tetroxide was not the fixative, the preparations were subsequently stained with OsO_4. After embedding, both cross and longitudinal thin sections of the giant axons were cut. These sections were examined in the electronmicroscope either at the Marine Biological Laboratory or at Carnegie-Mellon University. From the electron micrographs the following measurements were made: (i) radial thickness of the periaxonal space, (ii) length and width of the clefts between Schwann cells, (iii) frequency of clefts between Schwann cells.

(3) The original 1947 method to estimate R_s as well obtain the most direct value for membrane capacity was to apply a step of constant current from an axial electrode to a guarded external electrode and measure the transient potential between them. This is still the simplest and most direct in concept. However, as equipment and experiments developed in power and speed the current step has not been made instantaneous on the µ sec scale and a correction is needed. This is relatively simple for an approximately exponential current rise but of uncertain accuracy otherwise--such as

for the slow initial rise which is sometimes hard to avoid.

(4) The several methods for measurement of cytoplasmic resistivity without cell invasion are indirect and have given inconsistent and widely varying results in many forms which seem not to have been explained in any single case. Recent investigations of axons and ganglia within a single small internal electrode have resulted in even wider spread of data and some uncertainty of the contributions of the electrode impedances. Although there was a wide spread of values for squid axoplasm, they were in the range of some earlier conclusions. Another approach was to measure the resistance between two small electrodes as they were moved toward each other from the ends of an axon immersed in sucrose. Ion loss across the membrane made this impractical so the same procedure was used on axoplasm extruded into a glass capillary.

Major Findings: (1) The results of SDS gel electrophoresis of whole extruded squid axoplasm indicate the location of three high molecular weight bands. These bands are all sufficiently separated from other bands so that gel slices were not contaminated by nearby bands. The bands are referred to as at 100,000 D, 135,000 D, and 150,000 D as originally determined for smooth muscle mesosin. The tubulin band at 52,000 D agrees with the molecular weight of purified tubulin from several sources. The amino acid composition of gel slices of the tubulin band also is in agreement with similar analyses of purified tubulin.

The amino acid analysis of acrylamide gels results in large quantities of ammonia which blots out the basic amino acids to varying degrees and this is evident in both axoplasm and R.V. Rice's smooth muscle analyses. The relatively close correspondence of tubulin composition from gel slices as compared to purified tubulin gives us confidence in comparing the composition of mesosin chains from gel slices of the two species.

The amino acid analyses of the three higher bands from squid axoplasm show a similarity between the three chains, except perhaps for the proline and tyrosine residues. A more important comparison is evident when histograms of the bands from smooth muscle and axoplasm are superimposed on each other. A more convenient comparison can be obtained from polar coordinate plots of the amino acid composition of each of the three bands from the two different sources.

Until each of the chains of mesosin is more completely characterized and compared to the lower molecular weight chain we cannot know if the higher molecular weight chains are parents of the smaller chain. Proteolysis could produce the smaller chain and in fact the three larger chains of mesosin could be the result of proteolysis. Thus we cannot yet know if mesosin is a single large protein or several proteins of very similar composition.

(2) Examination of electron-micrographs of Loligo and Myxicola axons show that the most likely source of the series resistance is in the cracks and channels between the Schwann cells. We can estimate the resistance of the channels between the Schwann cells under the simple assumption that the channels observed in the electron-micrographs are filled with ambient

electrolyte. These estimates are roughly 0.6 ohm cm^2 for Loligo axons and 2.3 ohm cm^2 for Myxicola axons. Since both of these estimates neglected the resistance across the basement membrane and protein fiber layer, these are probably underestimates of the total resistance across the periaxonal tissue layer. The periaxonal tissue layer is approximately seven times thicker for Myxicola than for the Loligo axons. Both voltage-clamp experiments and electron micrographs show a wider periaxonal space in Myxicola axons than in squid axons. Recently, electron micrographs of squid axons have been available from Dr. M. Henkart of NICHD for study of the Schwann sheath. This study has given information on axons fixed by a different technique and other hands. Measurements tend to confirm the above results.

(3) A method of unknown origin in which an effective step time is calculated from the experimental record has been shown to apply rigorously to this system and so give R_s from an arbitrary current form. Alternatively early records of V vs. 1 must approximate a straight line for arbitrary currents and is particularly simple for a ramp. This work is complete in manuscript form "On the Measurement of Series Resistance in Giant Axon Preparations" except for an illustration.

