

ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH

1965-1966

NATIONAL INSTITUTE OF NEUROLOGICAL
DISEASES AND BLINDNESS

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

United States

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

ANNUAL REPORT *of program activities* ^{letter} ₃₄

July 1, 1965 through June 30, 1966

TABLE OF CONTENTS

	<u>Page</u>
THE DIRECTOR'S REPORT	1
Report - Special Assistant for Biometry	1-a
NDB(OD)-65-1258	3-a
Human Reproduction and the Menstrual Cycle	
Direct Training	1-b
EXTRAMURAL PROGRAMS	1-c
INTRAMURAL RESEARCH	
Table of Organization	1-d
Scientific Director's Report	1-e
NDB(I)- 66/OAD 1310	3-e
Mathematical Study of Lens Shapes of Chick Embryos	
Clinical Director's Report	1-f
<u>Medical Neurology Branch, Summary Report</u>	1-g
NDB(I)-62 MN/OC 915(c)	9-g
Histochemistry Applied to the Study of Neurologic Disease	
NDB(I)-62 MN/OC 917(c)	13-g
Biochemistry Applied to the Study of Neurologic Disease	
NDB(I)-63 MN/OC 1034(c)	17-g
Myopathies	
NDB(I)-63 MN/OC 1037(c)	23-g
Microbiology Applied to the Study of Neurologic Disease	

7C
346
112774
1965-66

	<u>Page</u>
NDB(I)-63 MN/OC 1039(c) Amyotrophic Lateral Sclerosis (ALS) and Other Lower Motor Neuron Diseases	25-g
NDB(I)-65 MN/OC 1189(c) Episodic Weakness	31-g
NDB(I)-65 MN/OC 1190(c) Myasthenia Gravis	35-g
NDB(I)-65 MN/OC 1191(c) Immunological Abnormalities of Neurologic Disease	39-g
NDB(I)-65 MN/OC 1192(c) Electron Microscopic Studies of Skeletal Muscle and Neurons	43-g
NDB(I)-65 MN/OC 1193(c) Radioautography Applied to the Study of Neurologic Disease	47-g
NDB(I)-66 MN/OC 1311(c) Site of Action of Intra-Ventricular Tubocurarine	49-g
NDB(I)-61 MN/NR 806(c) Axial Transverse Encephalography	51-g
NDB(I)-62 MN/NR 922(c) An Atlas Pathologic Pneumoencephalographic Anatomy	53-g
NDB(I)-62 MN/NR 925(c) The Small Sella Turcica	55-g
NDB(I)-63 MN/NR 1047(c) Isotope-Ventriculography and Isotope- Cisternography	57-g
NDB(I)-63 MN/NR 1048(c) Prognostic Significance of Parasellar Carotid Calcifications	59-g
NDB(I)-65 MN/NR 1195(c) Selective Arteriography of Spinal Cord Arterio- venous Aneurysms	61-g
NDB(I)-65 MN/NR 1196(c) Clinical Comparison of Radioactive Tracers Used for Brain Scanning	65-g

	<u>Page</u>
NDB(I)-65 MN/NR 1197(c) Radio-Isotope Brain Scanning for Evaluating the Function of Cerebrospinal Fluid Shunts	67-g
NDB(I)-65 MN/NR 1198(c) Brain Scanning(Monograph)	69-g
NDB(I)-65 MN/NR 1199(c) Virus Induced Experimental Brain Tumors in Dogs	71-g
NDB(I)-65 MN/NR 1200(c) Secondary Radiation from Irradiated Residual X-ray Opaque Material in the Spinal Canal	73-g
NDB(I)-65 MN/NR 1201(c) A Repeating Surgical Monostapler	75-g
NDB(I)-65 MN/NR 1202(c) Salivary Gland Scanning with Technetium 99m (^{99m} Tc) Pertechnetate	77-g
NDB(I)-65 MN/NR 1203(c) A Bilateral Angiographic Computer Evaluation of the Superficial Veins and Sinuses of the Brain	79-g
NDB(I)-66 MN/NR 1283(c) The Empty Sella Turcica	81-g
NDB(I)-66 MN/NR 1284(c) The Tetrascanner	83-g
NDB(I)-61 MN/AP 807(c) A Comparative Pharmacological Study of Fast and Slow Muscles	87-g
NDB(I)-62 MN/AP 926(c) Electromechanical Coupling in Muscle and Drug Activity	89-g
NDB(I)-63 MN/AP 1049(c) Mechanical Properties of Muscle in Relation to Drug Action	91-g
NDB(I)-63 MN/AP 1050(c) The Relationship Between the Functional Activity and the Mechanical Properties of Normal and Modified Striated Muscle	93-g
NDB(I)-65 MN/AP 1194(c) Genetic Studies of Abnormal Plasma Cholinesterase in Relation to Drug Metabolism	95-g

	<u>Page</u>
<u>Surgical Neurology Branch, Summary Report</u>	1-h
NDB(I)-54 SN/OC 100(c) Epileptogenic Mechanisms in the Brain of Man and Other Primates	11-h
NDB(I)-54 SN/OC 101(c) Functional Representation in the Temporal Lobe of Man and Higher Primates	17-h
NDB(I)-55 SN/OC 200(c) Involuntary Movements	21-h
NDB(I)-56 SN/OC 303(c) The Study of the Functional Anatomy and Pathology of the Human Visual System	27-h
NDB(I)-56 SN/OC 304(c) Effect of Lesions Upon the Function and Structure of the Human Central Nervous System	29-h
NDB(I)-61 SN/OC 802(c) Single Cell Discharges in Motor Cortex in Response to Stimulation of Basal Ganglia and Cerebellum	31-h
NDB(I)-62 SN/OC 906(c) The Effects of Cold and the Relation of Temperature to Functions of the Central Nervous System	33-h
NDB(I)-62 SN/OC 907(c) Trauma to the Nervous System. Experimental and Clinical	37-h
NDB(I)-62 SN/OC 913(c) Single Cell Discharges from Cerebral Cortex of Man	41-h
NDB(I)-63 SN/OC 1025 (c) Tumors of the Nervous System	43-h
NDB(I)-65 SN/OC 1205(c) Neurosurgical Monitoring	45-h
NDB(I)-65 SN/OC 1206(c) Microbial Characteristics in a Neurosurgical Environment	47-h
NDB(I)-60 SN/NA 702(c) Experimental Hypothermia	55-h

	<u>Page</u>
NDB(I)-60 SN/CN 706(c) Clinical, Biochemical and Genetical Studies of Mental Retardation, Progressive Cerebral Degeneration and Cerebral Palsy in Children	59-h
NDB(I)-60 SN/CN 707(c) Study of Pathological Lesions in the Central Nervous System Occurring During Prenatal, Intra- natal and Early Postnatal Life	63-h
NDB(I)-60 SN/CN 708(c) Cytogenetical Study of Human Chromosomes Especially in Patients with Congenital Mal- formations and Mongoloids and Their Families	67-h
NDB(I)-63 SN/CN 1026(c) Clinical and Biochemical Studies in Epilepsy	73-h
NDB(I)-65 SN/CN 1204(c) Studies on the Incorporation of an Amino Acid Analogue -TRIFLUOROLEUCINE - Into Protein of Mammals	77-h
NDB(I)-63 SN/NP 1027(c) Changes in Chloride and Water Content of Brain Tissue After Chemical Injury of the Blood-Brain Barrier	79-h
NDB(I)-63 SN/NP 1030(c) Observations on Differential Penetrability of Simultaneously Administered Tracers in Blood- Brain Barrier (BBB) Damage	83-h
NDB(I)-64 SN/NP 1207(c) Effect of Systolic Blood Pressure on the Dynamics of Brain Edema	87-h
NDB(I)-66 SN/NP 1285(c) Cellular Morphology of the Lemon Shark Brain	89-h
NDB(I)-66 SN/NP 1286(c) Enzyme Levels in Experimental Brain Edema	91-h
NDB(I)-66 SN/NP 1287(c) Comparative Protein and Enzyme Profiles of the Cerebrospinal Fluid, Extradural Fluid, Nervous Tissue, and Sera of Elasmobranchs	93-h
NDB(I)-57 SN/CP 401(c) Psychological Evaluation of Temporal Lobe Operations	95-h

	<u>Page</u>
NDB(I)-63 SN/CP 1032(c) Psychological Effects of Subcortical Lesions Used for Relief from Abnormal Movements	99-h
NDB(I)-63 SN/CP 1033(c) Phonemic Aspects of Dysnomia	101-h
NDB(I)-66 SN/CP 1245(c) EEG Learning Correlates Using Intracranial Depth Electrodes	103-h
<u>Ophthalmology Branch</u> , Summary Report	1-i
NDB(I)-56 O/OPS 300(c) Design and Construction of Ophthalmic Instruments; Research in Psychophysical Methods of Evaluating Vision	9-i
NDB(I)-60 O/OPS 700(c) Activity of Visual Receptor Organs	13-i
NDB(I)-63 O/OPS 1012(c) Electrophysiologic and Psychophysical Studies of Tapeto-Retinal Degenerations - Clinical and Experimental	17-i
NDB(I)-63 O/OPS 1016(c) Electrophysiological Studies of Mammalian Retina	19-i
NDB(I)-66 O/OPS 1288(c) Spectral Sensitivities of Receptor Cells in the Lateral Eye of <u>Limulus</u>	21-i
NDB(I)-56 O/CH 302(c) Electron Microscopic Studies on Tissues of the Eye, Such as Epithelium, Fibers, and Capsule of the Lens, and the Conjunctiva	23-i
NDB(I)-61 O/CH 801(c) Autoradiographic Studies of Cellular Prolifer- ation in Ocular Tissues	25-i
NDB(I)-65 O/CH 1212(c) Retina and Optic Nerve Pathology in Autoimmune Encephalomyelitis of Rhesus Monkeys of Different Ages	29-i
NDB(I)-65 O/OC 1216(c) Thioacetamide-Induced Cataract in Trout	33-i

	<u>Page</u>
NDB(I)-65 O/CH 1223(c) Studies on the Enhancement of Contrast in Isolated Particles and Thin Sections	35-i
NDB(I)-66 O/CH 1289(c) Fine Structure of Toad Retinal Capillaries	37-i
NDB(I)-66 O/CH 1290(c) Studies on the Function of the Retinal Pigment Epithelium	39-i
NDB(I)-66 O/CH 1291 (c) Studies on the Endothelium of the Corneal Homograft	43-i
NDB(I)-63 O/OCH 1017(c) Physical Chemistry of Corneal Collagen	47-i
NDB(I)-65 O/OCH 1210(c) Chemistry of Rhodopsin	49-i
NDB(I)-65 O/OCH 1217(c) Physical Biochemistry of Model Gel System	51-i
NDB(I)-66 O/OCH 1292(c) Physical Chemistry of Neucleic Acids	53-i
NDB(I)-66 O/OCH 1293(c) Chemistry of Vitreous Proteins	55-i
NDB(I)-65 O/CB 1209(c) Mode of Action of Rhodopsin	57-i
NDB(I)-65 O/CB 1211(c) Studies on Glycoprotein Synthesis	59-i
NDB(I)-66 O/CB 1294(c) Synthesis of Sugar-Containing Polymers in Retina	61-i
NDB(I)-66 O/CB 1295(c) Aminosugar Metabolism in the Retina	63-i
NDB(I)-56 O/OPH 301(c) Study on the Pharmacodynamics of Various Agents Affecting the Intraocular Pressure	67-i
NDB(I)-59 O/OPH 600(c) The Role of Vasculature in the Maintenance of Intraocular Pressure	71-i

	<u>Page</u>
NDB(I)-63 O/OPH 1018(c) A Study on Enucleated, Arterially Perfused Human and Cat Eyes	75-i
NDB(I)-65 O/OPH 1214(c) The <u>In Vitro</u> Effect of Corticosteroids on the Intraocular Muscles of the Cat and Monkey	77-i
NDB(I)-65 O/OPH 1215(c) Studies on Extraocular Muscles. (a) A Comparative Pharmacologic-Histologic Study of the Rabbit. (b) The <u>In Vitro</u> Effect of Sympathomimetic Agents on the Cat, Monkey, and Rabbit	79-i
NDB(I)-58 O/OC 500(c) Investigation of the Effects of Topically Administered Steroids on the Intraocular Pressure Examination of Aqueous Humor Dynamics	81-i
NDB(I)-63 O/OC 1022(c) Methotrexate Therapy of Selected Patients with Uveitis	83-i
NDB(I)-65 O/OC 1213(c) The Effect of Experimental Choroidal Ischemia on the Retina	85-i
NDB(I)-65 O/OC 1218(c) Ocular Manifestations in Carcinoid Syndrome	87-i
NDB(I)-65 O/OC 1219(c) The Ocular Complications of Chloramphenical Therapy	89-i
NDB(I)-65 O/OC 1220(c) Ocular Manifestation of Ehlers-Danlos Syndrome	91-i
NDB(I)-65 O/OC 1221(c) Clinical Aspects of Fabry's Disease (Glycolipid Lipidosis)	93-i
NDB(I)-65 O/OC 1222(c) Ocular Manifestations of Chlorpromazine and Other Phenothiazines	95-i
NDB(I)-66 O/OC 1296(c) Maintenance of Anterior Chamber Depth During Anterior Segment Surgery	97-i

	<u>Page</u>
NDB(I)-66 O/OC 1297(c) Peripheral Retinopathy of Cystinosis	99-i
NDB(I)-66 O/OC 1298(c) Suppression of Corneal Graft Rejection with Protein Synthesis Inhibitors	101-i
<u>Electroencephalography Branch</u> -Summary Report	1-j
NDB(I)-61 EEG/OC 800(c) Depth Recording in Man	7-j
NDB(I)-65 EEG/OC 1225(c) Nocturnal Sleep. Implanted Electrode Study	9-j
NDB(I)-65 EEG/CN 1226(c) Electrical Constants of Neurons in the Motor Cortex of Cat	11-j
NDB(I)-65 EEG/CN 1227(c) Cortical Neurons; Conductance Changes During IPSPs and Evaluation of Anodal Break and Post-Inhibitory Rebound	15-j
NDB(I)-66 EEG/CN 1299(c) Epilepsy - A Critical Review of the Literature for 1964-1965	19-j
NDB(I)-66 EEG/CN 1300(c) Basic Mechanisms in Epilepsy	21-j
NDB(I)-66 EEG/CN 1301(c) The Thalamus. Data on its Functional Anatomy and on Some Aspects of Thalamo-Cortical Integration	23-j
NDB(I)-66 EEG/CN 1302(c) Impedance Measurements of Motoneurons by Means of Separated Double Microelectrodes	25-j
<u>Laboratory of Neuroanatomical Sciences</u> -Summary Report	1-k
NDB(I)-60 LNS/FN 712 The Ascending and Descending Auditory Connections in the Primates	9-k
NDB(I)-60 LNS/FN 713 A Study of the Auditory Vestibular Afferent and Efferent Systems Including the Receptors in the Chinchilla and Cat	11-k

	<u>Page</u>
NDB(I)-65 LNS/FN 1229 Fine Structure of Afferent and Efferent Nerve Endings in the Cochlear Nucleus of Normal and Experimental Animals	13-k
NDB(I)-60 LNS/EN 711 Regeneration in the Peripheral Nervous System	15-k
NDB(I)-63 LNS/EN 1054 "Trophic" Functions of the Peripheral Nervous System	17-k
NDB(I)-66 LNS/EN 1303 Histochemical Characteristics of Muscle Sensory Systems	19-k
NDB(I)-61 LNS/NC 808 The Innervation of Smooth Muscle	21-k
NDB(I)-61 LNS/NC 809 The Uptake of Ferritin by the Ependyma of the Rat Brain	23-k
NDB(I)-63 LNS/NC 1051 The Distribution Within the Brain of Ferritin Injected Intraventricularly	25-k
NDB(I)-65 LNS/NC 1230 Localization of Monoamines in Brain	27-k
NDB(I)-65 LNS/NC 1231 The Fine Structure of the Olfactory Bulb, I, Glomerulus	29-k
NDB(I)-65 LNS/NC 1232 The Structural Effects of the Monoamine Oxidase Inhibitor Pargyline on the Nerve Endings of Rat Vas Deferens	31-k
NDB(I)-66 LNS/NC 1304 Pinocytosis in the Brains of Dead Rats	33-k
NDB(I)-66 LNS/NC 1305 Normal Fine Structure of Nerve Endings in the Nucleus of the Trapezoid Body and Ventral Cochlear Nucleus	35-k
NDB(I)-65 LNS/EE 1233 Morphogenesis of the Inner Ear	37-k
NDB(I)-65 LNS/EE 1234 Ocular Morphogenesis	41-k

	<u>Page</u>
NDB(I)-65 LNS/EE 1235 Histogenesis of the Neural Portions of the Visual System as Related to the Onset of Function	45-k
<u>Laboratory of Neuropathology</u> - Summary Report	1-1
NDB(I)-62 LN/EN 942 Histochemical Study of Nerve Cells	3-1
NDB(I)-62 LN/EN 943 The Effect of Aging on the Central Nervous System	5-1
NDB(I)-62 LN/EN 944 Hematologic Control of Primates	7-1
NDB(I)-63 LN/EN 1063 The Reaction of Mesodermal Cells in the Central Nervous System During Senescence	9-1
NDB(I)-63 LN/EN 1065 Perfection of the Perfusion Technique for Fixation <u>In Situ</u>	11-1
NDB(I)-63 LN/EN 1066 Acute Degenerative Changes in the Central Nervous System	13-1
NDB(I)-62 LN/EN 1237 Cytologic Characteristics of Microglia Cells	15-1
NDB(I)-66 LN/EN 1306 A Comparative Study of Acute Retrograde Neuronal Changes	17-1
NDB(I)-66 LN/EN 1307 Extranuclear Expulsion of Nucleoli	19-1
<u>Laboratory of Neurophysiology</u> -Summary Report	1-m
NDB(I)-58 LNP/SC 501 Generation of Impulses in Nerve Cells	3-m
NDB(I)-62 LNP/SC 934 Basic Mechanisms of Synaptic Transmission	7-m
NDB(I)-62 LNP/SC 973 Integrative Mechanisms in the Central Auditory Pathway	11-m
NDB(I)-65 LNP/SC 1239 Photoreceptors in the <u>Limulus</u> eye	13-m

	<u>Page</u>
<u>Laboratory of Biophysics-Summary Report</u>	1-n
NDB(I)-62 LB/CB 935	5-n
Ionic Permeabilities of Excitable Membranes. Electrical Experiments and Analyses	
NDB(I)-62 LB/CB 939	9-n
Ionic Permeabilities of the Squid Giant Axon Membrane. Electrical Experiments and Analyses with Alterations of Environments	
NDB(I)-62 LB/I 940	13-n
Ionic Permeabilities of the Squid Giant Axon. Effects of Chemical Agents	
NDB(I)-62 LB/MA 936	17-n
Ionic Permeabilities of Nerve Membranes: Theoretical Investigations	
NDB(I)-62 LB/ME 938	21-n
Alternating Current Bridge Measurements of Squid Axon Membrane Dielectric Properties	
NDB(I)-65 LB/ME 1240	23-n
Molecular Biophysics-Physical Properties of Membranes and Simple Membrane-Like Systems	
<u>Laboratory of Neurochemistry-Summary Report</u>	1-o
NDB(I)-61 LNC/AAC 810	5-o
Metabolism of Free and Protein-Bound Amino Acids in Neural Tissues	
NDB(I)-61 LNC/AAC 811	9-o
Electrolytes and Energy Metabolism in Cerebral Cortex <u>In Vitro</u>	
NDB(I)-61 LNC/EC 813	13-o
Enzymological Aspects of Neural Function	
NDB(I)-61 LNC/LC 815	15-o
Metabolism of Complex Lipids of Nervous Tissue	
NDB(I)-61 LNC/LC 816	19-o
Structural and Metabolic Studies of Gangliosides	
NDB(I)-61 LNC/LC 817	21-o
Immunochemical Studies in Multiple Sclerosis	
NDB(I)-65 LNC/LC 1241	23-o
Investigation of the Anti-Cancer Activity of Derivatives of Tetrolic Acid	

	<u>Page</u>
NDB(I)-66 LNC/LC 1308 Sphingolipid Metabolism in Tissue Culture Cells	25-o
NDB(I)-66 LNC/LC 1309 Studies on the Metabolism of Sphingolipids in Tumor Tissue	27-o
NDB(I)-62 LNC/PM 941 Studies on Bioelectrogenesis	29-o
NDB(I)-65 LNC/PM 1242 The Mechanism of Lipoprotein Synthesis	31-o
NDB(I)-65 LNC/PM 1243 Pilot Studies and Consulting Services in Neurochemistry	35-o
<u>Laboratory of Molecular Biology</u> -Summary Report	1-p
NDB(I)-62 LMB/OC 947 Structure and Alteration of DNA and Chromosomes	7-p
NDB(I)-63 LMB/OC 1074 Genetic Analysis of Virus-Induced Mutants of <u>Escherichia coli</u>	11-p
NDB(I)-65 LMB/OC 1208 The Participation of Ribonucleic Acid in the Regulation of Cellular Metabolism	13-p
NDB(I)-65 LMB/OC 1244 Control and Differentiation of Protein Synthesis and Function	15-p
<u>Laboratory of Perinatal Physiology</u> -Summary Report	1-q
NDB(CF)-59 LPP 603 Social Behavior, Reproduction, and Population Dynamics in Free-Ranging Rhesus Monkeys	11-q
NDB(I)-65 LPP 1259 Neuropathological Effects of Umbilical Cord Compression	15-q
NDB(I)-65 LPP 1260 A Correlational Study of Brain Lesion Effects on a Behavioral Test Battery	17-q
NDB(I)-65 LPP 1261 The Superior Colliculus in the Goat	19-q

	<u>Page</u>
NDB(I)-65 LPP 1262 Reproduction Behavior of Caged Rhesus Monkeys	21-q
NDB(I)-65 LPP 1263 Ocular Pathology and Experimental Allergic Encephalomyelitis in Young Monkeys	23-q
NDB(I)-65 LPP 1264 Studies on Auto-Immune Diseases and Related Immunological Processes	25-q
NDB(I)-66 LPP 1384 Localization of Learned Motor Skill Functions in Corpus Callosum in the Monkey	27-q
NDB(I)-66 LPP 1385 Pathology of the Stillborn	29-q
NDB(I)-66 LPP 1386 Experimental Placental Insufficiency in the Rhesus Monkey	31-q
NDB(I)-66 LPP 1387 Experimental Placental Abruptio in the Rhesus Monkey and its Relation to Brain Damage	33-q
NDB(I)-66 LPP 1388 Perinatal Asphyxia in the Monkey and its CNS Consequences	35-q
NDB(I)-66 LPP 1389 Experimental Hydranencephaly in the Monkey	37-q
NDB(I)-66 LPP 1390 The Effects of Oxygen in High Concentrations and Vasopressor Substances upon the Fetus	39-q
NDB(I)-66 LPP 1391 Rate of Absorption of Donor Red Cells Following their Administration into the Peritoneal Cavity of the Fetus	41-q
NDB(I)-66 LPP 1392 Nembutal Prophylaxis of Brain Damage	43-q
NDB(I)-66 LPP 1393 Acid Base Balance Studies of Amniotic Fluid in Rhesus Monkeys	45-q
NDB(I)-66 LPP 1394 Acid Base Balance Study of Amniotic Fluid in Relation to Fetal Distress in Rhesus Monkeys	47-q

	<u>Page</u>
NDB(I)-66 LPP 1395 Hyperbilirubinemia and Kernicterus in the Rhesus Monkey	49-q
NDB(I)-66 LPP 1396 Neuropathology of Lead Encephalopathy in the Rhesus Monkey	51-q
NDB(I)-66-LPP 1397 Comparative Studies of Ascending Spinal Projections	53-q
NDB(I)-66 LPP 1398 Development of Neurohistological Methods	55-q
NDB(I)-66 LPP 1399 Comparative Studies of Retinal Projections	57-q
NDB(I)-66 LPP 1400 Functional Properties of Cells within the Juxtastriate Area 18 of the Rhesus Monkey	59-q
NDB(I)-66 LPP 1401 Investigations on the Biochemistry of the Necrobiosis of Mouse Brain	61-q
NDB(I)-66 LPP 1402 Investigations on the Chemistry of Necrobiosis of the Cat Brain	63-q
NDB(I)-66 LPP 1403 Effect of a Prefrontal Cortical Lesion on a Non- Spatial Delayed Response Task	65-q
NDB(I)-66 LPP 1404 Effects of Brain Lesions on Conditioned Vocalization in Monkeys	67-q
NDB(I)-66 LPP 1405 Lactate Concentration in Amniotic Fluid	69-q
NDB(I)-66 LPP 1406 Birth Weight and Gestational Age	71-q
NDB(I)-66 LPP 1407 The Pathogenesis and Transmission of Pulmonary Acariasis	73-q
NDB(I)-66 LPP 1408 Mycoplasma Antibody Titers in the Sera of Normal and Clinically Ill <u>M. mulatta</u>	75-q

	<u>Page</u>
NDB(I)-66 LPP 1409 Embryonic Tooth Development in the Rhesus Monkey	77-q
NDB(I)-66 LPP 1410 Reproduction and Behavior in the Ecology of the Rhesus Monkey	79-q
NDB(I)-66 LPP 1411 Social Behavior in Confined Rhesus Monkeys	81-q
NDB(I)-66 LPP 1412 Group Behavior, Reproduction, and Population Dynamics in Free-Ranging Rhesus Monkeys	83-q
NDB(CF)-61 LPP 820 Effects of Controlled Early Experience on Development of the Siamese Kitten	85-q
NDB(CF)-61 LPP 821 Early Development of Emotional Learning in Monkeys	87-q
NDB(CF)-61 LPP 822 The Effects of Infantile Trauma and Social Isolation During Rearing Upon Adult Reproductive Behavior in the Rhesus Monkey <u>Macaca mulatta</u>	89-q
NDB(CF)-63 LPP 1075 The Effects of Neonatal Damage to the Inferior Colliculus upon the Physiological and Behavioral Development of the Rhesus Monkey (<u>Macaca mulatta</u>)	91-q
NDB(CF)-63 LPP 1076 Infantile Trauma: The Role of Genetic and Environmental Factors	93-q
NDB(CF)-63 LPP 1077 Studies on the Development of Sleep and Wakefulness Patterns in the <u>Macaca mulatta</u>	95-q
NDB(CF)-63 LPP 1080 Experimental Ketosis in the Pregnant Rhesus Monkey	97-q
NDB(CF)-63 LPP 1081 Electrophoretic Analysis of the Hemoglobins of a Large Colony of <u>Macaca mulatta</u>	99-q
NDB(CF)-63 LPP 1082 <u>In Vitro</u> Metabolism of Ketone Bodies by Gestational and Fetal Tissues of the <u>Macaca mulatta</u>	101-q

	<u>Page</u>
NDB(CF)-63 LPP 1083 Electrophoretic Analysis of Cord Blood Hemo- globins and Infant Hemoglobins in the <u>Macaca mulatta</u>	103-q
NDB(CF)-63 LPP 1088 Mechanisms of Vasomotor Response Observed After i.v. Administration of a Muscle Relaxant in the Cat	105-q
NDB(CF)-63 LPP 1089 Quantitative Study of the Growth of the Skull and Brain in the Cat from Birth to Seventeen Weeks of Age	107-q
NDB(I)-65 LPP 1265 Responsiveness of Denervated Diaphragmatic Muscle to Immunological Stimulation	109-q
NDB(I)-65 LPP 1266 The Circulation of the Lungs	111-q
NDB(I)-65 LPP 1267 The Effect of Temperature Change on Muscle Activity in the Extremities after Upper Motor Neuron Lesions	113-q
 COLLABORATIVE & FIELD RESEARCH	
Associate Director's Report	1-r
<u>Special Chronic Disease Studies</u> -Summary Report	5-r
NDB(CF)-62 OAD 969 Slow, Latent, and Temperate Virus Infections of the Central Nervous System of Man and Animals	7-r
Sub Project I : Attempts to Isolate Transmissible Agents from Subacute and Chronic Diseases of the Nervous System	8-r
Sub Project II : Workshop-Symposium on Slow, Latent, and Temperate Virus Infections	15-r
Sub Project III: Fluorescent Antibody Technique in Localizing Neurotropic Virus Antigen in Whole Animals	17-r
Sub Project IV : Studies on the Ecology, Epidemi- ology and Pathogenesis of Arbo- virus Infections	21-r

Studies of Child Growth, Development, and Behavior
and Disease Patterns in Primitive Cultures

- Sub Project I : Study of the Developmental Pattern- 23-r
ing of the Human Nervous System
(A Cybernetics of Human Develop-
ment)
- Sub Project II : Human Evolutionary Study in 23-r
Isolated Primitive Groups
- Sub Project III : Studies of Isolated Micronesian 23-r
Populations
- Sub Project IV : Studies of Isolated New Guinea 24-r
Population
- Sub Project V : Studies in Isolated New Hebridean 24-r
and Solomon Islands Populations
- Sub Project VI : Studies on Australian Aborigines 24-r
- Sub Project VII : Studies on Central and South 24-r
American Indians
- Sub Project VIII: Developmental, Genetic and Disease 25-r
Pattern Studies in Other Primitive
Populations of Asia, Africa and
Polynesia
- Sub Project IX : Experimental Developmental Neuro- 25-r
pediatrics of Infantile Program-
ming: An Empirical Approach to
the Languages of Information Input
into the Nervous System
- Sub Project X : Ciphers and Notation for the Cod- 25-r
ing of Sensory Data for Neurological
Information Processing
- Sub Project XI : Racial Distribution of Neuro- 25-r
anatomical Variations in the
Structure of the Human Brain