The impedance loci which were introduced in 1928 would be circular arcs for a simple membrane-enclosed electrolyte have been found to show varying amounts of unexplained departure from a simple arc at high frequencies for many cells. It has seemed obvious for some time that impedance measurements in the megahertz frequency range were needed to supplement the EM estimates of R_s. Further, the question was raised in 1952 as to the possibility of a capacity-membrane-component accompanying R_s. But equipment has not been available. Only recently it was noticed that one of the first 1937 impedance loci up to 5 MH_2 published in 1938 showed about half of a second dispersion. With reasonable assumptions for now-lost factors these old data points have been matched to about 1% and analyzed to give a resistance of 1.6 ohm cm^2 and a capacity equivalent to about 12 plasma membranes in series. These values are so close to electrical transient and EM results as given them an independent prediction and confirmation. This work is in final manuscript form, "Electrical Properties of the Squid Axon Sheath."

(4) The result from extruded axoplasm gives an axoplasm resistivity of 1.4 and .2 times sea water. This value includes several earlier determinations and it is suggested that this variability comes from the long process of isolating and cleaning the axon and extruding the axoplasm. A manuscript on this work "Resistivity of Axoplasm, I Resistivity of Extruded Squid Axoplasm" is in preparation.

Significance to Biomedical Research and the Program of the Institute:

(1) Attention is drawn to the close similarity (if not identity) of three chains of 100,000 D, 135,000 D and 150,000 D which consistently appear in SDS gels of both smooth muscle and squid axoplasm. These results taken together with numerous morphological reports of 10 nm filaments found not only in smooth muscle and axoplasm but in many other cell types suggest that the 10 nm intermediate filament may be a ubiquitous protein filament. This

intermediate filament appears to contain a protein which has a unique amino acid composition.

(2) Understanding the role of these adjunct structures with respect to membrane function can possibly form a basis for modeling disease entities such as epilepsy.

(3 & 4) Reasonably accurate voltage clamp current measurements require: (a) Values of the resistance R_s discovered in 1947 in series with axon plasma membrane capacity for compensation including better values for axoplasm resistivity and general methods to correct for rise time of current step used in current clamp to determine resistance between potential electrodes. (b) Knowledge of the nature and effects of the Schwann sheath impedance to confirm geometric estimates of R_s and the effects of any reactance on it and to give additional basis to estimate ion concentration changes and their effects.

Proposed Course of Project: No further work is planned on squid axoplasm resistivity. Some guidance may be given for more complete measurements on ganglia and a primitive attempt at analysis has been begun on the basis of earlier work on tissues.

No further improvements or corrections for current step wave form in R_s measurements seem to be needed at present.

The badly needed additional data on the axon sheath at higher frequencies will not be attempted unless and until the original equipment can be rehabilitated and improved or time and facilities are available.

Keyword Descriptors: Loligo axons, Myxicola axons, Axoplasm proteins, Mesosin, Schwann Sheath, Periaxonal tissue layer, Electron Microscopy, current clamp, series resistance, impedance measurements, axoplasm resistivity.

Honors and Awards: None

Publications: L. Binstock, W.J. Adelman, J.P. Senft, H. Lecar: Determination of the resistance in series with membranes of giant axons. J. Membrane Biol. 21: 25-47, 1975.

Project No. Z01-NS-01944-04 LB
1. Laboratory of Biophysics
2. Section on Neural Systems
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Organization of a Molluscan Central Nervous System

Previous Serial Number: NDS(I)-72 LNP/GP-1944

During FY 1975 this project was incorporated with Project No. Z01-NS 02151-01 LB.

Project No. Z01-NS 02151-01 LB
1. Laboratory of Biophysics
2. Section on Neural Systems
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
December 1, 1974 through June 30, 1975

Project Title: Information Processing in Simple Nervous Systems

Previous Serial Number: None and incorporating Z01-NS 01944-04 LB.

Principal Investigators: D. L. Alkon, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: To study the mechanisms by which simple neural networks process information with particular emphasis on mechanisms of learning. Information processing at several levels is of interest:

(1) Sensory transduction by photoreceptors and hair cells.
(2) Synaptic interactions between primary sensory receptors.
(3) Synaptic interactions between primary and higher order neural elements.
(4) Intersensory communication: e.g. synaptic interaction between the visual and gravitational sensory pathways.
(5) Changes of synaptic interaction produced by conditioning paradigms administered to the intact animals as well as to the isolated nervous systems.
(6) Membrane properties modified by conditioning.
(7) Identification of critical developmental stages of the neural networks studied as well as stages critical for learning.