	<u>Page</u>
<u>Biometrics Branch</u> -Summary Report	1-s
NDB(CF)-61 B 833 Model Reporting Area for Blindness Statistics	19-s
NDB(CF)-62 B 965 Study of Association Between Factors of Pregnancy, Labor, and Delivery and the Occurrence of Blind- ness in Children	25-s
NDB(CF)-62 B 968 Monograph on Neurological and Sense Organ Diseases: A.P.H.A. Vital and Health Statistics Monographs Series	27-s
NDB(CF)-63 B 1134 Study of Perinatal Factors Associated with Strabismus in Children	29-s
NDB(CF)-63 B 1137 A Study of Awareness, Attitudes and Performance in Reporting Persons to Blindness Registers	33-s
NDB(CF)-64 B 1187 Study of Association between Factors of Pregnancy, Labor and Delivery and the Occurrence of Deafness in Children	37-s
NDB(CF)-64 B 1188 Study of Blindness Statistics in Egypt	39-s
<u>Epidemiology Branch</u> -Summary Report	1-t
NDB(CF)-54 E 102 Population Survey and Case-Finding Study of ALS-PD Complex in the Mariana Islands Other than Guam, and the Caroline Islands	15-t
NDB(CF)-54 E 103 Clinical Studies on ALS-PD Complex of Guam	17-t
NDB(CF)-55 E 201 Epidemiological Aspects of ALS/PD Complex on Guam	21-t
NDB(CF)-61 E 832 Studies on the Natural History of Multiple Sclerosis	23-t
NDB(CF)-62 E 957 Amyotrophic Lateral Sclerosis and Parkinsonism- Dementia Complex: Further Pathologic Studies	25-t

	<u>Page</u>
NDB(CF)-62 E 958 A Serological and Hematological Genetic Survey of of Chamorros in Guam	27-t
NDB(CF)-62 E 959 Further Studies on the CA ₁ C Variant of Carbonic Anhydrase Enzyme	29-t
NDB(CF)-62 E 961 Five-Year Registry Study of ALS/PD Cases on Guam	31-t
NDB(CF)-63 E 1090 Pedigree Studies in Umatac	33-t
NDB(CF)-63 E 1091 Serological and Hematological Investigations Among the Trukese in Micronesia	35-t
NDB(CF)-63 E 1092 Dermatoglyphic Evaluation of the Chamorros and Carolinians	37-t
NDB(CF)-63 E 1093 Blood Group Investigations on the Carolinians and Chamorros of Saipan	39-t
NDB(CF)-63 E 1101 Clinical Aspects of Diabetes Mellitus in Guam	41-t
NDB(CF)-63 E 1103 Neurological Diseases Other than ALS-PD on Guam	43-t
NDB(CF)-63 E 1106 Screening of Edible and Medicinal Legumes for Neurotoxic Constituents	45-t
NDB(CF)-63 E 1107 Food, Medicinal, and Toxic Plants of Mitogawa, Japan	47-t
NDB(CF)-63 E 1108 Study of the Toxic Constituents of <u>zen mai</u> (<u>Osmunda japonica</u> Thunb.)	49-t
NDB(CF)-63 E 1109 Study of Toxic Fungi in Cycad and Other Plant Material Used for Human or Animal Food in Various Areas of the World	51-t

	<u>Page</u>
NDB(CF)-63 E 1110 Plants from the Island of Guam: Food, Medicinal and Toxic	53-t
NDB(CF)-63 E 1111 Neurotoxin(s) in Fronds of Cycad Plants	55-t
NDB(CF)-63 E 1112 Chemistry of the Cycad (Related to Carcinogenic Hepatotoxic Components)	59-t
NDB(CF)-63 E 1113 Effects on Animals Following Administration of Cycad Plant Material and of Toxic Compounds Isolated From Cycads	61-t
NDB(CF)-63 E 1114 Effects of Fresh Cycad Kernel Meal on Open Wounds	63-t
NDB(CF)-63 E 1117 Epidemiological Studies of Multiple Sclerosis in New Orleans, Louisiana	65-t
NDB(CF)-65 E 1248 The Fine Structure of Cerebral Fluid Accumulation, VII. Reactions of Astrocytes to Cryptococcal Polysaccharide Implantation	67-t
NDB(CF)-64 E 1250 The Reaction of the Nervous System to Cryptococcal Infection. An Experimental Study with Light and Electron Microscopy	69-t
NDB(CF)-64 E 1251 Amyotrophic Lateral Sclerosis, Histopathological Considerations	71-t
NDB(CF)-65 E 1252 Reaction of Ependyma to Cryptococcal Polysaccharide Implantation	73-t
NDB(CF)-64 E 1253 Influence of Small Systematic Errors on the Results of Tonometric Screening	75-t
NDB(CF)-64 E 1254 Comparison of Measurements of Parameters of Chronic Simple Glaucoma Among Monozygous and Dizygous Twins	77-t

	<u>Page</u>
NDB(CF)-64 E 1255 Tonometry Survey in an Italian-American Population	79-t
NDB(CF)-64 E 1256 Tonometry Survey Among an American Indian Population at Colorado River Reservation, Arizona	81-t
NDB(CF)-64 E 1257 U.S. Mortality Experience with Malignant Neoplasms of the Eye	83-t
NDB(CF)-66 E 1312 Comparison of Components of Refraction and Ocular Motility Among Monozygous and Dizygous Twins	85-t
NDB(CF)-66 E 1313 Ophthalmic Survey of Population Groups of the Solomon Islands	87-t
NDB(CF)-66 E 1314 Influence of a Synthetically Derived Aglycone of Cycasin on Ocular Tissues	89-t
NDB(CF)-66 E 1315 Tonometry Survey Among American Indian Population of Salt River Reservation, Arizona	91-t
NDB(CF)-66 E 1316 Hyperacute Allergic Encephalomyelitis. Electron Microscopic Observations	93-t
NDB(CF)-66 E 1317 The Fine Structure of Cerebral Fluid Accumulation. IX. Edema Following Silver Nitrate Implantation	95-t
NDB(CF)-66 E 1318 The Fine Structure of Cerebral Fluid Accumulation. X, A Review of Experimental Edema in White Matter	97-t
NDB(CF)-66 E 1319 Determining Autoimmunity in Chronic Neurological Diseases by the Use of Peripheral Lymphocytes	99-t
NDB(CF)-66 E 1320 Incidence of ALS and PD Among Guamanians Living in the States	101-t

	<u>Page</u>
NDB(CF)-66 E 1321 Japanese B Encephalitis Studies on Guam	103-t
NDB(CF)-66 E 1322 Ear Infections and Hearing Loss on Guam	105-t
<u>Special Projects Branch</u> (See Associate Director's Report)	1-r
<u>Perinatal Research Branch-Summary Report</u>	1-v
NDB(CF)-57 PR/ID 402 Epidemiology of Infectious Diseases in the Collaborative Study on Cerebral Palsy, Mental Retardation, and Other Neurological and Sensory Disorders of Infancy and Childhood	57-v
NDB(CF)-61 PR/ID 835 Clinical Investigations in Human Volunteer and Other Populations of Virus Effects and Production of Prototype Human Antisera and Vaccines	63-v
NDB(CF)-62 PR/ID 972 Experimental Animal, Tissue Culture, Histo- pathological and Serological Investigations of the Role of Viruses and Other Micro-Organisms in the Perinatal Period.	67-v
NDB(CF)-65 PR/ID 1238 Isolation of Infectious Agents from Tissues and Chromosomal Studies	71-v
NDB(CF)-65 PR/ID 1270 Toxoplasmosis: Serological and Clinical Studies	73-v
NDB(CF)-65 PR/ID 1271 Maternal Rubella and Pregnancy Outcome	75-v
NDB(CF)-66 PR/ID 1326 Rubella Vaccine Development Program	77-v
NDB(CF)-63 PR/OC 1144 An Instrument for the Conduct of a Restrospective Study of Seizures, Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood	79-v

NDB(CF)-63 PR/OC 1146	81-v
Revision and Expansion of Previous Project Entitled: A Commentary on the Appropriateness of the Use of Certain Tabular Data, for Formulating Generalizations Concerning Populations in the Same Cities as Those in which the Collaborative Study on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood Is Being Conducted	
NDB(CF)-66 PR/OC 1368	83-v
Cesarean Section in Labor	
NDB(CF)-66 PR/OC 1369	85-v
Spontaneous Premature Rupture of the Membrane	
NDB(CF)-66 PR/OC 1370	87-v
Fetal Hazard after Rupture of the Membrane	
NDB(CF)-66 PR/OC 1371	89-v
The Epidemiology of Neonatal Seizures	
NDB(CF)-66 PR/OC 1372	91-v
The Birthweight-Placental Weight Relationship -- A Statistical Analysis	
NDB(CF)-66 PR/OC 1373	93-v
The Effects of the Duration of Labor on the Fetus	
NDB(CF)-66 PR/OC 1374	95-v
Complications of Pregnancy in Relation to the Duration of Labor	
NDB(CF)-66 PR/OC 1375	97-v
Early Symptomatology and Pregnancy Outcome	
NDB(CF)-66 PR/OC 1376	99-v
Organic Heart Disease in Pregnancy	
NDB(CF)-66 PR/OC 1377	101-v
Reproductive Wastage in Bronchial Asthma	
NDB(CF)-66 PR/OC 1378	105-v
The Prediction of Birthweight-Multivariate Analysis	
NDB(CF)-66 PR/OC 1379	107-v
Fetal Heart Rate in Relation to Pregnancy Outcome	

	<u>Page</u>
NDB(CF)-66 PR/OC 1380 Acute and Chronic Glomerulonephritis in Pregnancy	109-v
NDB(CF)-66 PR/OC 1381 Complications of Pregnancy and Their Association with Change in the Placental Weight/Birthweight Relationship	111-v
NDB(CF)-66 PR/OC 1382 The Effects of Maternal Diabetes	113-v
NDB(CF)-66 PR/OC 1383 Certain Socio-Cultural Parameters in Relation to Pregnancy Outcome	115-v
NDB(CF)-66 PR/OB 1327 Maternal Deaths in the Collaborative Project	117-v
NDB(CF)-66 PR/OB 1328 Evaluation of the Possible Teratogenic Effect of Antihistaminic Drugs Taken During Pregnancy	119-v
NDB(CF)-66 PR/OB 1329 Estrus, Ovulation and Menstruation; A Critical Historical Review	121-v
NDB(CF)-66 PR/OB 1330 The Length of the Premenstrual Phase by Age of Women	123-v
NDB(CF)-66 PR/OB 1331 Obstetric Factors in Twin Pregnancies	125-v
NDB(CF)-66 PR/OB 1332 Down's Syndrome and the Incidence of Infectious Hepatitis During Pregnancy	127-v
NDB(CF)-66 PR/OB 1333 Distribution of Abortions by Chronologic and Gynecologic Age of the Gravida	129-v
NDB(CF)-66 PR/OB 1334 The Span of Human Gestation	131-v
NDB(CF)-63 PR/PN 1155 Apgar Score - An Index of Infant Morbidity	133-v
NDB(CF)-63 PR/PN 1156 Neonatal Hyperbilirubinemia and Subsequent Neurological Sequelae	135-v

	<u>Page</u>
NDB(CF)-63 PR/PN 1161 Prematurity	137-v
NDB(CF)-63 PR/PN 1162 Apgar Scores	139-v
NDB(CF)-63 PR/PN 1163 An Investigation into the Relationship Between Congenital Heart and Great Vessel Anomalies and Selected Perinatal Factors as Recorded in the Collaborative Perinatal Research Project	141-v
NDB(CF)-63 PR/PN 1164 Early Signs as Predictors of Death and Neurological Abnormality Among Premature Infants Weighing 1000-2000 Grams	143-v
NDB(CF)-65 PR/PN 1272 Hyaline Membrane Disease	145-v
NDB(CF)-66 PR/PN 1335 Mortality and Morbidity Among Infants Weighing 1000-2000 Grams	147-v
NDB(CF)-66 PR/PN 1336 Effects on the Child of Convulsive Disorder in the Mother	149-v
NDB(CF)-66 PR/PN 1337 An Investigation into Relationships Between History of Signs, Symptoms and Behavior Early in Pregnancy and Pregnancy Outcome	151-v
NDB(CF)-66 PR/PN 1338 The Association of Mental Subnormality with Head Circumference, Congenital Malformations, and Other Conditions of the Newborn Term Infant	153-v
NDB(CF)-66 PR/PN 1339 Sudden Unexpected Death	155-v
NDB(CF)-63 PR/BS 1166 Evaluation of the Gross and Fine Motor Functions of Children Eight-Months of Age in Relation to the Neurological Results of the Examination of the Children at One-Year of Age	157-v

	<u>Page</u>
NDB(CF)-63 PR/BS 1167 Explorative Study for the Use of a Speech and Language Screening Examination for 3-Year Old Children in the Home Situation	159-v
NDB(CF)-63 PR/BS 1168 Indexes of Socio-Economic Status of Study Mothers in Relation to Outcomes of Pregnancy	161-v
NDB(CF)-63 PR/BS 1169 Antecedents and Correlates of Retarded Motor Development	163-v
NDB(CF)-63 PR/BS 1170 Socio-Economic Status Grouping and Their Relationship to Illnesses and Disorders as Reported on the OB-3, "History Since Last Menstrual Period," and OB-8, "Repeat Prenatal History"	165-v
NDB(CF)-63 PR/BS 1171 An Evaluation of the Offspring of Unwed Mothers in Terms of the Complications of Birth and Delivery, Results on the 4-Month Examination, 8-Month Examination and One-Year Examination, as Compared to a Matched Group of Married Mothers and Their Offspring	167-v
NDB(CF)-65 PR/BS 1273 Hypothesized Performance on 8-Month Old Children, Considered Premature at Birth, on the COLR Form of the Bayley Scales of Mental and Motor Development	169-v
NDB(CF)-63 PR/EG 1172 Pregnancy Outcome and Prior Pregnancy Record	171-v
NDB(CF)-63 PR/EG 1173 A Study of Socioeconomic, Medical and Genetic Factors in Major Congenital Malformations	173-v
NDB(CF)-63 PR/EG 1174 Birthweight in Relation to Selected Socio- Economic Variables	175-v
NDB(CF)-63 PR/EG 1175 Determination of the Zygosity of Twins Born to Mothers in the Collaborative Study	177-v

	<u>Page</u>
NDB(CF)-63 PR/EG 1176 A Genetic and Socioeconomic Study of Habitual Aborters	179-v
NDB(CF)-63 PR/EG 1177 Genetic and Socioeconomic Factors in Early and Late Fetal Death	181-v
NDB(CF)-63 PR/EG 1178 A Socioeconomic Index for the Population of the Collaborative Project	183-v
NDB(CF)-63 PR/EG 1179 The Association of Blood Groups to Amyotropic Lateral Sclerosis	185-v
NDB(CF)-63 PR/EG 1180 A Survey of Schizophrenia Among the Relatives of Schizophrenic Patients	187-v
NDB(CF)-63 PR/EG 1183 A Genetic Study of Parkinsonism and the Dystonias Produced by Ataraxic Drugs	189-v
NDB(CF)-63 PR/EG 1184 Population Dynamics of Tay-Sachs' Disease	191-v
NDB(CF)-65 PR/EG 1274 Genetic Bases of Neonatal Reflexes	193-v
NDB(CF)-65 PR/EG 1275 Study of Family Size with Respect to Rh Blood Type and Other Variables	195-v
NDB(CF)-65 PR/EG 1276 Sequential Aspects of Occurrence of Spontaneous Abortion in Family Histories	197-v
NDB(CF)-65 PR/EG 1277 A Study of the Properties of B.Woolf's Statistic for Combined Analysis of 2x2 Tables	199-v
NDB(CF)-65 PR/P 1278 Biologic Pattern Data Processing	201-v
NDB(CF)-65 PR/P 1279 The Influence of Race and Other Factors on Pulmonary Hyaline Membranes	207-v

	<u>Page</u>
NDB(CF)-65 PR/P 1280 Umbilical Cord Inflammation	209-v
NDB(CF)-65 PR/P 1281 Significance of a Single Umbilical Artery	211-v
NDB(CF)-66 PR/P 1340 Intrauterine Pneumonia in Relation to Birth- weight and Race	213-v
NDB(CF)-66 PR/P 1341 Placental Shape According to Race, Birth- weight, Gestational Age and Placental Weight	215-v
NDB(CF)-66 PR/P 1342 The Length of the Umbilical Cord	217-v
NDB(CF)-66 PR/P 1343 A Simplified Anatomic Classification of Abortions	219-v
NDB(CF)-66 PR/P 1344 Predictors of Organ Weight at Autopsy - A Multivariate Analysis, I. Heart Weight	223-v
NDB(CF)-66 PR/P 1345 Influencing Factors in Sudden Unexpected Death	225-v
NDB(CF)-66 PR/P 1346 An Epidemiological Assessment of Primary Endo- cardial Fibroelastosis	227-v
NDB(CF)-66 PR/P 1347 A Case of Marchiafava Bignami Disease with Clinical Recovery	229-v
NDB(CF)-66 PR/P 1348 Embryologic Analysis of Multiple Congenital Anomalies	231-v
NDB(CF)-66 PR/P 1349 Alzheimer's Disease: A Family Study	233-v
NDB(CF)-66 PR/P 1350 Birthweight, Gestational Age and Type of Delivery in Rhesus Monkeys	235-v
NDB(CF)-66 PR/P 1351 Fetal Vessel Ligation in Rhesus Monkey Placentas	237-v

NDB(CF)-66 PR/P 1352	239-v
The Neuropathological Study of a Series of Selected Monkey Brains from Animals in the Perinatal Period	
NDB(CF)-66 PR/P 1353	241-v
A Sequential Study of Ultrastructural Changes in an Experimentally Produced, Traumatic, Brain Lesion	
NDB(CF)-66 PR/P 1354	243-v
Automated Microspectrophotometry Employing the LINC Computer	
NDB(CF)-66 PR/P 1355	247-v
Factors Influencing Quantitative DNA Staining	
NDB(CF)-66 PR/SA 1356	249-v
The Association of Maternal Age and Parity to Pregnancy Outcome and to Certain Obstetric Complications	
NDB(CF)-66 PR/SA 1357	251-v
Prior Pregnancy Loss and Present Infant Outcome	
NDB(CF)-66 PR/SA 1358	253-v
Dilatation Curves	
NDB(CF)-66 PR/SA 1359	255-v
Neonatal Pneumonia in Liveborn Infants	
NDB(CF)-66 PR/SA 1360	257-v
Neonatal Serum Bilirubin Levels and Subsequent Neurologic Deficits	
NDB(CF)-66 PR/SA 1361	259-v
Marginal Sinus Rupture	
NDB(CF)-66 PR/SA 1362	261-v
The Effect of Birth Injuries	
NDB(CF)-66 PR/SA 1363	263-v
Early Signs of Neurologic Disorders	
NDB(CF)-66 PR/SA 1364	265-v
Retinal Hemorrhages in the Newborn	
NDB(CF)-66 PR/SA 1365	267-v
The Effects of Uterine Dysfunction and Mechanical Dystocia	

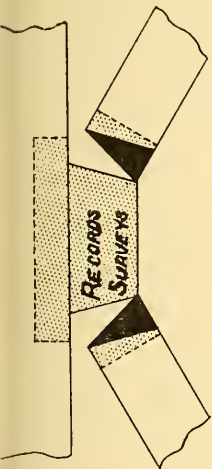
	<u>Page</u>
NDB(CF)-66 PR/SA 1366 The Incompetent Cervix	269-v
NDB(CF)-66 PR/SA 1367 The Relationship of Certain Demographic Variables to the Conditions and Complications of Pregnancy	271-v

HOW TO USE
THESE SEPARATORS

Use one page for
each separation.

Select appropriate
tab, add further
identification if
desired, and cover
it with scotch
tape.

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



PROJECTS LISTED NUMERICALLY

<u>PROJECT NUMBER</u>	<u>PAGE</u>	<u>PROJECT NUMBER</u>	<u>PAGE</u>
NDB(I)-54 SN/OC 100(c)	11-h	NDB(I)-60 LNS/FN 712	9-k
NDB(I)-54 SN/OC 101(c)	17-h	NDB(I)-60 LNS/FN 713	11-k
NDB(CF)-54 E 102	15-t	NDB(I)-61 EEG/OC 800(c)	7-j
NDB(CF)-54 E 103	17-t	NDB(I)-61 O/CH 801(c)	25-i
NDB(I)-55 SN/OC 200(c)	21-h	NDB(I)-61 SN/OC 802(c)	31-h
NDB(CF)-55 E 201	21-t	NDB(I)-61 MN/NR 806(c)	51-g
NDB(I)-56 O/OPS 300(c)	9-i	NDB(I)-61 MN/AP 807(c)	87-g
NDB(I)-56 O/OPH 301(c)	67-i	NDB(I)-61 LNS/NC 808	21-k
NDB(I)-56 O/CH 302(c)	23-i	NDB(I)-61 LNS/NC 809	23-k
NDB(I)-56 SN/OC 303(c)	27-h	NDB(I)-61 LNC/AAC 810	5-o
NDB(I)-56 SN/OC 304(c)	29-h	NDB(I)-61 LNC/AAC 811	9-o
NDB(I)-57 SN/CP 401(c)	95-h	NDB(I)-61 LNC/EC 813	13-o
NDB(CF)-57 PR/ID 402	57-v	NDB(I)-61 LNC/LC 815	15-o
NDB(I)-58 O/OC 500(c)	81-i	NDB(I)-61 LNC/LC 816	19-o
NDB(I)-58 LNP/SC-501	3-m	NDB(I)-61 LNC/LC 817	21-o
NDB(I)-59 O/OPH 600(c)	71-i	NDB(CF)-61 LPP 820	85-q
NDB(CF)-59 LPP 603	11-q	NDB(CF)-61 LPP 821	87-q
NDB(I)-60 O/OPS 700(c)	13-i	NDB(CF)-61 LPP 822	89-q
NDB(I)-60 SN/NA 702(c)	55-h	NDB(CF)-61 E 832	23-t
NDB(I)-60 SN/CN 706(c)	59-h	NDB(CF)-61 B 833	19-s
NDB(I)-60 SN/CN 707(c)	63-h	NDB(CF)-61 PR/ID 835	63-v
NDB(I)-60 SN/CN 708(c)	67-h	NDB(I)-62 SN/OC 906(c)	33-h
NDB(I)-60 LNS/EN 711	15-k	NDB(I)-62 SN/OC 907(c)	37-h

<u>PROJECT NUMBER</u>	<u>PAGE</u>	<u>PROJECT NUMBER</u>	<u>PAGE</u>
NDB(I) -62 SN/OC 913(c)	41-h	NDB(I) -62 LNP/SC-973	11-m
NDB(I) 62 MN/OC 915(c)	9-g	NDB(I) -63 O/OPS 1012(c)	17-i
NDB(I) -62 MN/OC 917(c)	13-g	NDB(I) -63 O/OPS 1016(c)	19-i
NDB(I) -62 MN/NR 922(c)	53-g	NDB(I) -63 O/OCH 1017(c)	47-i
NDB(I) -62 MN/NR 925(c)	55-g	NDB(I) -63 O/OPH 1018(c)	75-i
NDB(I) -62 MN/AP 926(c)	89-g	NDB(I) -63 O/OC 1022(c)	83-i
NDB(I) -62 LNP/SC 934	7-m	NDB(I) -63 SN/OC 1025(c)	43-h
NDB(I) -62 LB/CB 935	5-n	NDB(I) -63 SN/CN 1026(c)	73-h
NDB(I) -62 LB/MA 936	17-n	NDB(I) -63 SN/NP 1027(c)	79-h
NDB(I) -62 LB/ME 938	21-n	NDB(I) -63 SN/NP 1030(c)	83-h
NDB(I) -62 LB/CB 939	9-n	NDB(I) -63 SN/CP 1032(c)	99-h
NDB(I) -62 LB/I 940	13-n	NDB(I) -63 SN/CP 1033(c)	101-h
NDB(I) -62 LNC/PM 941	29-o	NDB(I) -63 MN/OC 1034(c)	17-g
NDB(I) -62 LN/EN 942	3-l	NDB(I) -63 MN/OC 1037(c)	23-g
NDB(I) -62 LN/EN 943	5-l	NDB(I) -63 MN/OC 1039(c)	25-g
NDB(I) -62 LN/EN 944	7-l	NDB(I) -63 MN/NR 1047(c)	57-g
NDB(I) -62 LMB/OC 947	7-p	NDB(I) -63 MN/NR 1048(c)	59-g
NDB(CF) -62 E 957	25-t	NDB(I) -63 MN/AP 1049(c)	91-g
NDB(CF) -62 E 958	27-t	NDB(I) -63 MN/AP 1050(c)	93-g
NDB(CF) -62 E 959	29-t	NDB(I) -63 LNS/NC 1051	25-k
NDB(CF) -62 E 961	31-t	NDB(I) -63 LNS/EN 1054	17-k
NDB(CF) -62 B 965	25-s	NDB(I) -63 LN/EN 1063	9-l
NDB(CF) -62B 968	27-s	NDB(I) -63 LN/EN 1065	11-l
NDB(CF) -62 OAD 969(I-IV)	7-r	NDB(I) -63 LN/EN 1066	13-l
NDB(CF) -62 PR/ID 972	67-v	NDB(I) -65 LMB/OC 1074	11-p

<u>PROJECT NUMBER</u>	<u>PAGE</u>	<u>PROJECT NUMBER</u>	<u>PAGE</u>
NDB(CF)-63 LPP 1075	91-q	NDB(CF)-63 B 1134	29-s
NDB(CF)-63 LPP 1076	93-q	NDB(CF)-63 B 1137	33-s
NDB(CF)-63 LPP 1077	95-q	NDB(CF)-63 PR/OC 1144	79-v
NDB(CF)-63 LPP 1080	97-q	NDB(CF)-63 PR/OC 1146	81-v
NDB(CF)-63 LPP 1081	99-q	NDB(CF)-63 PR/PN 1155	133-v
NDB(CF)-63 LPP 1082	101-q	NDB(CF)-63 PR/PN 1156	135-v
NDB(CF)-63 LPP 1083	103-q	NDB(CF)-63 PR/PN 1161	137-v
NDB(CF)-63 LPP 1088	105-q	NDB(CF)-63 PR/PN 1162	139-v
NDB(CF)-63 LPP 1089	107-q	NDB(CF)-63 PR/PN 1163	141-v
NDB(CF)-63 E 1090	33-t	NDB(CF)-63 PR/PN 1164	143-v
NDB(CF)-63 E 1091	35-t	NDB(CF)-63 PR/BS 1166	157-v
NDB(CF)-63 E 1092	37-t	NDB(CF)-63 PR/BS 1167	159-v
NDB(CF)-63 E 1093	39-t	NDB(CF)-63 PR/BS 1168	161-v
NDB(CF)-63 E 1101	41-t	NDB(CF)-63 PR/BS 1169	163-v
NDB(CF)-63 E 1103	43-t	NDB(CF)-63 PR/BS 1170	165-v
NDB(CF)-63 E 1106	45-t	NDB(CF)-63 PR/BS 1171	167-v
NDB(CF)-63 E 1107	47-t	NDB(CF)-63 PR/EG 1172	171-v
NDB(CF)-63 E 1108	49-t	NDB(CF)-63 PR/EG 1173	173-v
NDB(CF)-63 E 1109	51-t	NDB(CF)-63 PR/EG 1174	175-v
NDB(CF)-63 E 1110	53-t	NDB(CF)-63 PR/EG 1175	177-v
NDB(CF)-63 E 1111	55-t	NDB(CF)-63 PR/EG 1176	179-v
NDB(CF)-63 E 1112	59-t	NDB(CF)-63 PR/EG 1177	181-v
NDB(CF)-63 E 1113	61-t	NDB(CF)-63 PR/EG 1178	183-v
NDB(CF)-63 E 1114	63-t	NDB(CF)-63 PR/EG 1179	185-v
NDB(CF)-63 E 1117	65-t	NDB(CF)-63 PR/EG 1180	187-v