Methods Employed: The nudibranch mollusc Hermissenda crassicornis is the principal experimental preparation. Intracellular recording from several neural cells simultaneously has been the main technique used thus far. Means for simultaneously stimulating the visual and vestibular pathways (which has permitted conditioning) while recording intracellular potentials have been developed in our laboratory. Iontophonesis of

fluorescent dyes (e.g. Procion Yellow) has also been used extensively. An apparatus for monitoring and conditioning the intact animals has also been successfully employed.

Other methods planned will allow biochemical and genetic approaches to the problems of interest. These include mariculture, subcellular fractionation, protein phosphorylation analysis, uptake of neurotransmitter precursors, etc.

Major Findings:

Photoreceptor Physiology: The nature of response to light of these receptors has been closely investigated. Two distinct components of this response were identified. Two distinct channels of information flow were found: one to higher order visual cells, the other to the neural cells of the vestibular system. Within the last six months it was found that signals of specific photoreceptors in response to light can be transformed (through synaptic input from the vestibular pathway) when light is paired with rotation.

Hair Cell Physiology: First intracellular recordings from hair cells. First stimulation of hair cells with a vibration stimulus while recording intracellular potentials. This technique permitted analysis of transduction mechanisms in hair cells. It was recently adopted by another laboratory (the LNP, NINCDS) as the basis for a major research effort to extend this analysis of transduction mechanisms. Within the past year responses of hair cells to a gravitational stimulus (produced by generating a centrifugal force with a rotating table) have been studied. This technique revealed at least two distinct components within the hair cell response and provided evidence that the transduction site is at or on the hairs themselves. Elements of this technique are also now being adopted by another laboratory (the LNP, NINCDS) to perform similar analyses in Hermissenda. First identification of lateral inhibition between hair cells.

Neural Networks. The most precisely understood neural networks in neurophysiology now include, as the result of work in this laboratory, the vestibular and visual pathways identified in Hermissenda. The synaptic relation of each cell with every other cell has now been fairly well established.

Conditioning: A training paradigm analogous to conditioning in higher animals has been successfully applied to the intact animal as well as (within the last six months) to the isolated nervous system of Hermissenda. Because this paradigm involves stimulation of the two well-characterized visual and vestibular pathways, cellular mechanisms underlying short-term learning are now accessible to study. Recent experiments indicate that long-term changes in sodium activation may be crucial for the learning process.

Significance to Biomedical Research and the Program of the Institute: Knowledge of mechanisms by which simple neural networks process information will aid our understanding of neural networks of higher organisms. It is possible with simple neural networks to ask questions which cannot be asked with present technology in more complex nervous systems.

The major findings reported here range from receptor physiology to cellular mechanisms underlying a form of learning. The nervous system of Hermissenda, then, has proven to be an excellent model for neural information processing on many levels. Further studies, as suggested below, hopefully will lead to hypotheses involving biochemical processes which may be probed pharmacologically in clinical situations.

Proposed Course of the Project: Precise analysis of synaptic interactions between cells within the aforementioned neural networks of interest will be continued using the techniques of intracellular recording and iontophoresis. It is also planned to utilize electron microscopic visualization of cell contacts aided by axonal absorption of hydrogen peroxidase and/or cobalt.

Hair cell receptor physiology will be further studied with the introduction of an improved turntable which permits variable acceleration.

Cellular mechanisms responsible for the type of learning identified in the intact animal and its isolated nervous system will be further analyzed. Particular attention will be given to the role of sodium inactivation.

Biochemical and pharmacologic studies will be initiated to determine neurotransmitters within the visual and vestibular pathways. Studies will also be conducted to characterize subcellular and/or biochemical loci for the neural changes produced by the conditioning paradigms used.

Mariculture has now been successfully applied to raising Hermissenda from eggs through metamorphosis. It is planned to combine all of the foregoing approaches with mariculture. The existence of clearly identified neural networks in Hermissenda together with mariculture offer for the first time the possibility of using genetic mapping to study principles of neural organization, development and learning.

Honors and Awards: Guest Lecturer at the Institute of Biophysics, Camogli, Italy (July, 1974); Guest Lecturer at the Zoologic Institute, University of Munich, Germany (Sept, 1975)

Key Words: Photoreceptors, Hair cells, Information Processing, Learning, synaptic, interactions, mollusc, neural organization.

Publications:

Alkon, D.L., 1974. Associative training of Hermissenda, J. Gen Physiol. 64:70.
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