<u>PROJECT NUMBER</u>	<u>PAGE</u>	<u>PROJECT NUMBER</u>	<u>PAGE</u>
NDB(CF)-63 PR/EG 1183	189-v	NDB(I)-65 O/OCH 1210(c)	49-i
NDB(CF)-63 PR/EG 1184	191-v	NDB(I)-65 O/CB 1211(c)	59-i
NDB(CF)-64 B 1187	37-s	NDB(I)-65 O/CH 1212(c)	29-i
NDB(CF)-64 B 1188	39-s	NDB(I)-65 O/OC 1213(c)	85-i
NDB(I)-65 MN/OC 1189(c)	31-g	NDB(I)-65 O/OPH 1214(c)	77-i
NDB(I)-65 MN/OC 1190(c)	35-g	NDB(I)-65 O/OPH 1215(c)	79-i
NDB(I)-65 MN/OC 1191(c)	39-g	NDB(I)-65 O/OC 1216(c)	33-i
NDB(I)-65 MN/OC 1192(c)	43-g	NDB(I)-65 O/OCH 1217(c)	51-i
NDB(I)-65 MN/OC 1193(c)	47-g	NDB(I)-65 O/OC 1218(c)	87-i
NDB(I)-65 MN/AP 1194(c)	95-g	NDB(I)-65 O/OC 1219(c)	89-i
NDB(I)-65 MN/NR 1195(c)	61-g	NDB(I)-65 O/OC 1220(c)	91-i
NDB(I)-65 MN/NR 1196(c)	65-g	NDB(I)-65 O/OC 1221(c)	93-i
NDB(I)-65 MN/NR 1197(c)	67-g	NDB(I)-65 O/OC 1222(c)	95-i
NDB(I)-65 MN/NR 1198(c)	69-g	NDB(I)-65 O/CH 1223(c)	35-i
NDB(I)-65 MN/NR 1199(c)	71-g	NDB(I)-65 EEG/OC 1225(c)	9-j
NDB(I)-65 MN/NR 1200	73-g	NDB(I)-65 EEG/CN 1226(c)	11-j
NDB(I)-65 MN/NR 1201(c)	75-g	NDB(I)-65 EEG/CN 1227(c)	15-j
NDB(I)-65 MN/NR 1202(c)	77-g	NDB(I)-65 LNS/FN 1229	13-k
NDB(I)-65 MN/NR 1203(c)	79-g	NDB(I)-65 LNS/NC 1230	27-k
NDB(I)-65 SN/CN 1204(c)	77-h	NDB(I)-65 LNS/NC 1231	29-k
NDB(I)-65 SN/OC 1205(c)	45-h	NDB(I)-65 LNS/NC 1232	31-k
NDB(I)-65 SN/OC 1206(c)	47-h	NDB(I)-65 LNS/EE 1233	37-k
NDB(I)-65 SN/NP 1207(c)	87-h	NDB(I)-65 LNS/EE 1234	41-k
NDB(I)-65 LMB/OC 1208	13-p	NDB(I)-65 LNS/EE 1235	45-k
NDB(I)-65 O/CB 1209(c)	57-i	NDB(I)-65 LN/EN 1237	15-l

<u>PROJECT NUMBER</u>	<u>PAGE</u>	<u>PROJECT NUMBER</u>	<u>PAGE</u>
NDB(CF)-65 PR/ID 1238	71-v	NDB(I)-65 LPP 1266	111-q
NDB(I)-65 LNP/SC 1239	13-m	NDB(I)-65 LPP 1267	113-q
NDB(I)-65 LB/ME 1240	23-n	NDB(CF)-65 PR/ID 1270	73-v
NDB(I)-65 LNC/LC 1241	23-o	NDB(CF)-65 PR/ID 1271	75-v
NDB(I)-65 LNC/PM 1242	31-o	NDB(CF)-65 PR/PN 1272	145-v
NDB(I)-65 LNC/PM 1243	35-o	NDB(CF)-65 PR/SB 1273	169-v
NDB(I)-65 LMB/OC 1244	15-p	NDB(CF)-65 PR/EG 1274	193-v
NDB(I)-66 SN/CP 1245(c)	103-h	NDB(CF)-65 PR/EG 1275	195-v
NDB(CF)-65 E 1248	67-t	NDB(CF)-65 PR/EG 1276	197-v
NDB(CF)-64 E 1250	69-t	NDB(CF)-65 PR/EG 1277	199-v
NDB(CF)-64 E 1251	71-t	NDB(CF)-65 PR/P 1278	201-v
NDB(CF)-65 E 1252	73-t	NDB(CF)-65 PR/P 1279	207-v
NDB(CF)-64 E 1253	75-t	NDB(CF)-65 PR/P 1280	209-v
NDB(CF)-64 E 1254	77-t	NDB(CF)-65 PR/P 1281	211-v
NDB(CF)-64 E 1255	79-t	NDB(CF)-65 OAD 1282(I-XI)	23-r
NDB(CF)-64 E 1256	81-t	NDB(I)-66 MN/NR 1283(c)	81-q
NDB(CF)-64 E 1257	83-t	NDB(I)-66 MN/NR 1284(c)	83-q
NDB(OD)-65 1258	3-a	NDB(I)-66 SN/NP 1285(c)	89-h
NDB(I)-65 LPP 1259	15-q	NDB(I)-66 SN/NP 1286(c)	91-h
NDB(I)-65 LPP 1260	17-q	NDB(I)-66 SN/NP 1287(c)	93-h
NDB(I)-65 LPP 1261	19-q	NDB(I)-66 O/OPS 1288(c)	21-i
NDB(I)-65 LPP 1262	21-q	NDB(I)-66 O/CH 1289(c)	37-i
NDB(I)-65 LPP 1263	23-q	NDB(I)-66 O/CH 1290(c)	39-i
NDB(I)-65 LPP 1264	25-q	NDB(I)-66 O/CH 1291(c)	43-i
NDB(I)-65 LPP 1265	109q	NDB(I)-66 O/OCH 1292(c)	53-i

<u>PROJECT NUMBER</u>	<u>PAGE</u>	<u>PROJECT NUMBER</u>	<u>PAGE</u>
NDB(I)-66 O/OCH 1293(c)	55-i	NDB(CF)-66 E 1318	97-t
NDB(I)-66 O/CB 1294(c)	61-i	NDB(CF)-66 E 1319	99-t
NDB(I)-66 O/CB 1295(c)	63-i	NDB(CF)-66 E 1320	101-t
NDB(I)-66 O/OC 1296(c)	97-i	NDB(CF)-66 E 1321	103-t
NDB(I)-66 O/OC 1297(c)	99-i	NDB(CF)-66 E 1322	105-t
NDB(I)-66 O/OC 1298(c)	101-i	NDB(CF)-66 PR/ID 1326	77-v
NDB(I)-66 EEG/CN 1299(c)	19-j	NDB(CF)-66 PR/OB 1327	117-v
NDB(I)-66 EEG/CN 1300(c)	21-j	NDB(CF)-66 PR/OB 1328	119-v
NDB(I)-66 EEG/CN 1301(c)	23-j	NDB(CF)-66 PR/OB 1329	121-v
NDB(I)-66 EEG/CN 1302(c)	25-j	NDB(CF)-66 PR/OB 1330	123-v
NDB(I)-66 LNS/EN 1303	19-k	NDB(CF)-66 PR/OB 1331	125-v
NDB(I)-66 LNS/NC 1304	33-k	NDB(CF)-66 PR/OB 1332	127-v
NDB(I)-66 LNS/NC 1305	35-k	NDB(CF)-66 PR/OB 1333	129-v
NDB(I)-66 LN/EN 1306	17-l	NDB(CF)-66 PR/OB 1334	131-v
NDB(I)-66 LN/EN 1307	19-l	NDB(CF)-66 PR/PN 1335	147-v
NDB(I)-66 LNC/LC 1308	25-o	NDB(CF)-66 PR/PN 1336	149-v
NDB(I)-66 LNC/LC 1309	27-o	NDB(CF)-66 PR/PN 1337	151-v
NDB(I)-66 OAD 1310	3-e	NDB(CF)-66 PR/PN 1338	153-v
NDB(I)-66 MN/OC 1311(c)	49-g	NDB(CF)-66 PR/PN 1339	155-v
NDB(CF)-66 E 1312	85-t	NDB(CF)-66 PR/P 1340	213-v
NDB(CF)-66 E 1313	87-t	NDB(CF)-66 PR/P 1341	215-v
NDB(CF)-66 E 1314	89-t	NDB(CF)-66 PR/P 1342	217-v
NDB(CF)-66 E 1315	91-t	NDB(CF)-66 PR/P 1343	219-v
NDB(CF)-66 E 1316	93-t	NDB(CF)-66 PR/P 1344	223-v
NDB(CF)-66 E 1317	95-t	NDB(CF)-66 PR/P 1345	225-v

<u>PROJECT NUMBER</u>	<u>PAGE</u>	<u>PROJECT NUMBER</u>	<u>PAGE</u>
NDB(CF)-66 PR/P 1346	227-v	NDB(CF)-66 PR/OC 1371	89-v
NDB(CF)-66 PR/P 1347	229-v	NDB(CF)-66 PR/OC 1372	91-v
NDB(CF)-66 PR/P 1348	231-v	NDB(CF)-66 PR/OC 1373	93-v
NDB(CF)-66 PR/P 1349	233-v	NDB(CF)-66 PR/OC 1374	95-v
NDB(CF)-66 PR/P 1350	235-v	NDB(CF)-66 PR/OC 1375	97-v
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NDB(CF)-66 PR/P 1352	239-v	NDB(CF)-66 PR/OC 1377	101-v
NDB(CF)-66 PR/P 1353	241-v	NDB(CF)-66 PR/OC 1378	105-v
NDB(CF)-66 PR/P 1354	243-v	NDB(CF)-66 PR/OC 1379	107-v
NDB(CF)-66 PR/P 1355	247-v	NDB(CF)-66 PR/OC 1380	109-v
NDB(CF)-66 PR/SA 1356	249-v	NDB(CF)-66 PR/OC 1381	111-v
NDB(CF)-66 PR/SA 1357	251-v	NDB(CF)-66 PR/OC 1382	113-v
NDB(CF)-66 PR/SA 1358	253-v	NDB(CF)-66 PR/OC 1383	115-v
NDB(CF)-66 PR/SA 1359	255-v	NDB(I)-66 LPP 1384	27-q
NDB(CF)-66 PR/SA 1360	257-v	NDB(I)-66 LPP 1385	29-q
NDB(CF)-66 PR/SA 1361	259-v	NDB(I)-66 LPP 1386	31-q
NDB(CF)-66 PR/SA 1362	261-v	NDB(I)-66 LPP 1387	33-q
NDB(CF)-66 PR/SA 1363	263-v	NDB(I)-66 LPP 1388	35-q
NDB(CF)-66 PR/SA 1364	265-v	NDB(I)-66 LPP 1389	37-q
NDB(CF)-66 PR/SA 1365	267-v	NDB(I)-66 LPP 1390	39-q
NDB(CF)-66 PR/SA 1366	269-v	NDB(I)-66 LPP 1391	41-q
NDB(CF)-66 PR/SA 1367	271-v	NDB(I)-66 LPP 1392	43-q
NDB(CF)-66 PR/OC 1368	83-v	NDB(I)-66 LPP 1393	45-q
NDB(CF)-66 PR/OC 1369	85-v	NDB(I)-66 LPP 1394	47-q
NDB(CF)-66 PR/OC 1370	87-v	NDB(I)-66 LPP 1395	49-q

<u>PROJECT NUMBER</u>	<u>PAGE</u>	<u>PROJECT NUMBER</u>	<u>PAGE</u>
NDB(I)-66 LPP 1396	51-q	NDB(I)-66 LPP 1405	69-q
NDB(I)-66 LPP 1397	53-q	NDB(I)-66 LPP 1406	71-q
NDB(I)-66 LPP 1398	55-q	NDB(I)-66 LPP 1407	73-q
NDB(I)-66 LPP 1399	57-q	NDB(I)-66 LPP 1408	75-q
NDB(I)-66 LPP 1400	59-q	NDB(I)-66 LPP 1409	77-q
NDB(I)-66 LPP 1401	61-q	NDB(I)-66 LPP 1410	79-q
NDB(I)-66 LPP 1402	63-q	NDB(I)-66 LPP 1411	81-q
NDB(I)-66 LPP 1403	65-q	NDB(I)-66 LPP 1412	83-q
NDB(I)-66 LPP 1404	67-q		

ANNUAL REPORT

July 1, 1965 through June 30, 1966

National Institute of Neurological Diseases and Blindness
National Institutes of Health

The Director's Report

It is 15 years since the establishment of the National Institute of Neurological Diseases and Blindness. Today the Institute has an annual budget in excess of \$100 million and 675 full-time employees. Research projects in progress within the Institute total 218; an additional 1,615 research grants are being supported across the country, of which 300 are new projects launched this year. In order to maintain a continuing supply of trained scientists for this program, the Institute is supporting 275 training grants, providing a training environment for 1,600 individuals. In addition, training support is being provided for 240 special fellows, 124 postdoctoral fellows, and 111 development and career awards.

One might well say that the Institute has come of age. However, a review of the program indicates that there has not been any slackening of the growth of the Institute's program. In fact, evidence suggests that the field of neurology--the study of the brain, the nervous system, and the sense organs--is one which is experiencing an unprecedented growth, and is only beginning to manifest its potential capabilities.

The new intramural research laboratories, whose planning was launched almost at the inception of the Institute's program, are under way and should be finished and occupied in 1968. The new laboratory facilities of the Laboratory of Perinatal Physiology in San Juan, Puerto Rico, may be in their final stages of completion at approximately the same time. The acquisition of the new 600-acre island, Desecheo, near Puerto Rico, will provide a unique area in which new programs can be mounted.

Within the past five years 49 clinical research centers and program projects have been established across the Nation. Each of these has the potential of becoming the focal point for a vast interdisciplinary attack against some aspect of the categorical problems of the Institute. This year, as a further stimulus to such focused clinical investigation, six vision research outpatient centers have been established. These centers will make larger numbers of patients available for clinical and basic investigations of the eye, and serve further to extend our knowledge of these diseases and the interest in investigating them.

To cope with the vast output of new scientific knowledge, the Institute has established a coordinated network of scientific information centers. Closely linked to the National Library of Medicine, four of the Nation's leading university libraries are developing the resources for a continuing review of

the problems of clinical and basic neurology, of vision, and of speech, hearing, and human communication. This vast pioneering project offers real hope for the most rapid and efficient continuing review and monitoring of scientific progress in the field of the neurological sciences.

It is not possible to review briefly here all the impressive new advances of even the past year. Within the direct operation alone are at least three advances, any one of which represents a true landmark in scientific progress. The discovery of a transmissible factor in the etiology of kuru (previously considered a degenerative disease) and the definition of the specific enzyme defect in Gaucher's disease and Niemann-Pick disease are particularly noteworthy because they represent end points in the progress of science. Many of the other advances detailed in the reports of the direct operation and within the grants area, though less noteworthy, are equally important.

The Institute continues to maintain the broad base of its research effort on the individual project grant. We are relying increasingly upon the task forces of civilian consultants to advise us of those areas where special efforts may be required, or directed research appropriate. There are now four special subcommittees of the Council on cerebrovascular disease, vision, human communications, and physical rehabilitation. Four additional task forces are concerned with epilepsy, head injury, evaluation of therapy, and minimal brain dysfunction.

This year there have been five Council-sponsored conferences to provide for an exchange of scientific knowledge and to highlight areas of research need and opportunity. These have been in the area of head injury, cerebrovascular disease, neural mechanisms in restitution after brain damage, and brain mechanisms underlying speech and language disorders. Eleven other national conferences relating to brain injury, epilepsy, headache, toxins and poisons, brain tumors, mental retardation, and medical information centers have received support from the Institute. Monographs or special reports have been published on epilepsy and minimal brain dysfunction, and the results of two previously held conferences, one on slow virus infections and the other on the thalamus.

The following statements briefly review the Institute's approaches to many of its major areas of defined responsibility. Charts I and II show the growth of the Institute's research and training programs and allocation of funds by disease categories.

CEREBROVASCULAR DISEASE

This year has seen significant program developments as a result of the stimulus provided by the recommendations of the President's Committee on Heart Disease, Cancer, and Stroke. The number of stroke centers has been increased from 3 to 14 this year, and a major network of such centers now exists across the country. These centers will provide a focal point for research in the prevention, diagnosis, and treatment of strokes, for the evaluation of therapy, and for the training of professional and paramedical

personnel. Through regular meetings of the program directors, informal coordination of their activities will be accomplished. A major concern is the development of more precise methods for the recognition of the abnormality of the cerebral vessels and impairment of the cerebral circulation as a means for the prediction and prevention of stroke. Within these and other centers, clinical training grants and clinical traineeships are now providing short-term training opportunities to medical practitioners who want to learn from specialists the latest development in diagnosis, prevention, and treatment.

Epidemiological studies of stroke are of great importance since detailed knowledge of the distribution of the disease could point the way to its causes. A cooperative study involving the University of Minnesota, and scientists in Fukuoka, Japan, has provided for the first time an accurate comparison of cerebrovascular disease in these two countries with widely different genetic and environmental factors. The establishment of a stroke epidemiology center, and the development under contract of studies recommended by the conference on epidemiology of cerebrovascular disease of last year, should provide these much-needed data.

For the evaluation of stroke therapy, the Institute has had a number of individual projects and cooperative studies. Since 1957 the cooperative study of intracranial aneurysms and acute cerebral hemorrhage carried on in 24 university-based centers, and including projects in Great Britain, Sweden, and Australia, has accumulated data on more than 6,000 cases of cerebral hemorrhage. These data are being analyzed to provide objective criteria for therapy.

Individual and clinical trials of various agents include papaverine hydrochloride, nicotinic acid, Vasodilan, and Arlidan. A cooperative study is now being launched to evaluate the long-term effects of hypotensive therapy in the prevention of stroke.

HEAD INJURY

The rising toll of deaths from automobile accidents, a large majority of which involve brain injury, has induced the Institute to move aggressively to develop a program to improve the methods of management and treatment of the brain-injured. Such a program has wide ramifications.

The major cause of death and disability from head injury is a secondary reaction of the brain involving brain swelling and increased intracranial pressure. Recent conferences have concentrated on the problem of the blood brain barrier and the factors which regulate the passage of fluid among the cerebral intracranial compartments. Increased support of the basic research required to determine the physical and chemical concomitants of brain edema will be essential. Practical methods for the handling of acute trauma will stem from these basic research developments, but continued evaluation of presently available therapeutic methods is an essential component of a total program. Finally, remediation of the long-term residuals of neurological damage has been a much overlooked aspect of the problem.

In collaboration with four of the major national societies, a conference to review the entire field of head injury was held in Chicago on February 23, 1966. The recommendations of this conference are appended, and these are being implemented as rapidly as personnel and resources can be mobilized for this purpose. In addition, a special subcommittee of the Council concerned with the physical rehabilitation of the neurologically damaged will address itself to the long-term needs of the brain damaged.

In a closely related effort, a small interagency task force is carrying out a review of the problems of those suffering from "minimal brain dysfunction." There is a serious need for the critical evaluation of physical and educational therapies being recommended for the cerebral palsied and brain-injured.

EPILEPSY

Aside from some minor advances in drug therapy, the management of epilepsy has changed little in the past 10 years. Major advances may well depend upon more precise knowledge of the nature and functions of the nerve membrane, the physical and chemical processes involved in synaptic activity, and the organizational patterns of this activity within the brain. Such concerns are central to the activities of a number of the intramural laboratories, including biophysics, neurophysiology, electroencephalography, surgical neurology, and neurochemistry. Steady advances in knowledge are reported from each of these laboratories.

Across the country there are now two major interdisciplinary centers concentrating on epilepsy, and within an additional laboratory the techniques of neurophysiology are being specifically applied to this problem. The study and development of anticonvulsants is the major effort of one large program project supported by the Institute. However, the field of drug development for epilepsy is not receiving the emphasis which it deserves.

In order to provide a strong emphasis on the problems of the convulsive disorders, the Surgeon General has appointed an advisory committee on the epilepsies. One section of this committee is working directly with this Institute in the formulation of an overall plan for research in these disorders.

In addition, this year the Institute has launched a pilot project, involving the cooperation of four extramural laboratories in the study of petit mal. The purpose of this project is to determine the feasibility of such a group effort, the extent to which comparable data can be obtained from several independent clinics and the methodology whereby this can be achieved, and the usefulness of such a network for the study of the natural history and treatment of these disorders. The evaluation of specific anticonvulsant drugs continues to be carried forward within a number of independent laboratories.

SLOW AND LATENT VIRUSES IN NEUROLOGICAL DISEASES

For the past seven years the Institute has been developing a program to search for viruses in chronic neurological disease. The focal point for this program has been within the collaborative and field research area of the direct operations. It has involved field studies in New Guinea, Guam, and other areas, and laboratory operations centered in Bethesda and in collaboration with the Patuxent Wildlife Research Center, Laurel, Maryland.

Using scrapie, the disease of sheep, as a model, scientists at the Patuxent laboratory have become experienced in techniques for the culturing and observation of latent viruses. A major emphasis has been on the inoculation of presumably infected human material into appropriate animal hosts. This year has seen a major development in this program--seven of eight chimpanzees inoculated with material from the brains of seven patients dying from kuru have developed a disease clinically and pathologically indistinguishable from kuru in humans. Of particular importance to neurological disease is the fact that this condition has shown an unusually long latency averaging over two years, and pathologically has more of the appearances of a "degenerative" disorder than those of the usual inflammation or infection of the brain.

Studies similar to those reported for kuru are being carried out in relation to a number of other neurological diseases, but to date no other positive findings have been reported. Considerable interest within the scientific community has been generated by these findings, and, in addition, by the report of the mission which the Institute sent to the Soviet Union in 1964, and by the international conference sponsored and organized by Institute scientists in 1965. The Institute is making every effort to exploit this important scientific development. The significance of these findings to such diseases as multiple sclerosis, amyotrophic lateral sclerosis, Parkinsonism, and the encephalitides of childhood remains to be seen, but in at least two of these disorders inclusion bodies resembling virus are reported to have been seen in electronmicroscopic examination of the brain tissue of afflicted individuals.

DISORDERS OF HEARING, LANGUAGE, AND SPEECH

Hearing disorders are recognized to be the most widespread disability today, accounting for approximately one fourth of all impairments involving over six million Americans. Also, frequently associated with hearing problems are the disorders of language and speech. To advance research into the fundamental nature of human communication (hearing, language, and speech) and its disorders, the NINDB allocated over \$10 million in FY 1965 to support approximately 250 grants to individuals and clinical centers.

Particularly important to the Institute in developing long-range plans was the organization last year of the NANDB Council Subcommittee on Human Communication and Its Disorders. This group was assigned the task of assessing the present state of knowledge in the field, determining the status of research and training, and identifying the areas needing special attention.

A further indication of special concern of the Council was the Conference on Brain Mechanisms in Language and Speech Disorders held at Princeton last year under Council sponsorship.

To further aid in program analysis and information exchange, a human communication information center was established this year at Johns Hopkins University. Here published material relating to the entire area of hearing, language, and speech will be reviewed, planning conferences held, and information will be disseminated to the scientific community.

The Institute now supports eight clinical research centers and program projects to provide focal points for a multidisciplinary attack on problems in the human communication area. These include investigations of fundamental processes of hearing, auditory communication, deafness, vertigo, and speech mechanisms. Included also is a Temporal Bone Bank Program. In this program, a reservoir of carefully prepared temporal bones is being developed from individuals who have had hearing problems and for whom medical records are available over a long period of time. In collaboration with the Institute's Collaborative Perinatal Program, definitive examinations of the pathology of German measles are now available. Important observations on other pathological processes, including otosclerosis, are also available.

The problem of early diagnosis of speech, hearing, and language disabilities continues to be a major concern in the Institute's program. Important techniques for diagnosis and treatment of hearing loss in children were highlighted in a survey published last year. Also special emphasis was given to the refinement of tests and testing techniques which would have ease of administration and standardization of interpretation to achieve their widespread acceptance. As an essential step toward standardization of diagnosis and classification, a series of essential workshops were supported last year by the Institute to develop and evaluate a proposed impairment code for disorders of hearing, voice, speech, and language comprehension and use. There continues to be serious problems resulting from lack of agreement regarding classification.

Within the Collaborative Perinatal Program, efforts are continuing to develop and standardize reliable screening examinations for the 3-year-old child.

In numerous laboratories within the intramural program in Bethesda and across the country, there are important advances in the understanding of the anatomy and function of the organs of hearing. Precise knowledge of the mechanism of hearing--the process whereby sound waves impinging on the eardrum are converted to meaningful information for the brain--is absolutely essential to understanding the impairments of the deaf and hard-of-hearing, and to the establishment of remedial or alternative methods of input.

In addition to this review of program activities and some of the specific problems in research advances, a study of the detailed reports from the individual program areas and laboratories shows the diversity of approaches to the nervous system, and the extremely rapid rate with which new knowledge regarding neurological and sensory disorders is being accumulated.

CAUSES AND PREVENTION OF CEREBRAL PALSY AND MENTAL RETARDATION

The Collaborative Project for the Study of Cerebral Palsy, Mental Retardation, and other Neurological and Sensory Disorders of Infancy and Childhood is achieving its objective of studying 60,000 pregnant women and their offspring. This study, paralleled by primate investigations in the Institute's laboratories in Puerto Rico, is the major focus of the Institute attack on cerebral palsy and mental retardation.

The recent epidemic of rubella, following closely upon the isolation of the causative agent, has been exploited within the Collaborative Project to provide remarkably precise information regarding the epidemiology and impact of this disorder. Institute scientists are providing leadership in the vaccine development program.

DISORDERS OF BRAIN AND MUSCLE CHEMISTRY AND STRUCTURE

Material advances in our knowledge of the fundamental nature of a number of genetically determined neuromuscular diseases have been accomplished this year. Studies of muscle diseases have made further advances, including specific diagnostic criteria of myotonic dystrophy, demonstration of a myotonic component in two specific protein defects, recognition of a new type of muscle disease, and a new classification of periodic paralysis leading to improved treatment regimens. The study of immunoglobulin patterns in these diseases is proving of particular interest. The active intramural research program and two clinical research centers all concentrating on these fundamental problems of muscle disease are serving as important focal points for the national research effort. Under the PL 480 program, a collaborating unit in Warsaw, Poland, is also active in these investigations.

Major advances have also been accomplished in relation to the lipoidosis--important causes of brain disease and mental retardation. The specific enzyme defect of Gaucher's disease and of Nieman Pick disease is now known--an essential step toward diagnosis and, hopefully, therapy.

The Institute is continuing to foster programs to achieve early diagnosis of neuromuscular diseases. A series of workshops involving university scientists and representatives of industry have evaluated the potential for extending a Guthrie-type screening procedure to other similar inborn errors of metabolism. A cooperative study has been set up to test this possibility.

PARKINSON'S DISEASE

The NINDB program in Parkinson's disease consists of grant support of many research projects in universities and medical centers, intramural research, and long-term support of training in neurology, neurosurgery, neuropharmacology, and the many other disciplines bearing on the problem. The Institute also encourages, in every way possible, focused medical and public interest through support of meetings, exhibits, and publications providing information on experience with patients and on new developments in research.

In order to mobilize a strong national effort against this disease, the Institute has established a Parkinson's Disease Research and Information Center at Columbia University. A large multidisciplinary research program has been developed. The scientists of this program also provide direction for reviewing the national research effort relative to this disorder, and disseminating the current information throughout the scientific community.

Among the research areas receiving emphasis are the development of better drugs for relief of symptoms; the improvement of surgical techniques to control tremor; the search for a possible viral cause of the disease; and the search for abnormalities of brain chemistry. The latter area was explored in detail at an Institute-supported conference at Columbia University entitled "Biochemistry and Pharmacology of the Basal Ganglia." This conference attracted more than 600 neurological scientists from this country and abroad, and drew together the most important work on the mechanisms and imbalances underlying normal and defective metabolism of brain cells.

Of particular current interest are research projects involving the metabolism of the biogenic amines; enzyme processes in brain stem nuclei, cells, and other subcellular components; and investigations of neuromelanin, a pigment pathologically modified in Parkinsonism patients. This pigment is greatly reduced in the basal ganglia of Parkinson patients; in fact, loss of melanin is the most common finding in post-mortem Parkinsonism tissue studies. Institute grantees at Columbia are conducting extensive cytochemical and electron microscope studies of this pigment, its distribution throughout the nervous system, and differences in its ultrastructure observed in tissues from Parkinsonism patients.

In the past year neurophysiologists working with microelectrodes implanted within brain structures have been able to trace the complex circuitry of the brain and nervous system with a far greater fineness of scale than was possible in the past. They have also been exploring the possibility of simultaneous use of fine catheters for the introduction of various drugs which act upon synapses (nerve junctions). These procedures will permit much greater accuracy in pinpointing the sites of action of drugs of proven therapeutic value for Parkinsonism, and will provide valuable information on the chemistry of the synaptic organizations involved in other movement disorders.

Tremor and muscle rigidity in Parkinson's disease result from an imbalance between two interacting motor control systems within the brain. Injury of one system by the disease may lead to overactivity of the other. One approach to the restoration of balance is by partial surgical interruption of the overactive pathway. Institute scientists and grantees have continued to develop refinements in the methods of producing the necessary tiny lesions in the thalamus, which now include simple cutting, electrocoagulation, freezing, injection of chemical agents, and ultrasound.

The epidemiology of Parkinson's disease is being investigated in an Institute-supported study of the Chamorro population in Guam. Chamorrans have an

extremely high rate of Parkinsonism associated with dementia, and also associated with amyotrophic lateral sclerosis. In long-term family studies, scientists on Guam have compiled a large volume of information on biological characteristics over a 5-year period, and are now analyzing the data with the help of the Department of Genetics at the University of Wisconsin. This study was expanded in the past year to include electron microscopic examination of autopsy material, and more extensive virological, serological, and genetic investigations. The study also includes comparison of incidence of the disease between Chamorrans living on Guam and those who have gone to live elsewhere.

A world-wide encephalitis epidemic during the years 1918 to 1922 produced symptoms of Parkinsonism in survivors for many years after the epidemic had passed. The infectious agent, which may have been a virus, was never detected. It is uncertain whether this infection or other agents are continuing to play a part in producing Parkinson's disease. However, many viruses that are known to gradually destroy nerve tissues and cause death after years of latent infection are being grown and studied in test tubes and in animals at the Institute's Patuxent laboratory. These studies may provide clues to the detection of unknown "slow viruses" implicated in multiple sclerosis, amyotrophic lateral sclerosis, and kuru, as well as Parkinsonism.

VISION RESEARCH

The Institute's major effort this year has been to broaden the base for continual expansion of clinical and basic research in eye diseases. To this end, eight multidisciplinary eye research centers have been established. Each of these comprises a broad program but has a major focus of interest.

This year, a new program has been launched--the outpatient clinical research unit. Within these outpatient units, essential research resources will be established. Clinical, laboratory, and epidemiological studies, utilizing the large numbers of afflicted individuals who do not require hospitalization for study, can be carried out within these newly created research environments. Six such units have been established on a pilot basis.

To strengthen program analysis and information activities in vision research, a feasibility study was authorized to plan for a Blindness Information Center, operating within the framework of the NINDB Information program. Also, a special Vision Research Subcommittee of the Council is conducting an extensive review of eye research needs, and is preparing a series of recommendations for action.

The growth of the Institute's research and training activities in eye disorders is summarized in Charts III and IV.

It is interesting to note (see Chart V) that non-medical and medical scientists are serving in about equal numbers as principal investigators on research projects. The Institute is undertaking a further analysis of the source and background of its research scientists.

Knowledge of eye disorders is advancing rapidly within this expanding program. At the Institute's laboratories at Bethesda, the attack on uveitis and the clinical evaluation of drugs on eye disorders have been intensified. Scientists also are investigating the basic process of nerve and tissue growth in the eye. In recognition of the increasing toll of blindness due to vascular disease of the eye in the older age group, a broad clinical and epidemiological study of such blindness has been launched.

The Institute's Model Reporting Area for Blindness is now providing consistent data for the first time on a large scale on the nature and distribution of the blinding diseases in this country. A similar program, under the PL 480 authority, has been developed in Egypt, where there is an extremely high prevalence rate for blindness.

Clinical studies at the Institute and elsewhere have brought improvements in the treatment of glaucoma, helped to clarify the inheritance factor in this disorder, and broadened our knowledge about the effects of drugs on the ocular blood vessels.

Last year Institute scientists were helpful in pinpointing the drug chloroquine as the cause of retinal damage. Patients receiving this drug were studied by the Institute over a period of several years. As a result, the investigators have now devised a retinal profile test to detect retinal changes in the early stages while these changes are still reversible.

New methods for screening donor corneas and keeping them alive so they can be transplanted were developed during the past year. A method of freezing at a precisely controlled rate to minus 190 degrees has kept corneal tissue alive in suspended animation over considerable periods of time. For some patients who cannot benefit from corneal transplants, the work of a Boston grantee and an Institute fellow offers new hope. They reported last fall the successful use of a buried corneal implant fashioned of transparent silicone rubber to restore partial vision in 30 patients suffering from one of the most severe corneal diseases, corneal edema.

The promising new technique of cryosurgery for retinal detachment has successfully undergone further trials this year. The use of the frozen tip may eventually replace other means of surgery because it may be less destructive and better controlled. Heredity and moderate to extreme myopia (nearsightedness) have been strongly implicated as predisposing or accompanying factors of retinal detachment, a common cause of partial or complete loss of vision.

The pharmacology and toxicology of drugs that can adversely affect the eye have been under intensive study. These include tranquilizers, steroids, antimalarial and anticollagen drugs, sulfonamides, antibiotics, and vitamins.

Much of the work of Institute grantees has been directed toward answering basic questions about the visual process and its relation to the nervous system. It is expected that a more thorough understanding of the normal visual process will illuminate many of the poorly understood defects and disorders of vision.

ACTIVITIES OF THE OFFICE OF THE DIRECTOR

Program Analysis Office

The Office of Program Analysis has continued its activities of the last year in developing inhouse capability for answering scientific questions and scientific research ongoing throughout the world relating to neurological problems, developing of an all-encompassing manpower file, and developing a preliminary survey for a management information system for the NINDB. As a part of the scientific information, the Neurological Information Network has grown enormously.

All available documents pertaining to NINDB-supported research has been put into an inhouse file and cross-indexed on an optical coincidence system, which allows extremely rapid retrieval of information relating to neurological research. A review of the categorical classification of this research is now under way. This review is being carried out to relate major budget categories to those subject areas which seem to be the most important in the public eye to facilitate answering questions asked by Congress and others, and to be able to satisfy the needs of the scientific community relating materially to problem areas. The development of this system has been accompanied by an increased demand for searches. The answering service of Program Analysis is rapidly expanding.

These inhouse information activities have resulted in several major reviews in the past year: (1) Drugs and Drug-Related Research of the NINDB, prepared by Dr. Bernard Conley; (2) a report on the Inborn Errors of Metabolism and research related to this which is being sponsored by the NINDB; (3) a review of the status of Mobility Aids for the Blind which use the sonar and radar principles; (4) Research in Vision and Diseases of the Eye, which was prepared for the Council Subcommittee on Vision and Diseases of the Eye; (5) Research on Hearing, Speech, and Diseases of the Ear, for the Council Subcommittee on Human Communication and its Disorders; and (6) an updated version of Dr. Robb's report on epilepsy for the Surgeon General's Epilepsy Advisory Committee. Also, total material was gathered for the review of chemotherapy of brain tumors for the Kentucky Brain Tumor Chemotherapy Conference held in December 1965.

A manpower file is being developed which will enable the Office of Program Analysis to identify research and clinical workers by name, location, specialty, and other scientific interests. This will be a continuing activity, updated by surveys, such as the Sherman Report of 1961, and will also expand and update the manpower survey initiated by Dr. Aura Severinghaus. It is expected that eventually this manpower evaluation will be consolidated into a large, central project for the purpose of determining and planning the future needs of the Institute.

The Neurological Information Network has continued to develop with the review and continuation of projects at Columbia University and UCLA, continued development at Johns Hopkins, and the signing of a contract for the development of the Vision Information Center at Harvard. With the establishment of

the four key centers in the Neurological Information Network, true network interaction has begun.

In the area of documentation, Columbia has agreed to index some 50 journals which normally contain a large number of articles on Parkinson's disease and related brain research. The National Library of Medicine, in turn, will incorporate the citations from these articles into its computer-based Medical Literature Analysis and Retrieval System (MEDLARS). The citations will then be available for publication in Index Medicus, the Library's monthly listing of references to the world's current biomedical literature.

At UCLA, programs have been written and are being tested to strip MEDLARS of pertinent citations and to merge them with other Brain Information Service citations which have been identified but are not listed in MEDLARS. These programs are for the use of all centers. The problems of glossary control and differences in indexing are, of course, more pressing now, but a coordinating committee's subcommittee is working on these particular problems for their early solution. All of the centers have been registered at the National Referral Center of the Library of Congress and this is generating a certain amount of question-and-answer activity in all of them. This question-and-answer activity is one of the major output developments in all the centers which will hopefully acquaint them with the actual user needs. A major book on the thalamus, presenting the proceedings of the Parkinson Center's international symposium, has been published and two other books are in press.

At UCLA, a special review on the anatomical and physiological basis for trigeminal neuralgia is under way and should be finished by the end of this year. The Parkinson Center's regular weekly bibliographic announcement is increasing its circulation constantly. All centers continue to put out many bibliographies on demand and are considering other forms of communication.

In addition to the Neurological Information Network, the Office of Program Analysis is undertaking the monitoring of Mayo Clinic's alerting service for cerebrovascular disease literature. This is the first step in the plan to examine, with the Joint Council Subcommittee, the entire cerebrovascular disease information problem for the purposes of developing a sound program.

Other explorations have been carried out with Excerpta Medica to see if this major medical information facility cannot be utilized in some way by the Institute's information network. Hopefully, in the next year this endeavor will come to fruition.

Other activities of the Office of Program Analysis include assisting in the planning of the Kentucky Chemotherapy Conference, which was supported by the Kentucky Branch of the Martin Cancer Society and co-sponsored by the National Institute of Neurological Diseases and Blindness; initiating the planning of the NINDB-sponsored conference on "Toxins and Poisons as a Cause of Mental Retardation"; and assisting in planning and preparation of materials for other minor conferences and workshops.

The Information Office

In fiscal year 1966, NINDB's Information Office continued to serve as the Institute's main resource for planning, developing, interpreting, and disseminating information to a variety of publics. These included the health-conscious private citizen, physicians and scientists, educators, the mass media, members of Congress, voluntary health agencies and foundations, State and local health officials, and others engaged or interested in the health professions.

Public Inquiries: Requests for information covered a wide range of neurological, sensory, and communicative disorders. The largest number of inquiries concerned eye disorders, spinal cord injury, stroke, epilepsy, multiple sclerosis, and neuromuscular diseases. Increasing interest was expressed in childhood disorders or conditions such as minimal brain dysfunction, dyslexia, and related neurosensory deficits, and effects of maternal rubella. Magazine articles and news stories on NINDB research generated a considerable number of these inquiries.

The total number of public inquiries received by the Information Office was 7,447. Over 1,200 of these required individually prepared letters in answer to specific questions. These included 89 Congressional inquiries. The remainder were answered with printed materials.

Press and Periodicals: Requests from reporters, feature writers and editors, television and radio broadcasters numbered 75. Services ranged from providing specific facts and figures by telephone or mail to developing comprehensive background material, such as the press kit containing a basic press release and six "backgrounders" for the Second Scientific Meeting of the Collaborative Perinatal Project.

In response to specific queries from the media, materials were supplied or interviews arranged (with NINDB Director and other staff) for articles and stories used in scientific and lay press represented by: AMA News, Medical Tribune, Medical World News, Modern Medicine, M.D. Medical News, U.S. Medical News, Scientific American, Science Service, Washington Post, Washington Star, Baltimore Sun, New York Times, etc.

The Information Office prepared 15 releases and announcements and arranged three press conferences on head injury, perinatal research, and a multiple sclerosis grant. Present at these conferences were representatives from the leading medical and scientific periodicals as well as lay press.

Publications: Almost 100,000 copies of leaflets and brochures were distributed as part of a far-reaching publications program to acquaint professional and lay audiences with the NINDB research attack. Among the new and revised publications were three new brochures in the "Research Profile" series (Director's reports to Congress) and seven Profiles updated. In the "Hope through Research" series, "Shingles" (Herpes Zoster) was published as the 13th in the series and several were revised. A new edition of "Who's Who in NINDB" was published, containing the curriculae vitae of more than 200 members of the Institute's

scientific and administrative staff. Six issues of the "NINDB Review" were published for distribution to voluntary health agencies and NANDB Council members.

A new Institute monograph series was initiated and three monographs published, as part of the Institute's scientific and technical communications program. These were: "Epilepsy: A Review of Basic and Clinical Research" (Monograph No. 1), "Slow, Latent, and Temperate Virus Infections" (Monograph No. 2), and "Minimal Brain Dysfunction in Children: Terminology and Identification" (Monograph No. 3). The Information Office provided editorial and proofreading assistance, supervised design and layout, and arranged for distribution of Monographs 1 and 3 and processed Monograph 2.

The production of the first in a series of reports from the Institute's Neurological Information Network was supervised by the Information Office. This report, "Parkinson's Disease: Present Status and Research Trends," was prepared by the Institute's specialized information center at Columbia University. Major editing was also provided for the report of the NINDB mission to U.S.S.R. on Viruses (for administrative use only).

Films: The Information Office and the Medical Arts and Photography Branch, DRS, produced a 20-minute color-sound motion picture, "Infections and Birth Defects" for NINDB's Section on Infectious Diseases (Collaborative Project). The new film, which describes the mission and the research approach used by that section, was shown first at the meeting of the Collaborative Study in March 1966.

Assistance was rendered in planning for the production, on contract, of a detailed shooting script for a film on current research on the early identification of neuro-motor-sensory deficits in young children. The script is to reflect present research findings and provide potential aid to parents, educators, nurses, and others, and to encourage referral to physicians for professional diagnosis.

Four Institute-supported films had close to 1,000 professional and lay showings arranged by the PHS Audio Visual Facility (CDC) and the American Medical Association film library. These films are: "Neurological Examination of the Newborn," "Neurological Examination of the One-Year-Old," "Year of Birth," and "Exploring the Human Nervous System." The last-named film, a previous prize-winner, this year captured the top scientific film award--"Orbit"--of the Australian and New Zealand Association for the Advancement of Science, in competition with 130 entries from 12 countries, making it the first time a U.S. film has won this honor.

Exhibits: The Information Office completed arrangements for and manned exhibits at 10 national meetings during the year. One exhibit, entitled "Progress in Deafness Research," was constructed and shown as a cooperative endeavor between the Deafness Research Foundation and NINDB. Two exhibits related to the Institute's extramural program. Showings were before the American Academy of Ophthalmology and Otolaryngology, the American Medical Association, the American Academy of Neurology, and the General Federation of

Women's Clubs. Also, at Dr. Van Buren's request, the Information Office arranged for Medical Arts to produce an exhibit entitled "Three Plane Variation Studies of the Human Diencephalon," which was shown at the annual meeting of the Harvey Cushing Society.

Photographs: Photographs were supplied for most of the 29 stories contributed by the Information Office for the NIH Record, and various pictures were supplied to the News Service for Professional Journals. With the approval of the Intramural Associate Director of Research, the Information Office shot a new series of photos illustrating investigations in clinical neurology, neurosurgery, and ophthalmology, as well as areas of basic research.

The Information Office also arranged for photographic scenes at grantee institutions. Pictures were taken by an NIH photographer at the Corneal Research Center and the Parkinson's Disease Center, both at Columbia's College of Physicians and Surgeons. Ear surgery and medical audiology scenes were shot at the Johns Hopkins Medical Institutions.

Speeches and Special Statements: Eighteen speeches were written, either for delivery by the NINDB Director or Assistant Director, other DHEW officials, and members of Congress. Suggested messages from President Johnson, requested by leading voluntary organizations marking special events, were written on eight occasions.

Reports and Special Documents and Projects: The Information Office wrote or assisted in preparing and processing various Congressional materials required by the Director during appropriations hearings. These included the opening statement, eight special reports, a document "Highlights of Research" summarizing 12 areas, and other essential materials.

Other major reports prepared that required considerable Information staff time brought the total reports to 18.

During the year, 45 journals were scanned on a weekly or monthly basis, and 41 weeklies were prepared for the Surgeon General using grantee or Institute scientists' findings. Additionally, 6 program development items were written.

Arrangements were completed for 11 Institute lectures, including the preparation and distribution of fliers.

Special Services: Throughout the year the Information Office represented the Institute and contributed information, services, or other assistance in various ways. For example: Served on DHEW's Committee to Review Publication Policy; participated in discussions and meetings of American Association of Medical Colleges concerned with medical school public information; supplied background materials on NINDB to various DHEW committees (Committee on Smoking and Health, Follow-up Committee of Inter-American Workshop on Mental Retardation, statement on blindness for Secretary's Office, etc.).

Cooperation with Voluntary Agencies and Foundations: Cooperation with national voluntary agencies and foundations dealing in neurological and

sensory disorders occupied an increasing share of the Information Office's public relations role. In fact, day-to-day operations reflect the continuing relationship by way of supplying copies of NINDB publications, answering public inquiries referred by the voluntaries, offering consultative assistance of various sorts. Examples of such cooperation are: (1) Deafness Research Foundation--supervised construction of jointly financed exhibit on "Deafness Research" and manned exhibits for DRF; (2) National Health Education Committee--updated information and statistics in eight disease categories for brochures on Health Education cost of disease series; (3) Research to Prevent Blindness--during Research to Prevent Blindness' first science writers' seminar, covered all sessions, arranged for an interview with NINDB Director, and answered numerous questions for science writers; (4) Epilepsy Association of America--attended first national meeting of newly merged former three Associations and discussed with leaders future cooperation with NINDB; (5) National Multiple Sclerosis Society--developed cooperative press release on Society's gift to NINDB (presented via DHEW Under Secretary Wilbur J. Cohen) and reviewed and discussed distribution of new MS diagnostic film; (6) National Association for Retarded Children--cooperated with Association's Mental Retardation Week program; (7) National Society for Crippled Children and Adults--co-sponsored monograph, "Minimal Brain Dysfunction," with the Society; (8) Muscular Dystrophy Associations of America--cooperated in plans for poster child and in international conference to be held this fall; (9) United Cerebral Palsy--supplied materials for use at national meetings; and (10) National Association of Hearing and Speech Agencies (formerly American Hearing Society)--spoke at national meeting. In addition, served as secretary to the National Committee for Research in Neurological Disorders and provided background materials for the Committee's meetings.

RECOMMENDATIONS
OF
HEAD INJURY CONFERENCE

I. EPIDEMIOLOGY

That epidemiological data be obtained relative to:

1. The general incidence and prevalence of head injuries of all kinds, i.e., those resulting from accident in industry, sports, and transportation.
2. The probable time and force pattern of the agent which may be obtained by police, ambulance drivers, physicians, or other observers.
3. The environment.
4. The type and location of wounding.
5. The time and place of immediate and definitive care.

II. GLOSSARY, CLASSIFICATION, AND STANDARDS OF MEASUREMENT

That a committee be appointed to consider the problem of classification of head injuries. A committee of engineers, physicians, biologists, and others should be appointed to formulate a head injury glossary, with definition of experimental, technical, and clinical terms. Quantitative methods of recording biological and physical phenomena (severity of injury) be devised which will be accepted by the various disciplines.

III. CLINICAL CENTERS

That consideration be given to establishment of a few centers devoted to head injury investigation. Such centers should be located in areas where proper clinical material is available, where laboratory facilities may be utilized, where ancillary techniques and services may be called upon, and where a high level of interest in problems of head injury already exists.

IV. POST-TRAUMATIC SEQUELAE

For improved understanding and rehabilitation of the neurologic consequence of trauma, sequential studies of sequelae are needed. The specific areas should include: (1) the development, remission, and stabilization of neurological deficits with particular interest in factors which favor recovery; (2) post-traumatic epilepsy with particular interest in prophylaxis, treatment, as well as ancillary laboratory studies of basic mechanism; (3) the symptoms of the post-traumatic syndrome with particular interest in factors that operate in mild as well as severe head injuries.

V. MILITARY OBSERVATIONS

The military experience affords a unique opportunity for observations in head injuries under a degree of control in age range, and in general

health, that does not exist in civilian populations. Further, the opportunity for continuous observation from the time of injury to the development of sequelae provides a means of understanding these complex phenomena. To realize the maximum yield of information, the design of prospective studies is essential. In short, advantage should be taken of the military experience in head injuries.

VI. BRAIN SWELLING

It is recommended that multidisciplinary research in the problem of brain swelling be pursued with special reference to: (1) the definition of the various compartments of the brain which swell and the etiology and pathophysiology of the various kinds of brain swelling; (2) the mechanism of the effect of the various kinds of swelling on neurological function in terms of nerve conduction, synaptic transmission, somal-dendritic polarization, etc.; (3) mechanism of action of hypertonic solution to clarify their actions on various types of swelling; (4) the effective mechanism of action of corticosteroids in preventing and treating various types of swelling; (5) basic studies with biochemical and electron microscopic techniques of the mechanisms involved in water and electrolyte transfer.

VII. BASIC STUDIES

Animal experimental work is necessary as human accident data are nearly valueless for the understanding of the mechanism of head injury. Such research should be supplemented by studies on normal humans as far as is possible without risk to volunteers. It is recommended that predictable and reproducible impact methods to cause head trauma in experimental animals be developed.

VIII. ENGINEERING AND MATHEMATICS

It is recommended that the active participation of engineers and physical scientists in research on head injuries be encouraged so that: (1) cross-fertilization may occur between physicians, engineers, and physical scientists; (2) the mechanisms of cranial and intracranial balance may be studied by modern experimental techniques such as X-ray, photo elasticity, etc.; (3) further quantitative studies may be made of the mechanical properties of the adult brain; (4) analytical models of the head may be constructed, consisting of fluid-filled shell. The response of such a system to transient loading simulate blows or penetrating wounds will then be calculated by modern mathematical techniques, including digital computers; (5) corresponding experimental models may be investigated under similar loading conditions and appropriate measurements of response be obtained. Theoretical results and experimental data will then be effectively correlated; (6) prophylactic and preventive measures, for example, helmet design, may be further improved by reference to quantitative data whenever available. In particular, design characteristics should be based on the results of fundamental studies as outlined in (4) and (5) above.

IX. RESEARCH PERSONNEL

For the implementation of the clinical and multidisciplinary research effects: (1) interest should be generated among scholars; (2) recruitment attempts should be pursued; (3) support should be provided for scientists in the field of head injury.

X. EDUCATION

It is recommended that: (1) an effort be made to insure that the latest data be widely disseminated to all those who are associated with the immediate handling of accidents and head injuries. This would augment the laudable efforts of established agencies; (2) an Information Analysis Center be set up for the collection and dissemination of information concerning head injuries.

FY 1954-66

NINDB GRANTS BY ACTIVITY

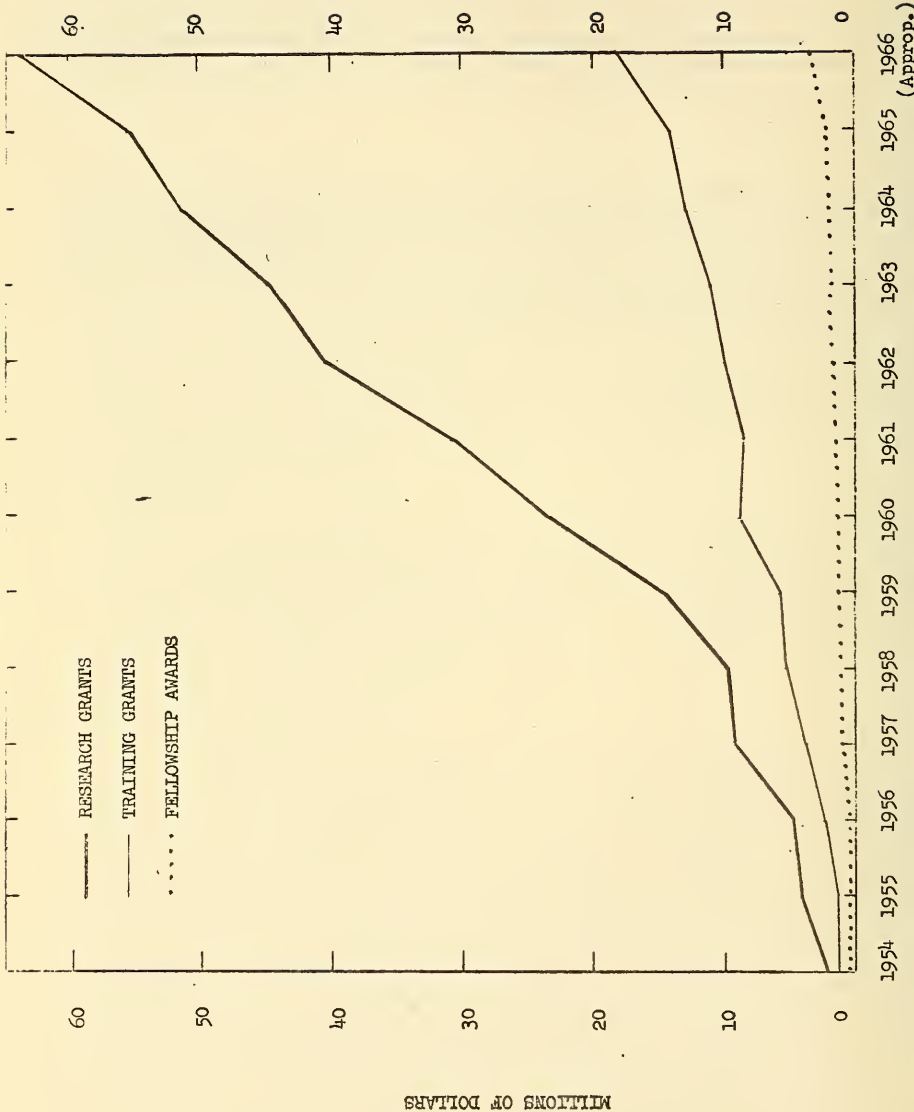


CHART I

1/20/66

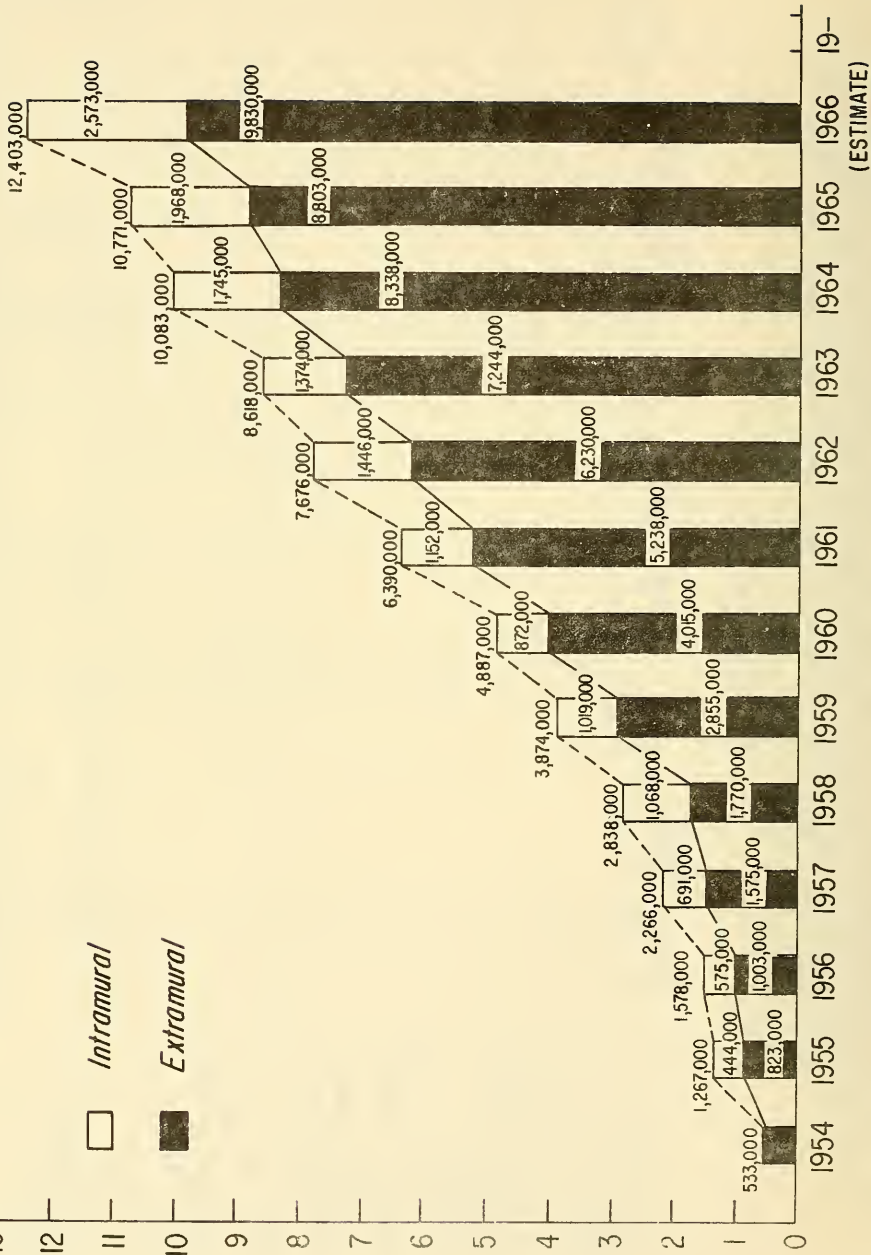
 FY 1967 President's Budget
 NINDB - Disease Category (Research) - In Thousands

Type of Disorder	1965 Actual			1966 Estimate			1967 President's Budget											
	Extramural No.	Intramural Amount	Total No.	Extramural No.	Intramural Amount	Total No.	Extramural No.	Intramural Amount	Total No.									
1. Chronic Neurol. Dis. of Childhood	114	\$9,831	86	474	71	2,421	59	2,030	9	486	68	2,516	107	\$9,900	98	\$4,461	205	\$14,361
2. Chronic Neurol. Dis. of Aging	63	1,947	8	197	88	2,807	90	4,400	14	553	104	4,953	97	4,760	14	574	111	5,334
3. Cerebrovascular Disorders	76	2,610	12	1,193	103	3,518	75	2,420	24	1,226	99	3,646	77	2,590	25	1,272	102	3,862
4. Epilepsy & Related Paroxysmal Disorders	81	2,325	22	501	117	2,953	80	2,620	33	515	113	3,135	82	2,800	34	534	116	3,334
5. Sclerosing Disorders	87	2,452	30	1,164	129	4,095	107	3,100	19	1,197	126	4,297	108	3,330	19	1,241	127	4,571
6. Muscular & Neurtomus. Disorders	112	2,931	17	385	9	191	22	576	12	443	10	197	13	485	10	205	23	690
7. Infectious Neurol. Disorders	73	1,524	33	870	106	2,394	73	1,640	36	895	109	2,535	72	1,760	38	929	110	2,689
8. Tumors of the Nervous System and Trauma	364	8,696	30	867	394	9,563	340	9,220	33	891	373	10,111	343	9,940	34	925	377	10,865
9. Other Neurological Disorders	333	8,803	54	1,968	387	10,771	337	9,830	59	2,573	396	12,403	343	10,570	62	2,670	405	13,240
10. Disorders of Vision	143	4,214	9	274	152	4,488	134	4,400	10	282	144	4,682	138	4,760	10	293	148	5,053
11. Dis. of Hearing & Equilibrium	28	994	6	163	34	1,157	25	1,100	6	168	31	1,268	26	1,170	7	174	33	1,344
12. Disorders of Speech & Higher CNS Functions	74	1,590	5	103	79	1,693	70	1,710	5	106	75	1,816	71	1,830	6	111	77	1,941
13. Other Sensory Disorders	33	1,429	6	110	39	1,539	30	1,440	6	114	36	1,554	30	1,540	7	118	37	1,658
14. Non-categorical	1,594	49,731	327	12,258	1,921	61,989	1,540	54,653	360	13,503	1,900	68,156	1,567	57,605	374	14,011	1,941	71,616
Subtotal	5,238	1,200		5,312				5,312				5,312		6,247				6,247
Gen. Res. Supporting Grants								3,050				3,050		3,050				3,050
Categorical Clinical Centers																		
Planning Grants	(4)	65			(4)	65		(3)	800		(8)	800		(5)	75		(8)	800
Scientific Eval. & Planning																		
Total	1,594	56,234	327	12,258	1,921	68,492	1,540	63,890	360	13,503	1,900	77,393	1,567	67,777	374	14,011	1,941	81,788

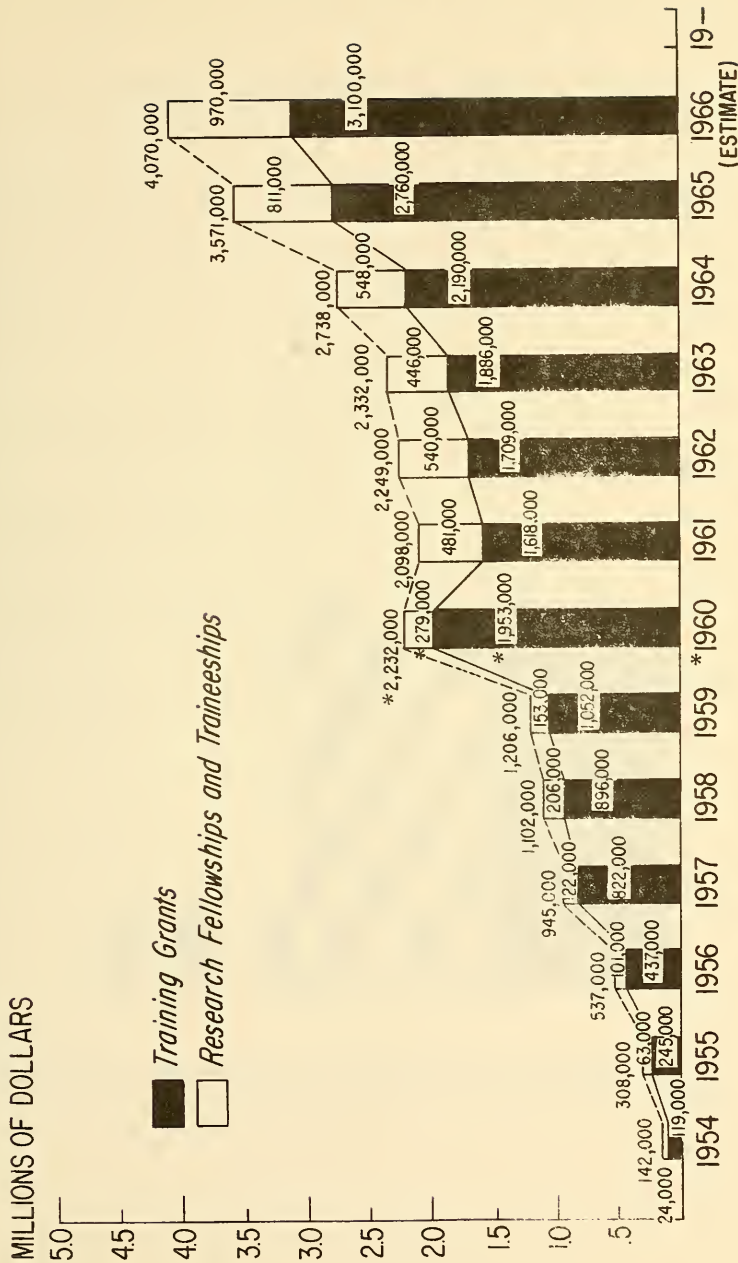
CHART II

EYE RESEARCH EXPENDITURES

MILLIONS OF DOLLARS



EYE RESEARCH TRAINING EXPENDITURES 1954 - 1966



* This apparent increase reflects a modification of financing and does not reflect the true level of activity during FY 1960.

NUMBER OF PRINCIPAL INVESTIGATORS

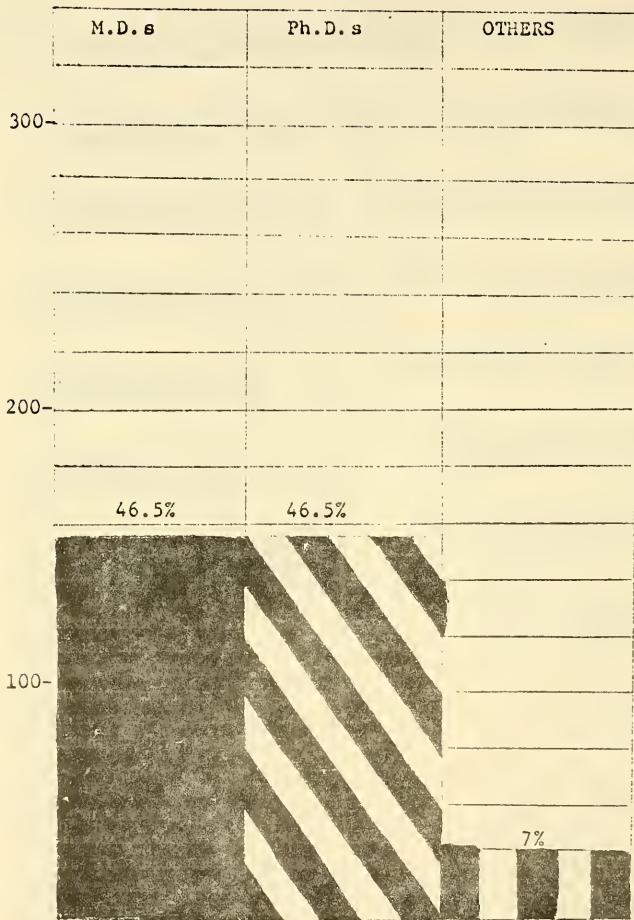


Figure 4. The distribution of 300 Principal Investigators by professional category conducting 387 NINDB-supported research projects in FY 65.

ANNUAL REPORT

July 1, 1965 to June 30, 1966

Office of the Special Assistant to the Director for Biometry
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

Intensified activity in a long-term research project has characterized the program of this Office for the past 12 months. This research program is reviewed in the concluding Individual Project Report - NDB(OD)-66-1258.

The biometric advisory functions of this office, which occupied a substantial part of the chief's time in the past, have been restricted to the following activities:

- (1) Development of a protocol for a proposed collaborative project to investigate the merit of thymectomy in the management of myasthenia gravis was discontinued early in the year. Evidence that the surgical procedure was safe and effective in experienced hands had increased to a level which effectively removed major objection to it. This office was therefore engaged only in a critical review of that evidence. The Myasthenia Gravis Foundation has withdrawn its promotion of the proposed collaborative research project.
- (2) The writer has served as biometric liaison between the Director of this Institute and the administration of the "Cooperative Study of Intracranial Aneurysms and Acute Subarachnoid Hemorrhage." Attendance at a recent meeting of participants in this study, followed by some contact with the administrators at the University of Iowa Medical School, gave grounds for questioning whether useful purposes were now being served by this liaison. Withdrawal from this role therefore seemed advantageous to more useful distribution of the limited resources of this office to the projects it serves.
- (3) The chief of this office is a member of the Institute's "Advisory Committee on Evaluation of Clinical Therapy." Full participation in the activities of this committee provides unusual opportunity for constructive contribution toward programs of the Institute, both intra-mural and extra-mural.
- (4) A "thoroughly objective appraisal" of the Model Reporting Area for Blindness Statistics was desired by the Institute to evaluate the mission, methodology and future direction of this activity. To this end the chief of this office served as chairman of a Review Panel consisting otherwise of three distinguished scientists serving as extra-mural consultants. After as intensive an inquiry as could be conducted within the time available, this Panel submitted its report recommending (1) continued effort to attain the goal of having representative States define an area from which valid inferences could be made, provided that (2) a thorough analysis would be made by independent action leading to validation

of the data and adequate definition of their limitations. The Panel thereby affirmed its belief that commendable research and planning goals within the mission of the Institute were attainable through this activity.

Approximately 13 per cent of his time has been given this year by the Chief of this Office to advisory and review conferences for accomplishment of the objectives enumerated above.

1. Office of the Director
2. Special Assistant for Biometry
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Human Reproduction and the Menstrual Cycle.

Previous Serial Number: Same

Principal Investigator: Alan E. Treloar, Ph.D.

Other Investigators: Borghild G. Behn, Ph.D.

Cooperating Units: University of Minnesota (Contract No. PH 43-65-1014)

Conducted by

a) Biostatistics Division, Dr. B.W. Brown, Jr.

b) University Health Service, Dr. D.W. Cowan

Man Years, Total: 4.8

Professional: 1.8

Other: 3.0

Project Description.

Objectives: a) To define quantitatively the pattern of temporal variation in the human menstrual cycle throughout the span from menarche to menopause.

b) Through current recording by date of all events, to secure accurate records of the interruptions of menstrual function through pregnancy, and to define reliably the gestational interval from onset of last menstrual period to delivery of the product of pregnancy.

c) To study the effects of the use of pregnancy control medications in the form of hormonal steroids (particularly the various "birth control pills") on menstrual cyclicity and duration of flow.

Methods Employed: Under a research plan to maintain continuous accurate records of all menstrual flow periods, enrollment of young women attending the University of Minnesota was commenced in 1934 by the chief of this office when he was in charge of the Biometry Department of the Graduate School of that University. Over 500 collaborators secured in the initial approach successfully launched the program. For the following four years, approximately the same numbers of new collaborators were secured each year from the entering class. Mail contact has been maintained as far as possible with all collaborators each year, when complete records and accessory report forms are collected as new calendar cards were issued. Daughters of these collaborators were invited a few years later to join the study as they reached menarche.

A new group of approximately 1200 university women has been enrolled in this study through the years 1962-4. Their records will enable a study of change to be made for the same sort of sample after a quarter-century time lapse. Accumulation of records from this new group will be adequate in a relatively few years to enable a dependable age-specific comparison to be reached with respect to the first third of menstrual life.

Approximately five years ago, collaborators experiencing menopause and hysterectomy reached a level sufficient in number to justify an analysis of the accumulated records being undertaken through the whole span of menstrual life. At that time the writer had joined the NIH staff, but the project was continued in most of its operations at the University of Minnesota where it remains active under the writer's general direction. Preparation of the data from the original collaborators and their daughters for analysis by punched card operations was started in 1961. Only the intervals between menstrual onsets, as interrupted by pregnancies, could be accommodated into a card designed at that time for available EAM equipment. Shortly thereafter, EDP equipment also became available. Computer runs with printed output from tested programs were achieved in the third year of these operations.

The first report on age-specific menstrual cycles, first made available to members of the Population Association of America in a symposium held last year at its annual meeting, has been accepted for publication. Another report dealing with the gestational intervals for the 2062 live births to the collaborators during the period 1934-61, and compared with results obtained from hospital records and private practice records, was presented to the second scientific meeting of this Institute's "Collaborative Study on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood". This report is undergoing final adjustments for early publication.

Major Findings: With approximately 26,000 person-years of experience available for these analyses, a composite picture of the age-specific of menstrual intervals covering the complete span from menarche to menopause has been developed. An accurate set of records for the gestational interval has been made available for the first time, and in sufficient numbers to be definitive of this variable. Both of these bodies of information are novel in that they replace fragmentary information by enough cases to generate distributions of great stability.

The menstrual interval studies have led to definition of three age zones in menstrual life. The first years of experience must be assembled for all collaborators in terms of their menstrual age. Defined as the menarchial zone of menstrual life, this experience satisfactorily unites with the "middle life" zone at chronological age 20 (approximately, and as a result). From this point onward for 20 years the usual age scale with its origin at birth proves adequate to define the pattern of menstrual intervals. At about age 40 the heterogeneity created by impending menopause for many collaborators necessitates termination of the chronological age zone in favor of a

menopausal age scale. This can be generated for any individual only when "absolute" menopause is reached; the scale of years proceeding backwards from menopause defines this terminal zone.

Frequency contours have been secured for this tripartite distribution of menstrual interval by single years of age within the three time scales. These contours, which proceed by deciles from 10 to 90 per cent, and by percentiles from one through five and from 95 through 99 per cent, are supplemented also by the quartile values (25 and 75 per cent). Except for the extreme boundaries (one, two, 98 and 99 percentiles), these empirical contours are so remarkably stable that attempting further smoothing by mathematical processes of graduation is without value.

The following conclusions concerning menstrual intervals have been reached from these analyses:

- 1). The commonly stated menstrual pattern of "regular, every 28 days" is so rare that this description must be dismissed as a fiction unless only very brief periods of time - a few months - are used.
- 2). After menarche, which is usually (but not always) initiated with very great irregularity in menstrual interval, there is a progressive decrease in irregularity as transition is achieved to a new pattern which characterizes the middle years of menstrual life. Duration of the menarchial zone is very variable; a few persons do not experience diminishing irregularity, while for others it persists well into if not through the twenties. When all experiences are combined in terms of menstrual age, the menarchial zone appears to extend for seven or eight years.
- 3). Relative stability in menstruation characterizes the middle years of experience commencing on the average at about age 20 years. The median menstrual interval per person tends to decrease slightly - by about 2 days - in a straight line manner from this age (20) to just before the menopausal experience is initiated around 40 years of age. The variability of menstrual intervals tends to decrease through these middle years; the average standard deviation of approximately 4 days at age 20 years falls in nearly straight line fashion to essentially three-fourths of that value at age 40 years. Inverting this, one may say that through this relatively stable zone of two decades of experience, there is a gradual gain in regularity when all persons are considered collectively.
- 4). For by far the majority of women, birth control by following the rhythm method is not practicable. The probability of failure using this technique reaches its lowest level in the middle and late thirties. It is in this period that neurological deficits in the offspring begin their increase. This period of sufficiently low variability is a critical one, but it is also of relatively short duration.

- 5). The menopausal zone, of approximately nine years on the average, is remarkably like a mirror image of the menarchial zone; its changing pattern is one of increasing variability. Age at menopause for 120 cases at present available ranges over 21 years, from 36 to 56. The average presently stands at approximately 48½ years, but this is expected to increase slightly as the distribution becomes enhanced by better balance in the more advanced ages.
- 6). Short menstrual intervals tend to increase in number along with the long intervals in both the menarchial and menopausal zones of menstrual life. Because of this the median interval changes slowly with time in these zones. Thus changes in the variability picture are reflected more in the mean than in the median.
- 7). Personal histories have been traced graphically for over 100 cases. A most impressive feature of them is the individuality of women with respect to menstrual interval. Each person has her own characteristics of mean interval and its change with time, and of change in her variability of interval through the years. There is no assurance that the pattern of menstrual intervals as observed over several years will not change in character either suddenly or with variable acceleration. Forecasting the day of next ovulation, if it is to be made without substantial risk of failure, must be based on more than past menstrual history; it must be supplemented by current observations on physical and psychological symptoms. Daily basal body temperature determinations seem to indicate an event just past and thus better serve purposes of defining existence of the pregnancy state.
- 8). There is no detectable correlation between age at menarche and age at menopause. This negates a traditional viewpoint in the gynecological literature. Age at menarche (as recalled a few years after this event) varied for the original collaborators (born approximately 1895 to 1920) from age 9 to 19, with a mean at 12.7 years. Mean age at menarche decreased slowly and progressively through these years over a span from 13.8 to 12.3 years; this change is statistically significant. Comparison of these results with those of the currently recorded age at menarche for the daughters is awaited with interest; the span of years covered is already approaching the same total range of roughly 18 years.

The gestational interval study as defined precisely in terms of currently recorded dates of last menstrual onset as well as of delivery, has yielded challenging results as follows:

- 1). Essential agreement has been established between results from within the menstrual history program with those obtained from a number of totally different sources for the white race. This concordance necessitates rejection of a view held by many obstetricians that "you can't believe what a pregnant woman tells you about her last menstrual period." Our currently recorded data agree splendidly with both hospital and private case records using recalled LMP dates.

- 2). Evidence from very divergent sources confirms that the traditional gestational interval (LMP to birth) for white single live births varies in fact over a range from approximately 28 to 49 weeks. The menstrual history study has provided records which make it reasonable to explain this wide range in terms of two propositions:
- a). Cyclic bleeding in step with previous menstrual history persists for a small proportion of women for one or more "apparent menses", these generating the short gestational intervals.
 - b). Abortions, mostly unknown, are followed by viable pregnancies without an intervening menstruation in enough cases to account perhaps fully for the long gestational intervals.
- 3). Using data supplied by the Perinatal Research Branch of this Institute, there is clear indication that whereas a diversity of sources for data on Caucasian races show a uniformity in variation pattern of gestational intervals, the American Negro race diverges significantly toward shorter and more variable gestational intervals. The difference of approximately six days on the average is supplemented by a different pattern of frequency distribution for this variable. The latter point suggests that perhaps post-pregnancy cyclic bleeding for the Negro is more frequent than for Caucasians.

Significance to Bio-Medical Research. The findings concerning menstrual cyclicity and gestational intervals as set forth above suggest that strong adherence to traditional concepts in these areas may have hampered scientific progress. Two distinct frontiers of research are affected by the facts revealed. The problems of birth control will be recognized to require extensive revision of the claims for adequacy of the rhythm method. The hormonal control systems as hypothesized for the menstrual function fail to give definitive evidence of failure of pregnancy. These hypotheses must therefore be shifted from a deterministic basis to a probabilistic basis. This type of change has commonly been experienced in the sciences as maturity is progressively achieved.

Proposed Course of Project. 1). It is anticipated that this research program will continue under its present direction for a number of years. It is anticipated this will be achieved with the continued cooperation of the University of Minnesota. However, the program is not dependent on that cooperation, but rather on continuance of its present direction and professional staffing in a setting advantageous to its full development. Many variables within the total scope of the available records have not yet been analyzed; contributions to important issues await development. New directions of data collection, particularly with respect to birth control via medications, will be developed. The study of a similar population selected 25 years after the original one will give valuable evidence respecting the nature of change in menarche and menstrual periodicity through that interval.

It is planned that extensions of the study should include new population groups of the following types:

- a) The American Negro, for whom currently documented evidence of menstrual characteristics may well show differences from the Caucasian as in gestational intervals.
 - b) The Eskimo as a race in a frigid climate, less removed from their earlier native state perhaps than any other race which can be reached readily.
 - c) The Polynesian natives contrasting with the Eskimo at least in being in a tropical setting.
 - d) A southern hemisphere Caucasian grouping such as the Australian which differs biologically from the United States whites chiefly in their southern hemisphere environment.
 - e) National or racial groups from other sectors of the globe for which this menstrual history study may be added to other related programs.
- 2). A unique opportunity presents itself for study of the effect of birth control medications on the course of menstrual cyclicality in the younger members of the original collaborator group. Provision for accumulation of data bearing on this issue has been made and analyses of results will be initiated in the near future. This will be accomplished as part of a plan now well developed to code comprehensively for computer analysis all data now available or to be available. Data collected for the original panel through calendar year 1965 have now been recoded and are being punched and analyzed under contract by the University of Minnesota in accord with this plan.
 - 3). Data from the "new series" of University student collaborators enrolled in 1962-4 will be coded in the same way next year. Analyses of these data will follow.
 - 4). Another eight years of intensive analytical work supplementing expanded data collection is envisaged for this study under its present direction.

Honors and Awards: None

Publication: Treloar, Alan E., Boynton, Ruth E., Behn, Borghild G., and Brown, Byron W. Jr.: Variation of the Human Menstrual Cycle Through Reproductive Life. International Journal of Fertility, 10: supplement (in press).

ANNUAL REPORT

July 1, 1965 through June 30, 1966

Direct Training

National Institute of Neurological Diseases and Blindness
National Institutes of Health

Funds under this activity provide for the support of the Institute's training programs for professional, technical, administrative, and clerical personnel, including job-related training at both government and nongovernment facilities.

The Institute, in cooperation with the Division of Hospitals, BMS, is currently supporting the training of an otolaryngologist at the University of Cincinnati.

In addition, two Commissioned Officers from the Intramural Research programs are receiving neurological and neurosurgical training at the Barnes Hospital, Washington University, St. Louis, Missouri, and at the State University of New York, Syracuse, New York, and two more are receiving postdoctoral academic training in immunology and in molecular biology.

Short-term courses are also supported as funds are available.

ANNUAL REPORT
Fiscal Year 1966
Extramural Programs
National Institute of Neurological Diseases and Blindness
National Institutes of Health

I. Purpose:

The Extramural Programs of the NINDB have as their primary objective the identification of research areas of national need in the neurological, neurosensory, and communicative fields and the stimulation and support of selected biomedical research and training in those areas. The research grant, training grant, and fellowship programs provide funds on a competitive basis for the manpower, resources and facilities necessary to meet this objective.

II. Programs:

A. Research Grants

The research grant program including regular research projects and clinical center-program projects provides funds for the initiation and support of research on basic and clinical problems related to the nervous system and neuromuscular systems; the special senses including vision, hearing, smell, touch, and pain; and communications including speech and language. In the neurological area special emphasis was given to the problems of epilepsy, multiple sclerosis, cerebrovascular disease, muscular dystrophy, cerebral palsy, and other neurological and neuromuscular disorders; in the area of vision, special attention was given to the problems of glaucoma, cataract, uveitis, infections and congenital abnormalities; in the area of hearing, special emphasis was given to otosclerosis, nerve deafness, middle ear and inner ear infections, and congenital disorders; and, in communicative disorders, special attention was given to aphasia, problems of voice production and modulation, speech development, and central nervous system information storage and organization. (See exhibits 1 a and 1 b.)

Through the research grant program the NINDB awarded in FY 66 a total of 1,615 research grants totaling \$58.1 million; of these, approximately 300 grants totaling \$11 million were awarded for needed new research undertakings, and \$47 million was provided to continue or supplement 1,315 successful on-going research projects.

Also through its research grants program, NINDB supported a total of 36 clinical center-program projects at a level of \$8.48 million; 22 in the area of neurological disorders and 14 in the area of sensory and perceptual disorders. (See exhibit 2.)

B. Training Grants

The training grant program provides funds on a competitive basis for the establishment, improvement, and support of training in the disciplinary areas related to the neurological, neuromuscular, sensory or

communicative disorders. The primary objective of the training grant program is to train needed additional teacher-investigators, scientist-physicians, and community health and public health personnel. In fiscal year 1966 the NINDB awarded 275 graduate training grants totaling \$15.3 million; 34 grants totaling \$1.3 million were awarded to establish new training programs; and \$14 million was awarded to continue and supplement 241 active and successful on-going programs. (See exhibit 3.)

In addition, 240 Special Fellowships, providing stipend support for highly specialized research training, were awarded to basic and clinical scientists preparing for careers in research and academic medicine. (See exhibit 4.)

C. Fellowship Program

Through its Fellowship Program, NINDB makes available stipend support in the form of Postdoctoral Fellowships, Career Awards and Career Development Awards. Postdoctoral Fellowships are awarded to scientists and scientist-clinicians desiring specific research training following the receipt of a doctoral award. Career Development Awards provide stipend support for able scientists developing careers in independent research and teaching, and are available primarily for investigators desiring experience and further training in a productive research environment, or for scientists undertaking independent research and in need of further experience to qualify for a senior position. Career Awards provide additional stable positions for experienced, independent investigators in pursuit of productive careers in research and teaching.

In fiscal year 1966, 124 postdoctoral fellowships totaling \$873,000 were awarded. Ninety-seven research career development awards totaling \$1,927,000 and 14 research career awards totaling \$347,000 were supported. (See exhibit 5.)

Exhibit 1 a

NUMBERS OF RESEARCH GRANTS SUPPORTED
IN FY 66 AND AMOUNTS ARRANGED BY DISORDER CATEGORY

TYPE OF DISORDER	No.	Amount	% of \$
<u>All Disorders</u>	<u>1615</u>	<u>\$58,178,000</u>	<u>100</u>
I. NEUROLOGICAL DISORDERS			
A. Chronic Neurological Disorders of Childhood	110	9,599,000	16.5
B. Chronic Neurological Disorders of Aging	60	2,269,000	3.9
C. Cerebrovascular Disorders	105	4,189,000	7.2
D. Epilepsy and Related Paroxysmal Disorders	70	2,443,000	4.2
E. Sclerosing Disorders	85	2,734,000	4.7
F. Muscular & Neuromuscular Disorders	100	3,025,000	5.2
G. Infectious Diseases	15	291,000	0.5
H. Accident and Injury	45	1,280,000	2.2
I. Tumors of Nervous System	35	873,000	1.5
J. General	375	10,414,000	17.9
<u>ALL NEUROLOGICAL DISORDERS</u>	<u>1000</u>	<u>37,117,000</u>	<u>63.8</u>

<u>ALL DISORDERS</u>	<u>No.</u>	<u>Amount</u>	<u>% of \$</u>
	1615	\$58,178,000	100
II. SENSORY & PERCEPTUAL DISORDERS			
A. Disorders of Vision	325	10,763,000	18.5
1. Cataract	25	814,000	1.4
2. Glaucoma	30	989,000	1.7
3. Retinopathy & Neurological Mechanism of Vision	125	3,433,000	5.9
4. Inflammatory & Parasitic	45	1,454,000	2.5
5. Metabolic & Degenerative	10	524,000	0.9
6. Strabismus & Neuromuscular	20	698,000	1.2
7. Injuries & Other Disorders including Tumors	20	640,000	1.1
8. General	50	2,211,000	3.8
B. Disorders of Hearing and Equilibrium	145	4,887,000	8.4
C. Disorders of Speech & Other Higher CNS Functions	40	1,745,000	3.0
D. Disorders of Other Senses	75	1,862,000	3.2
<u>ALL SENSORY & PERCEPTUAL DISORDERS</u>	<u>585</u>	<u>19,257,000</u>	<u>33.1</u>
III. <u>NON-CATEGORICAL</u>	<u>30</u>	<u>\$ 1,804,000</u>	<u>3.1</u>

Exhibit 1 b

NUMBERS OF RESEARCH GRANTS SUPPORTED
IN FY 66 AND AMOUNTS ARRANGED BY SCIENTIFIC DISCIPLINE

TYPE OF CLASSIFICATION

<u>All Classifications</u>	<u>No.</u> 1615	<u>Amount</u> \$58,178,000	<u>% of \$</u> 100
A. NEUROANATOMICAL SCIENCES			
1. Gross neuroanatomy & comparative neurology: Morphological	25	698,000	1.2
2. Gross neuroanatomy & comparative neurology: Experimental	40	815,000	1.4
3. Neurocytology & histology: Morphological	35	1,105,000	1.9
4. Neurocytology & histology: Experimental	55	1,687,000	2.9
5. Embryology, development & regeneration: Morphological	10	233,000	0.4
6. Embryology, development & regeneration: Experimental	35	814,000	1.4
7. Neuropathology: Morphological	10	407,000	0.7
8. Neuropathology: Experimental	35	873,000	1.5
9. Pathology of Sense Organs	45	1,629,000	2.8
<u>ALL NEUROANATOMICAL SCIENCES</u>	<u>290</u>	<u>8,261,000</u>	<u>14.2</u>
B. NEUROPHYSIOLOGICAL & NEUROPHYSICAL SCIENCES			
1. Neurophysiology	170	5,466,000	9.4
2. Neuromuscular Physiology	40	874,000	1.5
3. Biophysics, including Electrophysiology	120	3,433,000	5.9
4. Special Senses	115	3,783,000	6.5
<u>ALL NEUROPHYSIOLOGICAL & NEUROPHYSICAL SCIENCES</u>	<u>445</u>	<u>13,556,000</u>	<u>23.3</u>

TYPE OF CLASSIFICATION

	<u>No.</u>	<u>Amount</u>	<u>% of \$</u>
<u>All Classifications</u>	<u>1615</u>	<u>\$58,178,000</u>	<u>100</u>
C. BIOCHEMICAL SCIENCES			
1. Basic Biochemistry	105	3,201,000	5.5
2. Enzymology	35	989,000	1.7
3. Metabolism and Nutrition	100	2,734,000	4.7
4. Pharmacology & Experimental Therapeutics	95	2,385,000	4.1
 <u>ALL BIOCHEMICAL SCIENCES</u>	 <u>335</u>	 <u>9,309,000</u>	 <u>16.0</u>
D. SOMATO-NEUROLOGICAL RELATIONSHIPS			
1. Endocrinology	30	931,000	1.6
2. Hematology	30	640,000	1.1
3. Immunology	35	1,396,000	2.4
4. Microbiology	10	233,000	0.4
5. Parasitology	5	58,000	0.1
6. Respiratory	10	171,000	0.3
7. Whole Animal	5	180,000	0.3
8. Genetics	15	580,000	1.0
 <u>ALL SOMATO-NEUROLOGICAL RELATIONSHIPS</u>	 <u>140</u>	 <u>4,189,000</u>	 <u>7.2</u>
E. CLINICAL INVESTIGATIONS			
1. Clinical Neurology	155	13,614,000	23.4
2. Clinical Ophthalmology	50	2,385,000	4.1
3. Clinical Otology	35	1,047,000	1.8
4. Clinical Other	20	989,000	1.7
 <u>ALL CLINICAL INVESTIGATIONS</u>	 <u>260</u>	 <u>18,035,000</u>	 <u>31.0</u>
F. PSYCHOLOGICAL SCIENCES			
1. Psychology	70	2,094,000	3.6
2. Psychiatry	0	0	0.0
 <u>ALL PSYCHOLOGICAL SCIENCES</u>	 <u>70</u>	 <u>2,094,000</u>	 <u>3.6</u>

TYPE OF CLASSIFICATION

	<u>No.</u>	<u>Amount</u>	<u>% of \$</u>
<u>All Classifications</u>	<u>1615</u>	<u>\$58,178,000</u>	<u>100</u>
G. OTHER			
1. Epidemiology	5	407,000	0.7
2. Conferences	15	241,000	0.4
3. Publications	3	73,000	0.1
4. Consultations	7	233,000	0.4
5. Instrumentation	40	1,745,000	3.0
6. Other	5	35,000	0.06
<u>ALL OTHER</u>	<u>75</u>	<u>2,734,000</u>	<u>4.7</u>

Exhibit 2

PROGRAM PROJECTS - CLINICAL CENTERS
 EXPENDITURES - FY 66
 BY DISORDER CATEGORY

	<u>No. of Grants</u>	<u>Amount</u>
<u>NEUROLOGICAL DISORDERS (TOTAL)</u>	22	\$5,221,426
General	5	1,012,763
Chronic Neurological Disorders of Aging (Parkinsonism)	2	703,831
Cerebrovascular Disorders	10	2,561,918
Epilepsy and Related Paroxysmal Disorders	2	309,556
Muscular & Neuromuscular Diseases	2	450,007
Nerve Injury and Regeneration	1	183,351
 <u>SENSORY & PERCEPTUAL DISORDERS (TOTAL)</u>	 14	 \$3,265,831
General	5	1,042,330
Disorders of Vision	5	1,319,199
Disorders of Hearing	4	904,302
 <u>OVERALL TOTAL</u>	 <u>36</u>	 <u>8,487,257</u>

Exhibit 3

DISTRIBUTION, BY SCIENTIFIC FIELDS, OF
TRAINING GRANTS SUPPORTED IN FY 66

	<u>No.</u> <u>Grants</u>	<u>Amount</u> <u>Awarded</u>
Neurology	75	\$ 4,970,000
Ophthalmology	51	2,793,000
Otolaryngology	44	2,327,000
Auditory Physiology	2	52,000
Communicative Disorders	4	308,000
Medical Audiology	3	200,000
Neuroanatomy	7	314,000
Neurochemistry	3	250,000
Neuropathology	16	641,000
Neuropharmacology	5	275,000
Neurophysiology	14	725,000
Neuroradiology	5	181,000
Neurosurgery	13	563,000
Ophthalmic Basic Science	3	180,000
Otolaryngology and Audiology	2	233,000
Otolaryngologic Pathology	1	116,000
Pediatric Neurology	12	522,000
Preventive Medicine	1	51,000
Sensory Physiology	4	200,000
Speech Pathology	3	218,000
Speech Pathology - Audiology	1	53,000
Vision Psychophysiology	1	10,000
Other	5	175,000
<u>Total</u>	<u>275</u>	<u>\$15,357,000</u>

Exhibit 4

DISTRIBUTION, BY SCIENTIFIC FIELDS,
OF SPECIAL FELLOWSHIPS AWARDED IN FY 66

<u>Fields</u>	<u>No.</u>	<u>Amount</u>
Audiology & Sp. pathology	15	\$ 40,900
Biochemistry	5	35,900
Biophysics	10	106,000
Cerebrovascular	4	120,000
Immunology	2	19,000
Neurology	25	238,200
Neuroanatomy	13	161,000
Neurochemistry	8	96,100
Neurocytology	2	23,300
Neuroendocrinology	4	45,800
Neuro-ophthalmology	6	51,000
Neuropathology	12	117,600
Neuropharmacology	6	65,000
Neurophysiology	25	260,500
Neuroradiology	17	165,600
Neurosurgery	5	60,900
Ophthalmology	27	269,000
Ophthalmic pathology	12	121,200
Otolaryngology	5	33,800
Pediatric neurology	26	250,500
Sensory physiology (Oto.)	4	43,700
Sensory physiology (Vision)	7	75,000
 <u>Total</u>	 <u>240</u>	 <u>\$2,400,000</u>

DISTRIBUTION, BY SCIENTIFIC FIELDS, OF FELLOWSHIPS AWARDED IN FY 66

Fields	Research Career Awards		Research Career Development Awards		Postdoctoral Fellowships	
	No.	Amount	No.	Amount	No.	Amount
Audiology	1	26,926	5	95,969	2	14,500
Biochemistry					6	47,000
Biophysics					3	20,200
Cerebrovascular	2	56,283	13	239,162	8	57,900
Genetics		-		-		-
Neuroanatomy	2	36,717	4	69,168	9	66,500
Neurochemistry	1	25,917	6	110,181	8	54,600
Neurocytology			2	32,941	2	14,000
Neuroendocrinology	1	19,070			2	14,000
Neurology	2	60,465	2	52,100	5	35,000
Neuropathology			3	66,798	11	77,000
Neuropharmacology	2	45,121	14	247,285	8	54,600
Neurophysiology			17	369,914	24	165,200
Neuroradiobiology			1	16,796		
Neurosurgery			1	17,034	10	76,300
Otolaryngology			1	16,430	4	30,200
Otoneurology			2	46,064		
Ophthalmic pathology			7	154,806	4	31,000
Ophthalmology			3	74,413	6	36,500
Pediatric Neurology			1	26,500		
Physiology psychology	1	23,436	4	94,000	2	12,500
Physiological optics			2	40,957	2	13,000
Sensory physiology						
(Vision)	1	25,900	4	66,740	6	39,000
(Oto.)	1	26,961	3	57,971	1	6,000
Speech & Hearing Mech.			1	16,599	1	8,000
Virology & Epidemiology			1	17,172		
<u>Totals</u>	<u>14</u>	<u>346,796</u>	<u>97</u>	<u>1,927,000</u>	<u>124</u>	<u>873,000</u>

Table of Organization

Intramural Research

National Institute of Neurological Diseases and Blindness
(Personnel on hand May 1966)

Office of Associate Director:

Acting Associate Director - Karl Frank, Ph.D.
Clinical Director - Maitland Baldwin, M.D., M.S., F.R.C.S.
Administrative Officer - Glenn E. Hammond
Mathematician - Rosalind B. Marimont
Administrative Officer - Nick Kastelan, Jr.
Visiting Scientist - Edward A. Carmichael, M.B., C.B.
Surgeon - Robert E. Burke, M.D.
Surgeon - Robert J. Ruben, M.D.
Scientist - Donald R. Humphrey, Ph.D.
Surgeon - Gerald N. Gold, M.D.
Photographer - Clarence Kuritzky
Photographer - Wesley Pearson, Jr.
Special Assistant - Nancy F. Nusbaum
Secretary - Maxine O. Reynolds
Secretary - Margret L. Shipley
Clerk (Typing) - Doris R. Perry
Travel Clerk - Ida M. Isaacson
Photographer - Wallace J. Strange
Biological Laboratory Technician - George R. Duvall
Clerk-Stenographer - George Ann M. Johnson
Clerk-Stenographer - Virginia L. Gray
Budget Clerk - May I. Ferrari
Biological Laboratory Technician - Adrian P. Loftis
EEG Technician - Clyde E. Harris
Clerk-Stenographer - Rose L. Goldstein
Messenger - Frederick L. Brownholtz

Section on Technical Development:

Electronic Engineer - Jerome A. Rosenthal
Computer Programmer - William H. Sheriff, Jr.

Medical Neurology Branch:

Chief - W. King Engel, M.D.
Medical Officer - Jerome S. Resnick, M.D.
Surgeon - Arthur H. Wolintz, M.D.
Surgeon - Mahlon R. Barlow, M.D.
Surgeon - Allan C. Stam, Jr., M.D.
Surgeon - Dale E. McFarlin, M.D.
SA Surgeon - Arthur S. Grove, Jr., M.D.
Surgeon - Donald Mauser, M.D.
Surgeon - Jon D. Dorman, M.D.
Surgeon - Gerald S. Lazarus, M.D.
Medical Technologist - Guy G. Cunningham
Biologist - W. Frederick K. Seymour
Medical Technologist - Thomas F. Summers, Jr.
Biological Laboratory Technician - Donald W. Bishop
Clerk-Dictating Machine Transcriber - Patricia D. Williams
Clerk-Dictating Machine Transcriber - Lorraine A. Semer
Clerk-Dictating Machine Transcriber - Gertrude E. Wright
Laboratory Worker - Matthew P. Meadows

Section on Biophysical Applications:

Surgeon - Wayne E. Tobin
Secretary - Vernita Bergmeyer
Biological Aid - Dennis W. Cahill

Section on Neuroradiology:

Research Biologist - Giovanni Di Chiro, M.D.
Secretary - Carolyn E. Kline

Section on Clinical Applied Pharmacology:

Supervisory Research Pharmacologist - Richard L. Irwin, Ph.D.
Research Physiologist - Jay B. Wells, Ph.D.
Secretary (Dictating Mach. Trans.) - Emma P. Dick

Surgical Neurology Branch:

Associate Neurosurgeon - John M. Van Buren, M.D., M.Sc.
Medical Officer - Choh-Lub Li, M.D., M.S.
Medical Officer - Ayub Khan Ommaya, F.R.C.S.
Surgeon - Donlin M. Long, M.D.
Surgeon - Howard A. Richter, M.D.
Surgeon - Philip Yarnell, M.D.
Surgeon - Eugene Flamm, M.D.
Surgeon - George Ojemann, M.D.
Biologist - Rosemary C. Borke
Research Biologist - Fonda L.A. Ghiardi
Biologist - Levon O. Parker

Surgical Neurology Branch (contd.)

Clerk-Dictating Machine Transcriber - Helen M. Andregg
Clerk-Stenographer - Gertrude C. Grant
Secretary - Eleanor Lynn Schultz
Clerk-Stenographer - Michele F. Yaffe

Section on Primate Neurology:

Nurse Supervisor - Frances D.S. Lamberti
Assistant Nurse Supervisor - Mildred L. Haddox
Staff Nurse - Delta H. Trickett
Biological Laboratory Technician - Norman E. Mills
Staff Nurse - Barbara J. Loberg
Staff Nurse - Geraldine E. Fazioli
Clerk-Stenographer - Gertrude C. D'Addio
Clerk (Typing) - Celeste L. Sewell
Biological Laboratory Technician - Calvin S. Hawkins
Biological Aid - Leo Jacobs
Medical Aid - Morris M. Williams
Medical Aid - Charles E. Sartor
Medical Aid - John Tolliver, Jr.
Medical Aid - Howard F. Stewart, Jr.
Medical Aid - Octavie W. Jacobs

Section on Neuroanesthesiology:

Visiting Scientist - Tomio Ohta, M.D.
Biological Laboratory Technician - Wilbert F. Finney

Section on Clinical Psychology:

Supervisory Research Psychologist - Herbert C. Lansdell, Ph.D.
Research Psychologist - Paul Fedio, Jr., Ph.D.
Psychologist - Beverly Vest, Jr.
Audiologist - Jean S. Baker
Secretary - Lillian L. Wease

Section on Clinical Neuropathology:

Medical Officer - Igor Klatzo, M.D., M.Sc.
Staff Fellow - Lois E. Rasmussen, Ph.D.
Supervisory Medical Technician - Joseph T. Walker, Jr.
Biological Laboratory Technician - Ernestine G. Dye
Secretary - Helen L. Jacobs
Medical Technician - Thelma R. Fletcher
Physical Science Aid - Carol Peters

Section on Child Neurology:

Medical Officer - Anatole S. Dekaban, M.D.
Surgeon - Owen M. Rennert, M.D.
Chemist - Diane E. Mizel

Section on Child Neurology (contd.)

Medical Technologist - Rosemarie Thron
Secretary - Linda M. Ingram
Medical Technician - Jan K. Steusing

Electroencephalography Branch:

Chief - Cosimo Ajmone Marsan, M.D.
Medical Officer - Kristof Abraham, M.D.
Secretary (Dictating Machine Trans.) - Elizabeth A. Lozada
EEG Technician - Shirley Y Howard
EEG Technician - Ida Mae G. Hatter
Clerk-Stenographer - Janet L. Teti
Biological Aid - Diane J. Volz
Biological Aid - Myrtle A. Jones
Clerk-Typist - Barbara Gantz

Section on Clinical Neurophysiology:

Supervisory EEG Technician - Emily B. Smith
Biologist - Douglas M. Martin

Ophthalmology Branch:

Chief - Ludwig von Sallmann, M.D.
Associate Ophthalmologist - Vernon C. Wong, M.D.
Surgeon - Joseph Crawford, M.D.
Surgeon - William S. Gilbert, M.D.
Surgeon - Walter E. Morgan, III, M.D.
Surgeon - Robert H. Collier, M.D.
Secretary - Rita U. Durant
Clerk (Typing) - Harriett Murphy
Medical Aid - Gerald S. Hoover
Clerk-Stenographer - Dolores A. Foltz
Clerk-Stenographer - Elizabeth M. Randolph
Clerk-Typist - Ethel F. Butts

Section on Ophthalmology Physiology:

Head, Section on Ophthalmology Physiology - Michelangelo Fuortes, M.D.
Senior Surgeon - Peter Gouras, M.D.
Physicist - Ralph D. Gunkel, O.D.
Electronic Development Technician - James L. Jones
Medical Aid - Krista L. Mixon
Mathematical Technician - Naomi K. Boetker

Section on Cell Biology:

Research Chemist - Paul J. O'Brien, Ph.D.
Surgeon - Roger G. Mazlen, M.D.

Section on Cell Biology (contd.)

Chemist - Consuelo G. Muellenberg
Biologist - Rubye J. Williams

Section on Ophthalmology Chemistry:

Research Chemist - Marc S. Lewis, Ph.D.
Staff Fellow - Ralph H. Helmsen, Ph.D.

Section on Cytology and Histopathology:

Research Biologist - Arnaldo Lasansky, M.D.
Research Biologist - Bolivar J. Lloyd, Jr.
Chemist - Patricia A. Grimes
Medical Technician - Eleanor M. Collins
Biological Laboratory Technician - Julia Matthews
Biologist - Joseph H. Nash, Jr.
Biological Aid - Luther R. Dowell, Jr.

Section on Ophthalmology Pharmacology:

Supervisory Pharmacologist - Frank J. Macri, Ph.D.
Biological Laboratory Technician - Joseph G. Brown

Laboratory of Neuroanatomical Sciences:

Supervisory Research Biologist - Alfred J. Coulombre, Ph.D.
Photographer - Edwin H. Moodhe
Secretary - Ruth C. Jordan
Supervisory Biological Laboratory Tech. - Willie Perkins
Clerk (Typing) - Hilda M. Malcolm
Secretary - Marie Dickerson
Biological Laboratory Technician - Albert V. Cantu
Biological Laboratory Technician - Melvin H. Carroll

Section on Experimental Neurology:

Senior Surgeon - Lloyd Guth, M.D.
Staff Fellow - Herbert Yellin, Ph.D.
Biological Laboratory Technician - Janina Ziemnowicz
Biologist - William C. Brown
Biologist - Phyllis K. Watson

Section on Neurocytology:

Research Biologist - Keith C. Richardson, B.Sc., M.Sc.
Research Psychologist - Milton W. Brightman, Ph.D.
SA Surgeon - Nicholas Lenn, M.D.
Biological Laboratory Technician - Clara E. Mowry

Section on Functional Neuroanatomy:

Head, Section on Functional Neuroanatomy - Grant L. Rasmussen, Ph.D.
Research Psychologist - Robert H. Wurtz, Ph.D.
Medical Technologist - Edna P. McCrane
Biological Laboratory Technician - Frank D. Nolan
Biologist - Helen J. Osborne

Section on Experimental Embryology:

Surgeon - Edward R. Wolpow, M.D.
Surgeon - Paul N. Anderson, M.D.
Chemist - Jane L. Coulombre
Biologist - John L. Everly
Laboratory Worker - Kenneth M. Oglesbee

Laboratory of Biophysics:

Senior Research Biophysicist - Kenneth S. Cole, Ph.D., D.Sc.
Supervisory Research Physiologist - Robert E. Taylor, Ph.D.
Secretary - Grace Ream
Clerk-Stenographer - Marilyn K. Kaplan

Section on Instrumentation:

Electronic Engineer - Leonard Binstock
Electronic Development Technician - Herbert A. Walters

Section on Mathematical Biophysics:

Research Physicist - Richard Fitzhugh, Ph.D.
Research Physicist - Harold Lecar, Ph.D.

Section on Membrane Biophysics:

Surgeon - W. Knox Chandler, M.D.
Research Physicist - Gerald H. Ehrenstein, Ph.D.

Section on Cellular Biophysics:

Research Physiologist - Daniel L. Gilbert, Ph.D.
Surgeon - Irving M. Stillman, M.D., Ph.D.
Student Assistant - Z. Frank Wann

Laboratory of Neuropathology: (Section on Experimental Neuropathology)

Medical Officer - Jan Cammermeyer, M.D.
Biologist - Iris Mercado
Biological Laboratory Technician - Margaret G. Johnson
Editorial Assistant - Jane T. Phelps
Biological Aid - Sophia Grabinski

Laboratory of Neurochemistry:

Chief - Donald B. Tower, M.D., Ph.D.
Scientist - William L. Stahl, Ph.D.
Secretary - Vivian K. Arnold
Clerk (Stenography) - Sharon A. Ryan

Section on Amino Acid Chemistry:

Surgeon - Homer Knizely, Jr., M.D.
Chemist - Edmund L. Peters

Section on Physiology and Metabolism:

Research Chemist - Eberhard G. Trams, Ph.D.
Chemist - Carl J. Lauter

Section on Enzyme Chemistry:

Research Chemist - R. Wayne Albers, Ph.D.
Surgeon - George J. Siegel, M.D.
Chemist - George J. Koval
Physical Science Aid - Eunice L. Summers

Section on Lipid Chemistry:

Medical Officer - Roscoe O. Brady, Jr., M.D.
Research Chemist - Andrew E. Gal, Ph.D.
Research Chemist - Julian N. Kanfer, Ph.D.
Surgeon - John P. Kampine, M.D.
Chemist - Roy M. Bradley
Visiting Associate - Erik Martensson, M.D.
Chemist - Oscar N. Young
Biological Aid - Alexander Wheaton

Laboratory of Neurophysiology:

Medical Officer - Edward F. Evans, M.B., C.B., Ph.D.
Laboratory Worker Leader - Clifford A. Seay
Laboratory Worker - Harold E. Smith

Section on Spinal Cord:

Sr. Surgeon - Phillip G. Nelson, M.D., Ph.D.
Surgeon - Norman Robbins, M.D., Ph.D.
Medical Officer - Thomas G. Smith, Jr., M.D.
Staff Fellow - Barbara O. Alving, Ph.D.
Physicist - Margaret W. Chapman
Biological Laboratory Technician - William L. Beane
Secretary (Stenography) - Ramona C. Duvall

Laboratory of Molecular Biology:

Chief - Ernst Freese, Ph.D.
Research Chemist - Robert A. Lazzarini, Ph.D.
Research Biologist - Elisabeth G.M. Freese, Ph.D.
Surgeon - Michael M. Kaback, M.D.
SA Surgeon - Leo R. Berberich, M.D.
Research Chemist - Rudiger W. Schmitt, Ph.D.
Staff Fellow - Marvin Melzer, Ph.D.
Staff Fellow - Richard Wax, Ph.D.
Visiting Associate - Hans-Jurgen Rhaese, Ph.D.
Pharmacologist - Stuart L. Graham
Chemist - Stephen S. Sklarow
Secretary (Stenography) - Mary W. Taylor
Biologist - Cynthia L. McAlister
Clerk-Stenographer - Carole A. Bromley
Biologist - Edith M. Bellante
Biological Laboratory Aid - Blanche E. Lewis

Laboratory of Perinatal Physiology - Puerto Rico:

Medical Officer - Ronald E. Myers, M.D., Ph.D.
Research Chemist - Americo Rivera, Jr., Ph.D.
Veterinarian - Wendell Niemann, D.V.M.
Research Psychologist - Shun-ichi Yamaguchi, Ph.D.
Scientist - Sven O.E. Ebbesson, Ph.D.
Surgeon - Ronald A. Cyrulnik, M.D.
Administrative Officer - Elizabeth W. Snowden
Equipment Specialist - George M. Dold
Administrative Assistant - Orlando Nieves
Librarian - Jorge Rivera-Ruiz
Photographer - Pedro Sola-Amadeo
Biological Laboratory Technician - Michie A. Vane
Chemist - Carmen Luisa Freixas
Biologist - Carmen G. Ponce de Lugo
Biological Laboratory Technician - Maria L. Alvarado de Yamaguchi
Biologist - Rafael Rivera
Medical Technician - Raymond Vane
Biological Laboratory Technician - Luis C. Gonzales
Clerk (Typing) - Ruby C. Gittens
Clerk-Stenographer - Sara L. Mendez
Medical Aid - Calixto Jimenez-Lugo
Biologist - Maria A. Rivera de Rivera
Biologist - Luis R. del Rio
Supply Clerk - Carolos M. Rosario-Rivera
Biologist - Frank Resillez-Urioste
Biological Laboratory Technician - Carmelo Pagan-Blanchero
Secretary (Stenography) - Teresa A. del Rio
Clerk-Typist - Zara S. Powers
Biological Laboratory Aid - Luis Rivera-Baez
Clerk-Stenographer - Carmen O. DeGarcia

Laboratory of Perinatal Physiology - Puerto Rico (contd.)

Clerk-Stenographer - Mary Alice Mercado
Biological Aid - Gladys Diaz de Torres
Biological Aid - Maria de. Lopez
Physical Science Aid - Jose E. Oliver
Clerk-Stenographer - Paulina T. Papp
Photographer - Luis Villafane-Santiago
Biological Aid - Horacio Ramirez-Pastrana
Clerk-Typist - Luisa V. Sneed
Clerk-Stenographer - Estebania P. Perez
Guard - Manuel Solla-Hernandez
Messenger - Alfredo Perez
Facilities and Equipment Mechanic - Manuel Maldonado-Rosa
Animal Caretaker - Deomisiano Melendez
Carpenter's Helper - Avenancio Santiago-Monico
Animal Caretaker - Israel Arce-Adames
Animal Caretaker - Gregorio Figueroa
Laborer - Cayetano Guzman
Animal Caretaker - Demetrio Melendez
Animal Caretaker - Inocencio Robledo
Animal Caretaker - Policarpio Quinones
Animal Caretaker - Juan R. Encarnacion-Colon
Animal Caretaker - Confessor Martis
Animal Caretaker - Manuel Rosado-Rosario
Animal Caretaker - Emilio Tolentino
Laborer - Aristides Quinones
Animal Caretaker - Rafael Rivera-Correa
Laborer - Ismael Charriez-Ramos

Laboratory of Perinatal Physiology - Puerto Rico - Islands:

Supervisory Research Biologist - Carl Koford, Ph.D.
Research Biologist - John A. Morrison, Ph.D.
Research Biologist - Stephen Vessey, Ph.D.
Research Biologist - Halsey M. Marsden, Ph.D.
Construction and Maintenance Super. - Carlos R. Nagel
Clerk-Stenographer - Susan J. O'Higgins
Carpenter - Jacinto S. Rosado
Biological Laboratory Aid - Victor A. Bracero-Pagan
Carpenter Helper - Jose Angel Flores
Animal Caretaker - Angel Figueroa-Velez
Animal Caretaker - Israel Cordero
Animal Caretaker - Julio Ortiz
Laborer - Luis A. Santiago
Laborer - Francisco Santiago
Laborer - Jose Mario Ramos
Laborer - Hector Melendez
Construction & Maintenance Trainee - Trabert M. Felix, Jr.
Laborer - Luis I. Hernandez

ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR
OF THE
NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS
July 1, 1965 through June 30, 1966

The Intramural Program of the NINDB has consisted of four Branches (dealing with human patients) and six Laboratories for some years. Last year the Laboratory of Perinatal Physiology in Puerto Rico was transferred to the Intramural Program.

Personnel of the Intramural Program has increased from 285 to 303 as of June 1966. Of these 119 are professionals (GS-11 or higher or equivalent). Of these professionals 14 are leaving as of June 1966, but 22 new professionals are coming on duty July 1966. The average age of the professional staff has dropped this year from 37.2 to 37.1 years, suggesting a healthy influx of young staff members who bring recent education and fresh ideas to the Program. As of July 1966, 56 or 44% of the professional staff will be in limited tenure positions.

The Laboratories and Branches are supported not only by NIH-wide facilities but also by a number of service groups within the Intramural Program. An effective administrative staff provides for many of the personnel, supply, travel, budget, etc. needs of the Laboratories and Branches. Two animal rooms and a photographic Laboratory supply heavy demands and a mathematician in the Office of the Associate Director is available to all for advice and collaboration on appropriate research problems.

During the 12 months since July 1965 the Section on Technical Development continued to fulfill its traditional role of support of NIMH and NINDB research efforts. Continuing assistance was made available in shop help, special purpose instruments, and some modest instrumentation systems. The section continues to grow toward assistance in computer oriented research and digital instrumentation.

Use of the section's LINC computer has almost doubled since last year and has reached the saturation point --105 hours per week. The ability to take on new projects is now seriously limited by production runs on existing projects. Plans to relieve this pressure include:

- (1) Transfer where possible of existing production runs to CDPB, while limiting LINC functions to A to D conversion and on-line data editing.

- (2) Acquisition of additional computing equipment in fiscal 1967.
- (3) Help to laboratories toward setting up their own facilities where the workload justifies such a step.

The policy toward repair and maintenance of equipment has continued the same as in the previous year, with the section handling emergency problems but referring routine and long-term problems to the Instrument Branch. The electronic component stocking service continues with the occasional acquisition of government surplus components being used to supplement substantially the purchased stock.

Because of limitations in space, no significant expansion or change of plans is contemplated within the section until the move to new quarters is made sometime in fiscal 1968. At that time, it is planned to move the section to Building 36 while maintaining a small machine shop and electronic fabrication facility in Building 10. Staff will probably be expanded to include an additional technician for the Building 10 facility; and the additional space in Building 36 will probably permit the addition of a technician, a mechanical engineer, and a physicist to the staff.

Section personnel have contributed significantly to research projects in laboratories of the two Institutes. In all cases this work has been described in the reports of the laboratories involved.

Some progress has been made in recruiting for a permanent Scientific Director. It is hoped that by September 1966 the Acting Associate Director will be able to return to the Laboratory of Neurophysiology.

The number of research projects reported this year is 218, an increase of 26. Forty-nine projects were completed and/or terminated and 59 new projects were described. One-hundred and seven projects involve collaboration with other Laboratories and Branches within NINDB, within NIH and with outside organizations. Forty-two cross Institute lines and 49 show collaboration outside NIH.

In lieu of a recapitulation of scientific results reference is made to the summary reports by each Laboratory and Branch Chief.

Serial No. NDB(1) - 66/QAD 1310

1. Office of Associate Director
- 2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Mathematical study of lens shapes of chick embryos.

Previous Serial Number: None

Principal Investigator: Rosalind B. Marimont

Other Investigators: Edward Wolpov, M.D., and Alfred J. Coulombre, Ph. D.

Cooperating Units: Laboratory of Neuroanatomical Sciences, NINDB

Man Years:

Total: .1
Professional: .1
Other: 0

Project Description:

Objectives: To find an equation describing the profile of the chick embryo lens and thereby facilitate description and computation of various lens properties.

Methods Employed: Mathematical analysis.

Major Findings: All lens profiles studied are very accurately fitted by a member of the family of generalized ellipses.

$$\left(\frac{y}{a}\right)^k + \left(\frac{x}{b}\right)^k = 1$$

A value of $k=2$ (the true ellipse) fits most anterior profiles. The posterior profile has values of k from about 1.7 to 2, depending on age. A formula for volume of these figures for all values of k was found in terms of Gamma functions, and a chart plotted for easy evaluation of volumes. Volumes computed in this way agree within 1% with those done by numerical integration. Surfaces for k other than two are more difficult, and the integral may have to be evaluated numerically.

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

Having a mathematical expression for the lens profiles is a great advantage in both description and measurement. For example, any lens may be completely specified by 5 numbers - a , and the values of b and k for the anterior and posterior surfaces. The advantages of a set of 5 numbers over a photograph are obvious. Volumes and surfaces may be found very quickly, and therefore larger scale experiments may be planned. Any future studies of optical or mechanical properties will be greatly simplified by the use of the equations.

Proposed Course of Project: To complete calculation of a formula for the surface of the generalized ellipse for k not equal to 2, and to apply the results to other problems in the study of embryological development of the chick lens.

ANNUAL REPORT
July 1, 1965 through June 30, 1966
National Institute of Neurological Diseases and Blindness

The Clinical Director

Ophthalmology

From 1 July 1965 to 20 April 1966, 146 patients were admitted to the 13-West Nursing Unit accounting for 6,685 inpatient days. The outpatient census totalled 495 patients and 1,663 visits to the outpatient. Consultation requests from other Institutes totalled 1,279, exceeding by approximately 100 the figure reported for last year. The number of major operations rose from 26 to 63. Minor surgical interventions appeared to decrease because many of these are now performed in the treatment room.

Medical Neurology

For the clinical investigations, 233 patients were admitted for a total of 5,761 patient days and there were 1,290 outpatient visits. There were 300 muscle and brain biopsies obtained. The clinical neurologists responded to 384 consultation requests from other departments and performed the required myelograms, pneumoencephalograms and cerebral angiograms.

Surgical Neurology

During the period 16 April 1965 through 15 April 1966, 214 persons participated in the clinical investigations as inpatients totalling 7,047 patient days. 593 were examined as outpatients in a total of 754 visits. There were 154 major operative procedures, 15 minor surgical procedures and 129 physiological monitoring procedures in the new surgical suite.

Neurology Nursing Service

During the period 1 July 1965 to 1 May 1966, the Neurology Nursing Service provided 8,639 continuous nursing care hours and within the same period, 947-1/4 over-time hours. A staffing pattern analysis from May of 1965 through March of 1966 reflects a slight increase in vacancies as there were 7 in May of 1965 and there are now 12 in March of 1966. Despite this increase in vacancies, with a comparable decrease in the number of actual positions filled, there has been a steady increase in the number of nursing hours. It is noteworthy that from the patient census, the pediatric census alone shows an increase from 13.67 in July of 1965 to 21.49 in April of 1966.

In accordance with the Institute policy, the Clinical Director's report reflects only the patient care activities and not the program content. As before, it is a pleasure to thank the Director and staff of the Clinical Center without whose skilled support we could not continue to function.

ANNUAL REPORT

July 1, 1965 through June 30, 1966

MEDICAL NEUROLOGY BRANCH, IR

National Institute of Neurological Diseases and Blindness

W. King Engel, M.D.

Chief, Medical Neurology Branch

Clinical Investigation Program

Introduction: Our function is to apply the most promising basic research techniques to the clinical problems of the patients. The essentiality of an inter-related multi-dimensional attack on the chosen target diseases is to be emphasized. Added to the techniques of histochemistry and tissue culture have been biochemistry and immunology, but in quite modest forms due to limitation of space and personnel. The techniques of electron-microscopy and autoradiography were achieved only on a collaborative basis, due to acute lack of facilities for these important investigations. We are very appreciative of the collaboration received in these and other techniques. It is obvious that to have a balanced clinical investigative program, each of these six techniques must be provided for more adequately.

For the clinical investigations, 233 patients were admitted for a total of 5,761 patients days, and there were 1,290 out-patient visits. There were 300 muscle and brain biopsies obtained. The clinical neurologists carried a considerable service responsibility. They provided 384 consultations to other departments, and performed the indicated myelograms, pneumoencephalograms, and cerebral angiograms on those patients.

The two-year approved residency training program in clinical neurology has continued; medical students and residents from Howard University were taught clinical neurology weekly; and investigators and technicians came as guest workers to learn clinical research techniques in neurology and especially the application of enzyme histochemistry to human neuromuscular disease.

The collaborative research program in neuromuscular disease with the Department of Neurology, Warsaw Medical Academy has continued under the PL-480 program. During the past year, 20 of our 40 papers published (or in press) represented collaboration between the Medical Neurology Branch and other units.

Myopathies: A monograph on Current Concepts of Myopathies has been published, containing the research results and opinions of our group. Six additional chapters in different symposia and monographs described aspects of our histochemical techniques and detailed results in various neuromuscular diseases. In myotonic dystrophy the following have been delineated - temporomandibular joint dysfunction; virtually diagnostic specific atrophy of histochemical type I muscle fibers; and a unique hypercatabolism of serum gamma globulin (IgG) protein (with NCI). The cardiac lesion of this disease has been successfully treated by electro-cardioversion. Two other multisystem diseases with known protein defects, ataxia-telangiectasia (β 2A globulin deficiency) and acanthocytosis (β lipoprotein deficiency) were shown to have a myopathic component, increasing the number of myopathies associated with known metabolic defects. A new disease, late onset progressive rod myopathy, has been described. Similar rods were produced experimentally in tenotomized cat soleus. Contrary to other investigators, abnormalities of lactate dehydrogenase isoenzyme 5, myoglobin, and total body potassium (K^{40} method) were found to be not disease-specific in humans. In addition to biochemical changes, subtle histochemical abnormalities were found in muscle biopsies of clinically normal carriers of Duchenne dystrophy, but their interpretation must await histochemical studies of muscle of normal volunteers. Detailed correlation of electromyographic, repetitive stimulation, motor conduction velocity, and motor unit territory determinations with clinical and histochemical aspects of 500 completely studied patients having neuromuscular disease is in progress. Attempts are being made to obtain reproducible cultures of human muscle biopsies in vitro by maximow slide and diffusion-chamber techniques. In many cancer patients, generalized muscle weakness is a major cause of disability. Histochemically, we have shown carcinomatous (or cachectic) muscle atrophy preferentially to involve type II muscle fibers. The pathogenesis and prevention of type II fiber atrophy is being pursued with the hope of symptomatically benefiting patients weakened by cancer. In collagen-vascular disease (a major cause of strokes in young adults) blood vessels in muscle biopsies are being studied by histochemistry and electron-microscopy to analyse the generalized vascular abnormalities.

Episodic Weakness: Our new classification of the periodic paralyzes and non-dystrophic myotonias, based on the provocative and therapeutic effects of various ionic unbalancing tests, developed last year, has proved valuable in choosing the correct therapy for these patients, most of whom have responded quite well. Histochemical studies, which now make it doubtful that structural changes in the muscle fibers are responsible for the weakness in the initial part of the paralytic attack, are being paralleled by electron-microscopic studies. Refractory period

measurements of single muscle fibers and muscle fiber conduction velocity are being done between and during attacks in these patients, and we hope will soon be combined with forearm perfusion studies. In a patient with succinylcholine-induced paralysis, the low serum cholinesterase was found by electrophoresis to be associated with a newly recognized selective absence of the fastest two cholinesterase bands, and new pharmacologically atypical patterns of cholinesterase were described in her serum and the serum of her relatives.

Myasthenia Gravis: Histochemistry showed that every muscle biopsy from 45 myasthenia gravis patients was abnormal, with either denervation or preferential atrophy of type II fibers present in all. Histochemistry also demonstrated a new aspect of lymphorrhages, namely that each was around one or more abnormal muscle fibers. A detailed combined immunofluorescent and histochemical study showed that the muscle-binding factor (described by Strauss) was not bound to the neuromuscular junctions, contrary to previous assumptions of others. In collaboration with Strauss and colleagues (NCI), a combined clinical (including prostigmine and curare tests) and immunofluorescent study showed that 24% of patients with thymoma but without associated myasthenia gravis had the muscle-binding factor in their serum. A new toxic effect of colistin methanesulfonate (Coly-Mycin) consisting of neuromuscular blockade at therapeutic blood levels of the drug has been described in a patient with Sjogren's syndrome.

Amyotrophic Lateral Sclerosis (ALS) and Other Diseases Affecting the Lower Motor Neuron: In the clinical and pathologic spectrum of familial anterior horn cell disease, it has been demonstrated that within individual families infantile spinal muscular atrophy blends imperceptibly with the juvenile proximal form. In ALS, primary or associated biochemical and immunologic abnormalities are being sought. Distant cancer has been found in a few patients, but its possible pathogenic role remains unknown. Immunologic abnormalities of the serum could not be found by gel diffusion or passive cutaneous anaphylaxis. With a tissue culture screening system, no toxic or infectious agents in serum and spinal fluid were demonstrated. An intravenous form of the arginine tolerance test was developed and appears to be more useful than the oral test - its results in ALS are under study. With Dr. Fullmer (NIDR), abnormal collagenase activity has been demonstrated in skin from ALS patients and a few other neuromuscular diseases which, though probably abnormal, is not disease-specific. Electron-microscopic search of brain tissue for viral particles is in progress, and search for a transmissible agent is underway (these same studies are also being done in sub-acute inclusion body encephalitis) with NINDB-CFR and NCI). Contrasting with the assumptions of others, the carbohydrate intolerance found in about 25% of ALS patients is not disease-

specific, having been found in about the same percent of patients with myotonic dystrophy, progressive muscular dystrophy, and chronic peripheral neuropathy. More detailed studies of pancreatic function are underway to further characterize these changes. The apparatus we have designed and built for quantitating muscle strength has been shown to give reproducible results in normal controls and in patients. It serves as one aspect of evaluating ALS patients who are participating in our double-blind placebo-controlled therapeutic trials. As yet no drug tested has been found of therapeutic value. RNA metabolism has been studied with its tritiated specific precursor, uridine. The autoradiographic pattern of appearance in the nucleus and cytoplasm of motor neurons has been established in rabbits. The first pilot application of this technique to human neurologic disease has indicated a normal pattern in one case of infantile spinal muscular atrophy; the patterns obtained in ALS patients are being evaluated. A histochemical study of denervated and tenotomized cat muscles has shown a number of unexpected changes and emphasized the difficulties in relating experimental animal conditions to human neuromuscular diseases. Produced for the first time in animals were target fibers, rods, type I fiber atrophy, and type II fiber atrophy. Three new histochemical changes were described in patients - "type grouping", empirically considered a sign of chronic denervation; presence of target fibers to be nearly always in type I fibers; and occurrence of central cores to be exclusively in type I fibers, which reemphasized our earlier suggestion that central core disease may be a neurogenic disorder instead of a myopathy.

In ataxia-telangiectasia the following have been demonstrated - an unusual type of diabetes mellitus with marked hyperinsulinism; unexpected presence of immunoglobulin A in bone marrow and parotid cells; a profound defect of IgA synthesis and in 2 of 5 patients concomitant IgA hypercatabolism (with NCI); and impaired in vitro lymphocyte transformation (with NCI).

The first two patients having a new familial syndrome of recurrent peripheral neuropathy with α -lipoprotein deficiency (Tangier disease) have been discovered (with NHI). A new syndrome of familial hypertrophic interstitial neuropathy and unique cataracts has been described. Vincristine, an anti-metabolite, has been shown in patients to have a preferential effect on the fusimotor system and thus is being tried as an anti-spasticity or anti-rigidity agent (with NCI).

Methodology: The role and mechanisms of substantive tetrazolium compounds and of phenazine methosulfate in the false localization of histochemical reactions has been described. A new type of EDTA-activated myofibrillar ATPase activity in type I fibers has been found histochemically. A method for seeking the location of the pharmacologic effect (myoclonic jerks) of

d-tubocurarine perfused in cat ventricles has been achieved by use of the radioactive compound, and the penetration of the various nuclear masses has been studied. The rapid monitoring of tissue being processed for electron-microscopy by simultaneous histochemistry has been developed and proved to save considerable professional time.

Neuroradiology Section

Radiographic Diagnosis: Selective arteriography in patients with spinal cord arteriovenous aneurysms has allowed the demonstration of arteries feeding the malformation in five cases. This in turn has permitted surgical ligation of the main feeders in four paraplegic patients with resulting improvement in all of them. An Atlas of Pathologic Pneumoencephalographic Anatomy is now in press. The "empty sella" is a condition in which the normal intrasellar subarachnoidal space fills a good part of the sella turcica. These sellae, generally borderline large or frankly large, contain a large amount of cerebrospinal fluid but normal or smaller than normal pituitary glands. The knowledge of the large empty sella is important for: a) differential diagnosis from intrasellar tumors; b) explanation of certain hypopituitary syndromes and certain types of spontaneous cerebrospinal fluid rhinorrhea; and c) to avoid mistakes when contemplating transphenoidal (surgical and 90γ) or external radiation pituitary treatment. Further experience has been gained with the useful refinement of pneumoencephalography, "axial transverse encephalography". This is now an established routine diagnostic procedure. A cooperative project is now underway on the growth hormone effects in dwarfs. Repeated sella turcica measurements are being taken in the patients so treated. The reevaluation by computer techniques of the angiographic patterns of superficial cerebral veins in the two hemispheres in a large group of patients has been continued. As another aspect of studying cerebrovascular disease, the prognostic significance of parasellar carotid artery calcifications will be evaluated at the end of 1966, 10 years after the beginning of the study of the particular group of patients used for this project.

Radiation Dosimetry: The thermoluminescent LiF crystals have proven disappointing for evaluating secondary radiation from irradiated residual x-ray opaque material in the spinal canal. New attempts are being made with the recently introduced LiF-Teflon dosimeters.

Isotopic Diagnosis: The clinical comparison of isotopes for brain scanning has been expanded to now include RISA, ^{99m}Tc pertechnetate, RIAF (radio-iodinated antifibrinogen) and ^{197}Hg Neohydrin. The points which have been established with the

multiple isotope-multiple scan technique are: a) most space-occupying intracranial lesions can be diagnosed by several isotopes, but in some cases a diagnosis can be reached only or much better with one tracer; b) RISA has a "relative" specificity for metastatic lesions; c) ^{197}Hg Neohydrin has a "relative" specificity for glial tumors; d) RIAF may have specificity for sarcomas and certain large clots; and e) $^{99\text{m}}\text{Tc}$ pertechnetate is probably most useful as a quick screening tracer and it can be most effectively employed with an Anger-type camera. In over one year of extensive testing, the Tetrascanner has proven itself to be a very useful piece of equipment, combining the high resolution of the rectilinear scanners with a speed approaching that possible with stationary detecting machines (cameras). The Tetrascanner is the best available device for rapid high resolution three-dimensional brain scanning. The isotopic scanning of cerebrospinal fluid shunts is now an established procedure. Extensive additional experience has been gathered with the techniques of isotope-ventriculography and isotope-cisternography. These now may be considered as routine diagnostic procedures. The Rous sarcoma virus brain tumors in dogs, first induced in collaboration with Dr. Rabotti (NCI), are now being used by other investigators. Salivary gland scanning with $^{99\text{m}}\text{Tc}$ pertechnetate has been shown to be a useful diagnostic test for detecting the presence of abnormalities in the area of the salivary glands and for differentiating among salivary lesions (tumors, inflammatory processes, and primary and post-radiation atrophy). Preparation of the monograph Brain Scanning has continued.

Neuropharmacology Section

During the past year we have been able to clarify the ionic events which lead to contracture of slow muscle. Myogenic contracture can be initiated in slow muscle by the withdrawal of external Ca^{++} . Present evidence indicates that loss of membrane Ca^{++} is the initial ionic event, followed by increased permeability to Na^+ which leads to internal sodium accumulation. Contracture and an inflow of Na^{++} occur concurrently. Smooth muscle develops Ca^{++} deprivation contractures without depolarization. We have been able to develop muscle models relating ionic movement to tension development without consideration of depolarization. Further experimental verification of such models is needed. The present studies have furnished evidence that (1) Ca^{++} has a dual role in the function of slow skeletal muscle; (2) there exists a Na^+ current mechanism different from the one in fast muscle or nerves which is responsible for the action potential because a) it occurs in slow muscle that is not capable of a propagated potential and, b) it is not affected by procaine which inactivates the Na^+ current responsible for the action potential; (3) there is a K^+ requirement for complete relaxation in slow muscle. This requirement indicates that part of the active transport of Na^+ in slow muscle is K^+ dependent.

The studies with fast twitch-type rat skeletal muscle have related to the mechanical properties of both the series elastic elements and the contractile components. Load extension characteristics of the elastic elements permitted calculation of kinetic and potential energy during isometric contraction. The experiments do not support the prevailing concept of a passive undamped series elasticity in fast muscle. A study has been made in the rate of the elastic and contractile properties of a rapidly (anterior tibial) and a slowly (soleus) contracting fast muscle after immobilization by joint fixation. Disuse resulted in changes in the rate of energy expenditure which produced a significant increase in the intrinsic speed of the slowly contracting muscles. The elastic properties of the more slowly contracting soleus were changed by immobilization so that the elasticity more nearly resembled that of the more rapidly contracting anterior tibial muscle.

As part of a PL-480 project, a serpentarium has been established at the Physiology Department of Ein Shams University, Cairo, Egypt, to maintain adequate sources of snake venom. A polyvalent antivenom from immune horses has been prepared for human use. The venom of *Cerastes Cerastes* has been found to block nerve conduction and is not reversed by KCl or physostigmine; it has no direct effect on muscle. Walterinessia Aegyptia venom produces hypoglycemia apparently by stimulating insulin secretion from the islet β -cells.

Serial No. NDB(I)-62 MN/OC 915(c)

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Histochemistry Applied to the Study of
Neurologic Disease

Previous Serial Number: Same

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

Other Investigators: Michael H. Brooke, M.D.
Genevieve Drews, M.D.
George A. Karpati, M.D.

Cooperating Units: None

Man Years

Total: 2.5

Professional: 2.5

Other:

Project Description:

Objectives: To study the cellular and subcellular localization of a variety of histochemical reactions in normal human skeletal muscle and neurons and to see how they are altered in neurologic diseases. To develop new histochemical techniques and to analyse mechanisms of the reactions.

Methods Employed: Histochemical techniques for a number of reactions are being done on muscle and nerve tissue - they have been outlined in previous reports. Recently added ones include reactions for acid phosphatase (a lysosomal enzyme), esterase, neutral lipid, and EDTA-activated myofibrillar ATPase. The last originated in this laboratory.

Patient Material: Medical Neurology Branch patients as well as consultation patients and patient material submitted by outside physicians.

Major Findings: Nitro-blue tetrazolium (NBT) is the most commonly used indicator of dehydrogenase activity. However, it has been found to have a heretofore unrecognized selective

binding to lipoprotein membranes (mitochondria and sarcoplasmic reticulum triads) and thereby exert both false negative and false positive localization artifacts that are generally not taken into account.

Phenazine methosulfate (PMS), a commonly used facilitator of electron-transport, has been found to exert a selective "shunt" action by-passing NBT in certain types of muscle fibers and therefore cause false negative localization of activity. Further, this phenazine shunt action is dependent on the relative concentrations of the reagents used, in, for example, the lactate dehydrogenase and DPN-linked alpha-glycerophosphate dehydrogenase reactions. The presence of this shunt invalidates certain attempts at histochemical quantitation when PMS & NBT are used together.

Subcellular localization of histochemical reactions has been further detailed.

Reviews of our total experience in the histochemistry of muscle biopsies in neuromuscular disease has been published, by request, in seven monographs and symposia. The details contained therein are too numerous to be summarized here. Histochemical analyses of groups of patients with specific diseases are discussed under those projects (myopathies 1034, ALS 1039, episodic weakness 1189, and myasthenia gravis 1190).

A new enzyme - EDTA-activated myofibrillar ATPase - has been discovered histochemically and its localization studied.

Certain abnormalities in various neuromuscular diseases have been found to show preferential involvement of one histochemical fiber type (see Projects 1034, 1039, 1190).

Three new sorts of muscle fiber abnormality has been described:

- a) Type I fiber atrophy,
- b) Type II fiber atrophy, and
- c) non-specific fiber atrophy (of both I and II fibers).

The occurrence of these has been described (see Projects 1034, 1139 and 1190). Only with enzyme histochemistry can these different forms of atrophy be distinguished.

Neuron studies - see ALS Project (1039).

The development and determinants of muscle differentiation into two histochemical fiber types are being studied under normal and experimentally altered circumstances in the experimental animal.

Significance to Bio-Medical Research and the Program of the Institute: Histochemistry of skeletal muscle in neuromuscular disease is still in its infancy, and the present work represents the initial cataloging of the various abnormalities. In such unexplored territory it is not surprising that virtually every finding is new.

Of particular interest are the findings of an apparently selective involvement of one histochemical type of muscle fiber, suggesting that the particular characteristics of metabolism of that type of muscle fiber are more susceptible to a given disease process. This permits us to ask why they are more susceptible and conversely, what makes the others more resistant?

Neuron studies - see ALS Project (1039).

Proposed Course of Project: The detailed results of histochemical and cytochemical evaluation of muscle biopsies from about 1600 patients, averaging 10 different histochemical reactions each, are being compiled into an Atlas of Muscle Histochemistry, due to be completed in about a year.

Additional reactions are being added to the battery of techniques applied to each biopsy.

The basic mechanisms and false localizing factors of the reactions used are being analysed.

Further correlations between light-microscopic cytochemistry and electron-microscopy will be made in muscle and neuron biopsies in human neurologic disease.

We are also beginning to study biopsies from a large number of normal persons. This is necessary because our techniques of cytochemistry and electron-microscopy are showing very subtle changes, which may or may not be found in otherwise normal persons. The diagnostic importance of knowing what is "normal" cannot be underestimated.

Honors and Awards: None

Publications: Brooke, M.H., and Engel, W.K.: The selective binding of nitro blue tetrazolium within striated muscle fibers. Neurology 1966. In Press.

Brooke, M.H., and Engel, W.K.: The use of phenazine methosulfate in enzyme histochemistry of human muscle biopsies. Neurology 1966. In Press.

Serial No. NDB(I)-62 MN/OC 917(c)

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

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Biochemistry Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Genevieve Drews, M.D.
Richard J. Kossmann, M.D.
Irwin Brody, M.D.
Jerome S. Resnick, M.D.

Other Investigators: Thomas Waldmann, M.D.
Raymond D. Wochner, M.D.

Cooperating Units: Metabolism Branch, NCI

Man Years:

Total:	2.9
Professional:	1.9
Other:	1.0

Project Description:

Objectives: To seek and analyze biochemical abnormalities of neurologic disease.

Methods Employed: Serum and muscle biopsy extracts have been studied with a variety of techniques:

- a. creatine phosphokinase (CPK) isozymes - acrylamid gel electrophoresis, preferential inhibitors, histochemical reaction mix.
- b. esterase isozymes - starch gel electrophoresis, "selective" inhibitors, histochemical reaction mix.
- c. total body potassium - whole body counter of natural isotope K^{40} .

- d. other techniques set up - phosphorylase, glycogen, amylo 1,4 → 1,6 transglucosidase, lactate, pyruvate, arginine.
- e. turnover of various serum proteins by following the fate of isotopically labelled pure fractions of serum proteins from normal and abnormal persons.

Patient Material: Patients with neuromuscular disease.

Major Findings: Four isozymes of CPK have been identified on acrylamid gel electrophoresis and their patterns in various neuromuscular diseases are being evaluated.

The two isozymes of serum cholinesterase moving fastest toward the anode were demonstrated for the first time to be selectively absent in a patient with succinylcholine sensitivity. (She and members of her family also had qualitatively abnormal serum cholinesterase by pharmacologic testing - see Project 1194.

Total body K^{40} appears to be correlated with muscle mass and not with specific diseases. Carriers of muscular dystrophy did not have an abnormally low total body K^{40} , in contrast with reports of others.

Hypercatabolism, with normal synthetic rates of γ -globulin was found in patients with myotonic dystrophy but not in other dystrophies or in ALS. This hypercatabolism did not affect albumin, or macroglobulin, and may be related especially to the L-chain of γ -globulin.

Significance to Bio-Medical Research and the Program of the Institute: Elucidation of biochemical abnormalities, particularly in the realm of proteins, is vital to the understanding of neurologic diseases and in seeking means of therapy.

Proposed Course of Project: The above techniques will continue to be applied. Tolerance tests - namely pyruvate, and arginine - said to be abnormal in amyotrophic lateral sclerosis (ALS) - are being applied to ALS patients and various control patients (see ALS Project 1039). Further studies of serum and tissue protein metabolism are underway in various neurologic diseases.

Lack of adequate space and a biochemist seriously impair this project.

Honors and Awards: None

- Publications: Brody, I.A., Resnick, J.S., and Engel, W.K.:
Detection of atypical serum cholinesterase by
electrophoresis. Arch. Neurol. 13: 126-129,
Aug. 1965.
- Kossmann, R.J., Peterson, D.C., and Andrews, H.L.:
Studies in neuromuscular disease. I. Total body
potassium in muscular dystrophy and related
diseases. Neurology 15: 855-865, Sep. 1965.
- Wochner, R.D., Drews, G., Strober, W., and Wald-
mann, T.A.: Accelerated breakdown of immuno-
globulin G (IgG) in myotonic dystrophy: A
hereditary error of immunoglobulin catabolism.
J. Clin. Invest. 45: 321-329, Mar. 1966.

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Myopathies

Previous Serial Number: Same

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

Other Investigators: Jerome S. Resnick, M.D.
Michael H. Brooke, M.D.

Cooperating Units: None

Man Years:

Total: 1.8
Professional: 1.8
Other:

Project Description:

Objectives: To analyse patients with all sorts of myopathies in order to test a newly developed classification. To more fully elaborate the various clinical, histochemical, biochemical, and ultrastructural abnormalities of these patients. To treat by various methods to learn which are most effective within each disease category.

Patient Material: Medical Neurology Branch patients.

Major Findings: A new classification of the myopathies has been developed and is proving to be most useful for investigative purposes - one of its most important features is that it does not force one to make a specific diagnosis when evidence for such is incomplete.

The symposium on Current Concepts of Myopathies, containing the research results and opinions of our group, has been published as a separate monograph. Seven additional chapters in different symposia and monographs, by invitation, described aspects of our histochemical techniques and detailed results in various neuromuscular diseases. The histochemical findings are

too numerous to be summarized, but certain highlights related to myopathies may be mentioned (see also ALS Project 1039):

- (a) Selective atrophy of type II fibers (described from this Branch) occurs in cachexia (carcinomatous and other), 50% of myasthenia gravis patients without clinical cachexia, subacute inclusion body encephalitis, and probably is the histochemical aspect of corticosteroid atrophy. We have recently produced it in denervated cat gastrocnemius.
- (b) In adults, selective atrophy of type I fibers has been described and is virtually specific for myotonic dystrophy; it has been produced in tentomized cat gastrocnemius.
- (c) Certain parasites (of the sarcosporidia or toxoplasma group) within muscle fibers show a distinct preference for type II muscle fibers in the "normal" cat, suggesting a more favorable milieu within those fibers for the parasites.
- (d) Mitochondrial aggregates, with the same histochemical characteristics as originally described from this Branch (restricted to type II fibers and containing a high concentration of mitochondria but lacking succinate dehydrogenase and menadione-mediated α -glycerophosphate dehydrogenase) have now been found in a few additional patients who differed clinically from the original ones. This change continues to be very consistent but is not disease-specific.
- (e) Rather than the simplicity, the multiplicity of muscle fiber reactions in human neuromuscular disease has been emphasized by classifying histochemical and electron-microscopic changes on the basis of changes in ultrastructural elements of the fibers.
- (f) The difficulty of dividing the world of muscle abnormalities simply into myopathies and denervation atrophies and, in certain patients (or diseases), of determining the exact type of etiology has been emphasized, as exemplified by central core disease (perhaps a neurogenic rather than a myopathic disease) myasthenia gravis (perhaps a denervation disease), myotonic dystrophy (perhaps having a neurogenic aspect) and juvenile proximal spinal muscular atrophy (perhaps having a significant myopathic component). It was suggested that the idea that "myopathies" and "neurogenic" diseases are totally different from each other ought to be discarded.

- (g) An attempt was made to correlate in a semiquantitative fashion the histochemical and morphologic findings in six diseases using only the most typical cases of chronic familial peripheral neuropathy, amyotrophic lateral sclerosis, myotonic dystrophy, Duchenne dystrophy, facio-scapulo-humeral dystrophy, and limb girdle dystrophy.

In myotonic dystrophy the following have been delineated - temporomandibular joint dysfunction; virtually diagnostic specific atrophy of histochemical type I muscle fibers; and a unique hypercatabolism of serum gamma globulin (IgG) protein (with NCI) - see Clinical Biochemistry Project 917. The cardiac lesion of this disease has been successfully treated for the first time by electro-cardioversion.

Two other multisystem diseases with known protein defects, ataxia-telangiectasia (β 2A globulin deficiency) and acanthocytosis (β lipoprotein deficiency) were shown to have a myopathic component, increasing the number of myopathies associated with known metabolic defects.

A new disease, late onset progressive rod myopathy, has been described (see Electron-Microscopy Project 1192). Similar rods were produced experimentally in tenotomized cat soleus.

Contrary to other investigators, abnormalities of lactate dehydrogenase isoenzyme 5, myoglobin, and total body potassium (K^{40} method) were found to be not disease-specific in humans.

In addition to biochemical changes, subtle histochemical abnormalities were found in muscle biopsies of clinically normal carriers of Duchenne dystrophy, but their interpretation must await histochemical studies of muscle of normal volunteers.

Detailed correlation of electromyographic, repetitive stimulation, motor conduction velocity, and motor unit territory determinations with clinical and histochemical aspects of 500 completely studied patients having neuromuscular disease is in progress.

In many cancer patients, generalized muscle weakness is a major cause of disability. Histochemically, we have shown carcinomatous (or cachectic) muscle atrophy preferentially to involve type II muscle fibers. The pathogenesis and especially the prevention of type II fiber atrophy is being pursued with the hope of symptomatically benefiting patients weakened by cancer.

In collagen-vascular disease, which is a major cause of strokes in young adults as well as of disabling muscle disease, blood vessels in muscle biopsies are being studied by histochemistry and electron-microscopy to analyse the generalized vascular abnormalities in such patients.

Therapeutic agents have been used, with good results, in the inflammatory myopathies. However, in some cases the greatest benefit was obtained by reducing corticosteroid medication the patient was taking; presumably the drugs were producing a toxic effect in such patients. Clinical judgment indicates whether a drug is to be increased or decreased.

Clinical biochemistry, tissue culture, histochemistry, and electron-microscopy of myopathic biopsies - see other project reports.

Significance to Bio-Medical Research and the Program of the Institute: The clinical studies related to therapy will help plan a better treatment program for patients with myopathy. The new classification aids in the investigation and treatment of these patients. The biochemical, histochemical, tissue culture and electron-microscopic studies are providing more detailed information on the abnormalities occurring in the myopathies - information that is hoped to provide a basis for finding ways of treating and preventing these diseases.

Proposed Course of Project: The studies underway are part of a long-term project which will continue for several years. Electron-microscopic, biochemical, and immunologic studies will be intensified. An Atlas of Histochemistry of Muscle is being written summarizing our experience in more than 1600 patients and various experimental animals - with clinical correlations.

Honors and Awards: Nine of the following papers written were by invitation.

Publications: Brooke, M.H.: The histological reaction of muscle to disease. In Briskey, E.J. (Ed.): Physiology and Biochemistry of Muscle as a Food. Madison, Wis., University of Wisconsin Press, 1966. In Press.

Brooke, M.H., and Engel, W.K.: The histologic diagnosis of neuromuscular diseases: A review of 79 biopsies. Arch. Phys. Med. 47: 99-121, Mar. 1966.

Engel, W.K.: Histochemistry of neuromuscular disease - Significance of muscle fiber types. Neuromuscular Diseases in Proceedings of the VIII International Congress of Neurology, Vienna, Austria, 1965. Excerpta Med. (Amsterdam) II: 67-101, 1965.

Engel, W.K.: The multiplicity of pathologic reactions of human skeletal muscles. In Proceedings of the Vth International Congress of Neuropathology, Zurich, Switzerland, 1965. Excerpta Med. (Amsterdam) 1966. In Press.

Engel, W.K., and Brooke, M.H.: Histochemistry of the myotonic disorders. In Kuhn, E. (Ed.): Symposium Muskeldystrophie, Myotonie, Myasthenia Gravis. Berlin, Springer-Verlag, 1966. In Press.

Engel, W.K., and Brooke, M.H.: Muscle biopsy as a clinical diagnostic aid. In Fields, W.S. (Ed.): Recent Advances in Neurological Diagnostic Methods. Springfield, Ill., Charles C Thomas, 1966. In Press.

Engel, W.K., Brooke, M.H., and Nelson, P.G.: Histochemical studies of denervated or tenotomized cat muscle. Illustrating difficulties in relating experimental animal conditions to human neuromuscular diseases. Ann. N.Y. Acad. Sci. 1966. In Press.

Engel, W.K., McFarlin, D.E., Drews, G., and Wochner, R.D.: Protein abnormalities in neuromuscular diseases--Part 1. J.A.M.A. 195: 754-760, Feb. 1966.

Engel, W.K., McFarlin, D.E., Drews, G., and Wochner, R.D.: Protein abnormalities in neuromuscular diseases--Part 2. J.A.M.A. 195: 837-842, Mar. 1966.

Gold, G.N.: Temporomandibular joint dysfunction in myotonic dystrophy. Neurology 16: 212-216, Feb. 1966.

Serial No. NDB(I)-63 MN/OC 1037(c)

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Microbiology Applied to the Study of
Neurologic Disease

Previous Serial Number: Same

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

Other Investigators: Gerald N. Gold, M.D.

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	.1
Other:	1.0

Project Description:

Objectives: To study the growth characteristics, histochemical reactivities, immunologic properties, and electron-microscopic details of skeletal muscle and motor neurons grown in tissue culture. The muscle is either chick embryo or normal or abnormal biopsied human muscle.

Methods Employed: Standard tissue culture techniques are used as a basis, modified as necessary to obtain good growth from human skeletal muscle. The techniques applied to the material grown are described in other reports. Tissue culture screening for abnormality in serum and spinal fluid of ALS patients is described in Project 1039.

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

Major Findings: Histochemical and cytochemical studies of newly grown chick embryo skeletal muscle fibers show that they contain abundant amounts of the following materials: phosphorylase, glycogen, myosin ATPase, cytochrome oxidase, succinate dehydrogenase, lactate dehydrogenase, menadione-mediated

alpha-glycerophosphate dehydrogenase, and DPNH and TPNH diaphorases. The subcellular localization of the colored end-product of these reactions is like that in biopsied chick muscle, indicating a high degree of muscle fiber differentiation in vitro even though nerve supply is not present.

Preliminary studies show the same histochemical techniques are also applicable to spinal cord motor neurons grown in vitro.

Moderate growth of biopsied normal and abnormal human biopsies has been achieved, but is still less than the chick embryo muscle growth. In addition to maximow slides and roller tubes, diffusion chambers have recently been used.

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 abnormal muscle will be used to seek disturbed growth patterns and, more particularly, to determine ways of improving deficient growth. Agents found to improve deficient growth in vitro will then be tested for their usefulness in the patient to promote growth of abnormal muscle or to retard its degeneration. As a foundation for understanding phenomena in human muscle, chick embryo material has been studied. Study of motor neurons grown in vitro is part of a multilateral approach to diseases which preferentially involve human motor neurons.

Proposed Course of Project: Further studies of normal and abnormal human muscle in vitro will be done. Definitive studies, starting with histochemistry, will be applied to the cultured motor neurons. Lack of space will seriously curtail this project until the new building opens.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-63 MN/OC 1039(c)

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Amyotrophic Lateral Sclerosis (ALS) and
Other Lower Motor Neuron Diseases

Previous Serial Number: Same

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

Other Investigators: Gerald N. Gold, M.D.
Jon Dorman, M.D.
Gerald Lazarus, M.D.
Arthur Wolintz, M.D.
Wayne Tobin, M.D.
Robert I. Levy, M.D.
Harold M. Fullmer, M.D.
S. Gerald Sandler, M.D.

Cooperating Units: Laboratory of Metabolism, NHI
Laboratory of Histology and Pathology, NIDR
Medicine Branch, NCI

Man Years:

Total: 4.0
Professional: 4.0
Other:

Project Description:

Objectives: To find a means of arresting ALS and other diseases affecting the lower motor neuron.

Methods Employed: Double blind 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.

Pyruvate and arginine, oral and I.V. tolerance tests.

Carbohydrate metabolism studies - glucose, tolbutamide, and insulin tolerance tests.

Lipoprotein studies (with Dr. Levy, NHI).

Analysis of brain biopsies by histochemistry, electron-microscopy, and autoradiography, as described in other project reports, as well as by cholinesterase assay and terminal bouton staining.

Monkey spinal cord and motor cortex obtained by biopsy are being studied histochemically for comparison with human disease material.

Biochemical assays of serum fatty acids, of turnover of serum protein fractions, and of factors related to vitamin B₁₂ metabolism.

Search for virus by electron-microscopy, and by inoculation of tissue cultures and animals.

Growth of animal motor neurons in tissue culture to study their characteristics in vitro.

Patient Material: Medical Neurology Branch patients.

Major Findings: Because it is somewhat new to apply a variety of well-controlled basic science methods to ALS, the techniques developed for use in patient material are, in a sense, results. Definitive results of the various techniques will not be available for a year or two. A few preliminary items can be recorded.

Among the first 130 ALS patients, 10% were found to have an associated neoplasm, but additional patients are not showing this high incidence. The possible pathogenic role is being explored.

About 25% of the ALS patients have "diabetic" glucose tolerance tests. For comparison, our patients with other neuromuscular diseases were studied, with a similar incidence of glucose intolerance being found in muscular dystrophy, late-onset myopathy, chronic peripheral neuropathy, and myotonic dystrophy, identifying this as an abnormality not disease specific, in contrast with assumptions of others. The precise forms of carbohydrate intolerance is being elucidated in these conditions.

An I.V. arginine tolerance test has been developed and appears to be more reliable than the oral one. Its slope is being evaluated in ALS and other neuromuscular diseases.

In the clinical and pathologic spectrum of familial anterior horn cell disease, it has been demonstrated that within individual families infantile spinal muscular atrophy blends imperceptibly with the juvenile proximal form.

In ALS, immunologic abnormalities of the serum could not be found by gel diffusion or passive cutaneous anaphylaxis.

With a tissue culture screening system, no toxic or infectious agents in serum and spinal fluid were demonstrated in ALS.

With Dr. Fullmer (NIDR) abnormal collagenase activity has been demonstrated in skin from ALS patients and a few other neuromuscular diseases which, though probably abnormal, is not disease-specific.

Neither morphologic (E.M.) nor transmission evidence for an infectious agent has yet been found in our ALS material. Similar studies are also being done with brain from patients with sub-acute-inclusion body encephalitis and certain progressive dementias.

The apparatus we have designed and built for quantitating muscle strength has been shown to give reproducible results in normal controls and patients. It serves as one aspect of evaluating ALS patients who are participating in our therapeutic trials.

Repeated quantitative evaluation of muscle function, as well as of the history, neurologic exam, and total body potassium (K^{40} method) is giving a more detailed account of the natural course of ALS.

Several therapeutic regimens have been tried in double-blind placebo-controlled trials. None has proved consistently beneficial in ALS.

Turnover of several serum proteins have been found normal in ALS patients, as have blood fatty acid levels, including phytanic acid.

Analyses of brain biopsies by histochemistry and electron microscopy are in progress.

A histochemical study of denervated and tenotomized cat muscles has shown a number of unexpected changes and emphasized the difficulties in relating experimental animal conditions to human neuromuscular diseases. Produced for the first time in animals were: a) target fibers; b) rods; c) type I fiber atrophy; d) type II fiber atrophy. (see Project on Myopathies 1034)

Three new histochemical changes were described in patients: a) "type-grouping", empirically considered a sign of chronic denervation; b) presence of target fibers to be nearly always in type I fibers; and c) occurrence of central cores to be exclusively in type I fibers, which re-emphasized our earlier

suggestion that central core disease may be a neurogenic disorder rather than a myopathy.

The first two patients having a new familial syndrome of recurrent peripheral neuropathy with α -lipoprotein deficiency (Tangier disease) have been discovered (with NHI).

A new syndrome of familial hypertrophic interstitial neuropathy and unique cataracts has been described.

Primary (familial amyloidosis has been more clearly delineated as one cause of the "chronic familial peripheral neuropathy syndrome" and as a newly recognized cause of Sjogren's syndrome (keratoconjunctivitis sicca).

Vincristine, an anti-metabolite has been shown in patients to have a preferential effect on the fusimotor system and thus is being tried as an antispasticity or antirigidity agent (with NCI).

Significance to Bio-Medical Research and the Program of the Institute: New details are being accumulated on the morphologic and biochemical substrates of ALS and other diseases of the lower motor neuron. In familial neuropathy, a poorly defined group of disorders, a specific metabolic defect, α -lipoprotein deficiency, has been identified for the first time. It is hoped that identification of metabolic, immune, or infectious etiologies will lead to a means of treating and preventing these disorders affecting the lower motor neuron.

Honors and Awards: None

Publications: Gold, G., and Hogenhuis, L.A.H.: Hypertrophic interstitial neuropathy and cataracts. Neurology 16: 323, Mar. 1966.

Fullmer, H.M., Lazarus, G.S., Gibson, W.A., Stam, A.C., and Link, C.: Collagenolytic activity of the skin associated with neuromuscular diseases including amyotrophic lateral sclerosis. Lancet 1966. In Press.

Norris, F.H., and Engel, W.K.: Carcinomatous amyotrophic lateral sclerosis. In Brain, Russell, and Norris, Forbes (Eds.): The Remote Effects of Cancer on the Nervous System. New York, Grune & Stratton, 1965, pp. 24-34.

Tobin, W., and Sandler, S.G.: The effects of Vincristine on the Achilles reflex, H-reflex and conduction velocity of the posterior tibial nerve. Nature. In Press.

Tobin, W., and Sandler, S.G.: Vincristine-induced neuropathy: localization of the early neuro-physiologic lesion. Proc. of the Am. Ass. for Cancer Res. 1966. In Press.

Serial No. NDB(I)-65 MN/OC 1189(c)

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Episodic Weakness

Previous Serial Number: Same

Principal Investigators: Jerome S. Resnick, M.D.
W. King Engel, M.D.

Other Investigators: Wayne Tobin, M.D.

Cooperating Units: None

Man Years:

Total: 0.4
Professional: 0.4
Other:

Project Description:

Objectives: To define more clearly those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis. Attention is to be directed toward those conditions in which evidence suggests that the main site of intermittent dysfunction is somewhere within the following portions of the neuromuscular system: motor end plate region; sarcolemmal membrane; and sarcolemmal-sarcoplasmic reticulum-myofibrillar complex (excitation contraction coupling mechanism). Further experimentation is to be done with agents which are either provocative or therapeutic with respect to periodic paralysis syndromes with a view to obtaining more information regarding pertinent metabolic pathways.

Methods Employed: The techniques of clinical investigation (including electromyography and clinical biochemistry), muscle biopsy with samples for histochemical analysis, electron-microscopy, and biochemical study are delineated in other projects. Provocative loading tests and therapeutic trials to raise or lower potassium or sodium are used. Refractory period measurements of single muscle fibers and muscle fiber conductive velocity are being done between and during attacks of episodic weakness.

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 disturbance; thyrotoxic periodic paralysis; paramyotonia congenita; or myotonia congenita. (Patients with myasthenia gravis are part of another project.)

Major Findings: Initial success has been obtained in preventing hypokalemic attacks by parenteral thiamine, confirming a report by Japanese workers. Other patients will be studied in a double-blind fashion to further confirm this treatment. The mechanism of thiamine's benefit will be studied by exploration of carbohydrate and pyruvate metabolism.

A new classification of periodic paralyzes and myotonia congenita has been postulated on the basis of ionic unbalancing tests and is being tested for validity. It is designed to give insight into the pathogenesis of the attacks and the means of treating them. These tests have shown the identity of certain entities such as hyperkalemic periodic paralysis (adynamia episodica hereditaria) and paramyotonia congenita, considered by some to be different disorders. Myotonia has been demonstrated to be a new clinical aspect to hypokalemic periodic paralysis previously stated to be pathognomic of hyperkalemic periodic paralysis.

Muscle biopsies between and during attacks are being studied by histochemical, biochemical, and electron-microscopic techniques, as described under these projects.

A thyrotoxic form of hypokalemic paralysis has been cured, and the metabolic changes investigated, including aldosterone metabolism.

Significance to Bio-Medical Research and the Program of the Institute: The existence of patients with an endogenous metabolic defect which results in intermittent interruption of the normal neuromuscular excitatory pathway provides a unique model, study of which should provide clearer insight into the roles that carbohydrate metabolism and electrolytes play in muscular contraction. More effective therapeutic and prophylactic agents might thereby result for patients so afflicted. The identification of myotonia in hypokalemic periodic paralysis indicates it could be disastrous to further lower therapeutically the serum K simply because a periodic paralysis was associated with myotonia.

Proposed Course of Project: To complete studies mentioned under "major findings", with more emphasis on electron-microscopic analysis of the muscle, and radioisotopic studies of ions and metabolites affecting the attacks of weakness.

Forearm perfusion studies to challenge and analyse the muscle more directly are planned.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 MN/OC 1190(c)

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

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Myasthenia Gravis

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Dale E. McFarlin, M.D.

Other Investigators: Mahlon H. Barlow, M.D.
Gerald N. Gold, M.D.
Wayne E. Tobin, M.D.
Arthur J. L. Strauss, M.D.
John Vasko, M.D.

Cooperating Units: Laboratory of Immunology, NIAID
Surgery Branch, NHI

Man Years:

Total: 0.6
Professional: 0.6
Other:

Project Description:

Objectives: To apply histochemical, pharmacologic, electrophysiologic and immunologic techniques to investigate the pathogenesis of myasthenia gravis. Immunologic studies were included because: 1. Immunologic abnormalities have recently been demonstrated in the serum of myasthenic patients. 2. Thymic pathology in patients with myasthenia is well known and it is now felt that this gland has major immunologic functions.

Methods Employed: Sera from patients with myasthenia gravis were studied by a variety of immunologic techniques including agar diffusion, immunoelectrophoresis, latex agglutination, and immunofluorescence. Specimens of muscle, and in some cases thymus and thyroid, were studied using immunofluorescent, histochemical and routine histological techniques. The abnormalities were evaluated before and after thymectomy. Clinical studies, including prostigmine and curare challenging tests were done on myasthenics, on a group of patients with thymoma but without myasthenia, and on a patient with colistin methosulfonate-produced neuromuscular blockade.

Patient Material: Sera, muscle, thymus and other tissue were obtained from Medical Neurology Branch patients.

Major Findings: Histochemically, five types of abnormalities were seen in myasthenic muscle (35 patients): (a) Denervation atrophy - 63%, (b) Type II fiber atrophy - 50%, (c) Mild fiber necrosis - 30%, (d) Lymphorrhages - 23%, (e) Severe fiber necrosis - 7%. Every specimen was abnormal, and each had at least one of the first two changes.

By studying the same sections by immunofluorescent and then by histochemical cholinesterase techniques, it was shown that the serum muscle-binding factor of myasthenia gravis patients does not bind to the neuromuscular junction, contrary to assumptions of others.

In collaboration with Strauss and colleagues (NCI), a combined clinical (including prostigmine and curare tests) and immunofluorescent study showed that 24% of patients with thymoma but without associated myasthenia gravis had the muscle-binding factor in their serum.

Colistin methanesulfonate (Coly-Mycin) has been found to have a new toxic effect in a patient with Sjogren's syndrome--namely, neuromuscular blockade at therapeutic levels of the drug. The blockade is "competitive".

Significance to Bio-Medical Research and the Program of the Institute: (1) Techniques previously employed have revealed muscle pathology in less than fifty per cent of specimens from myasthenic patients; however, in the present study both a higher incidence and a wider variety of abnormalities were demonstrated, made possible by enzyme histochemical techniques. Characterization of the muscle pathology in myasthenia gravis may provide a clue as to the pathogenesis(es) of the neuromuscular junction defect. (2) Demonstration of non-reactivity between immunoglobulins from myasthenic patients and the neuromuscular junction is in contrast to the report of binding at "end plate-like" structures by one group of investigators who imply that a reaction between abnormal serum proteins and receptor substance is responsible for the pathophysiology of myasthenia gravis. The data obtained in this study suggest that relationship between immunological abnormalities and the physiological defect is by some means other than a direct binding at the end-plate.

Proposed Course of Project: The number of patients studied will be expanded and attention focused on post-thymectomy patients. A few cases of neonatal myasthenia will be studied, if available.

Techniques involving the study of cellular immunity are to be added, which should facilitate characterization of the thymic abnormality in myasthenia gravis.

Honors and Awards: None

Publications: Engel, W. K., and McFarlin, D.E.: Skeletal pathology in myasthenia gravis--histochemical findings. Ann. N.Y. Acad. Sci. 135: 68-78, Jan. 1966.

Gold, G., and Richardson, A.P.: An unusual case of neuromuscular blockage seen with therapeutic blood levels of colistin methanesulfonate (Coly-Mycin). Am. J. Med. 1966. In press.

McFarlin, D.E., Engel, W.K., and Strauss, A.J.L.: Does myasthenic serum bind to the neuromuscular junction? Ann. N.Y. Acad. Sci. 135: 656-663, Jan. 1966.

Strauss, A.J.L., VanderGeld, H.W.R., Smith, C.W., McFarlin, D.E., Barlow, M., and Cage, G.W.: Further studies on the specificity of the immune associations of myasthenia gravis and consideration of their possible pathogenetic implications. Ann. N.Y. Acad. Sci. 135: 557-579, Jan. 1966.

Serial No. NDB(I)-65 MN/OC 1191(c)

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Immunological Abnormalities of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: Dale E. McFarlin, M.D.
Mahlon H. Barlow, M.D.

Other Investigators: Thomas Waldmann, M.D.
Dean Wochner, M.D.
Warren Strober, M.D.
Jerome Block, M.D.
D.S. Schalck, M.D.

Cooperating Units: Metabolism Branch, NCI
Medicine Branch, NCI
University of Rochester, School of Medicine,
Rochester, N.Y.

Man Years:

Total:	1.3
Professional:	0.8
Other:	.5

Project Description:

Objectives: To apply immunological techniques to the study of diseases affecting the nervous system and neuromuscular system.

Methods Employed: Standard immunochemical techniques were employed. In addition, preliminary use of tissue culture techniques was begun. Fractional catabolic rates of serum proteins were studied in collaboration with NCI/ODIR. Immuno-logic assays of plasma insulin were done in collaboration with the University of Rochester School of Medicine.

Patient Material: Specimens of peripheral blood, bone marrow, muscle, peripheral nerve, thymus and other tissue were obtained from patients with neurological disease admitted to the Medical Neurology Branch.

Major Findings: A laboratory for immunological research was established. A bank of serum and various tissues was started.

In ataxia telangiectasia the following were found--

- (a) Profound defect of immunoglobulin A (IgA) synthesis, and in 2 of 5 patients a concomitant hyperkatabolism of IgA.
- (b) Unexpected presence of IgA in bone marrow and parotid cells.
- (c) An unusual type of diabetes mellitus with marked hyperinsulism.
- (d) Impaired in vitro lymphocyte transformation.

In familial amyloidosis no evidence of a serum protein abnormality or of plasma cell dysfunction was found.

Significance to Bio-Medical Research and the Program of the Institute: Elucidation of the defects of protein metabolism and hyperinsulism in ataxia telangiectasia may disclose the chemical abnormality responsible for impaired nervous system function in this disease, leading to a means of its correction. Absence of a disorder of plasma cell function in familial amyloidosis suggests that amyloid is produced by a different cell line. Clarification of this point would provide a basis of directing therapy aimed at inhibiting amyloid formation.

Proposed Course of Project: The present studies will be expanded. Specific metabolic and tissue culture studies are planned.

Honors and Awards: None

Publications: Barlow, M.H., McFarlin, D.E., Schalch, D.S.: An unusual type of diabetes mellitus with marked hyperinsulinism in patients with ataxia-telangiectasia. Clin. Res. 23: 530, Dec. 1965.

Block, J., Barlow, M.H., Oppenheim, J.: Impaired in vitro lymphocyte transformation in ataxia-telangiectasia. Brit. Med. Journ. 1966. In Press.

McFarlin, D.E., Strober, W., Wochner, R.D., and Waldmann, T.A.: Immunoglobulin A production in ataxia telangiectasia. Science 150: 1175-1177, Nov. 1965.

Serial No. NDB(I)-65 MN/OC 1191(c)

Strober, W., Wochner, R.D., Barlow, M.H., McFarlin, D.E., Waldmann, T.: Immunoglobulin metabolism in ataxia-telangiectasia. J. Clin. Invest. 1966.
In Press.

Serial No. NDB(I)-65 MN/OC 1192(c)

1. Medical Neurology Branch

2. Office of the Chief

3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Electron Microscopic Studies of Skeletal Muscle
and Neurons

Previous Serial Number: Same

Principal Investigators: Jerome S. Resnick, M.D.
W. King Engel, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.7
Professional:	0.7
Other:	1.0

Project Description:

Objectives: To study early fine cellular changes in 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 in certain diseases affecting neurons.

Methods Employed: A portion of biopsied muscle is tied to a glass rod to maintain resting length and immediately fixed in cold buffered osmium tetroxide for araldite embedding. Adjacent biopsy material is immediately frozen for cryostat sections and incubated in various histochemical media, as described in another project. Some stained cryostat sections are ready within an hour after the biopsy. These sections can be used as a guide to determine which of the biopsies contain light microscopic features of special interest and, in many instances, thereby serve as a guide for choosing material appropriate for electron microscopic study. Biopsy specimens of human brain tissue are fixed and embedded for electron-microscopy (EM), and are similarly controlled by simultaneous enzyme histochemistry.

Patient Material: Patients with various myopathies and neurogenic muscular weakness are the major sources of material. Congenital non-progressive neuromuscular dysfunction without evident pathologic changes on light microscopy and histochemistry (so-called benign congenital hypotonia) are also under study. When available, tissue from asymptomatic carriers of progressive genetic myopathies is taken for study. Brain biopsies are obtained from patients with amyotrophic lateral sclerosis and ones with certain forms of progressive dementia.

Major Findings: The EM proof of periodicity and Z-band origin of rods was obtained in a patient with late-onset rod myopathy, who is the first recognized example of this form of disease. Similar rods have been produced experimentally in the tenotomized cat soleus and their EM identity with those in human disease is being determined.

The minimal changes in muscular dystrophy carriers, changes in target fibers (of denervation), and in mitochondrial aggregates (in periodic paralysis), and in benign congenital hypotonia are being detailed by EM, as are three other new diseases.

The CNS changes in ALS, subacute inclusion body encephalitis, and certain progressive dementias are being detailed by EM, including the search for virons.

Significance to Bio-Medical Research and the Program of the Institute: Analysis of the changes in ultrastructure of the neuromuscular disorders is in its infancy. Identification of new forms of disease occur rapidly in this stage. However one must be careful not to name a condition on the basis of careful but severely limited EM or histochemical studies. Therefore, a broad experience with as many well-studied cases as possible is necessary, as is broad correlation of EM with histochemistry. It appears that certain morphologic changes can indicate suspected metabolic defects, as in contractile protein defects in rod myopathy. Experimental production of identical defects, e.g. rods, in animals if confirmed by EM, provide a new tool for analysing these changes. Similar expectations pertain to the other human neuromuscular disorders being studied, as well as the neuronal abnormalities. Regarding virons, morphologic identification of suspected particles would have to be confirmed by transmission.

Proposed Course of Project: Further studies that combine histochemical, immunologic, and autoradiographic techniques with electron-microscopy are being contemplated, so as to supplement the morphologic approach with pertinent studies of cellular

dynamics. The recent permission to buy our own microscope will greatly facilitate this work.

Honors and Awards: None

Publications: Engel, W.K., and Resnick, J.S.: Late-onset rod myopathy - A newly recognized, acquired and progressive disease. Neurology 16: 308-309, Mar. 1966.

Serial No. NDB(I)-65 MN/OC 1193(c)

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

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Radioautography Applied to the Study of
Neurologic Disease

Previous Serial Number: Same

Principal Investigators: Leon A.H. Hogenhuis, M.D.
W. King Engel, M.D.

Other Investigators: Stephen Spaulding

Cooperating Units: McGill University, Montreal, Canada

Man Years:

Total: 0.2
Professional: 0.2
Other:

Project Description:

Objectives: To apply radioautographic techniques with the use of purine, pyrimidine, amino acid and lipid precursors to the study of diseases affecting the nervous system.

Methods Employed: Radioautography was employed utilizing the excellent resolution afforded by the low energy β -radiation of tritiated compounds; the Kodak NTB 2 emulsion was used for processing the tissue with the dipping technique of Leblond and Kopperiwa.

Patient Material: Brain biopsy specimens from patients suffering from one of the clinical varieties of motor neuron disease (amyotrophic lateral sclerosis, bulbar paralysis, progressive muscular atrophy or infantile spinal muscular atrophy) were obtained from patients admitted to the Medical Neurology Branch with such motor neuron disorders. The central nervous system was exposed to a tritiated RNA precursor (uridine) by intracisternal injections of the compound prior to the brain biopsy.

Major Findings: An extensive study of the uptake pattern of the intracysternally administered labeled uridine in different parts of the CNS of a series of rabbits revealed a spatiotemporal optimum of one hour for uptake in the nucleus of large neurons and an optimum of two days for uptake in the cytoplasm of the large neurons. This technique was applied to humans after adjustments were calculated according to the observed kinetic pattern in the experimental animal. Preliminary results have confirmed the validity of these kinetics for the human. The technique was applied to one case of infantile spinal muscular atrophy, one adult case of familial amyotrophic lateral sclerosis with bulbar and supra bulbar signs and three adult cases of progressive muscular atrophy with bulbar and suprabulbar signs. Specimens from the ALS patients are in the process of being analysed for abnormalities in the uptake of the RNA precursor tritiated uridine. In the one patient with infantile spinal muscular atrophy, kinetics of uridine metabolism were not abnormal in medullary motor neurons.

Significance to Bio-Medical Research and the Program of the Institute: Abnormalities at the cellular and subcellular level can readily be studied with this autoradiographic technique and the site of the lesion in the affected structural elements of the central nervous system can be identified more specifically, especially in regard to RNA metabolism. This represents the first such study in ALS and perhaps in human neurologic disease.

Proposed Course of Project: Patients with this type of selective involvement of the large neurons will continue to be admitted for this study to participate in the research program outlined above. Clarification of the site of the lesion at cellular and subcellular level will induce further efforts to find a rationale for treatment of the still incurable, invariably fatal disorders, ALS and infantile spinal muscular atrophy.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-66 MN/OC 1311(c)

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

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Site of Action of Intra-Ventricular Tubocurarine

Previous Serial Number: None

Principal Investigator: E. Arnold Carmichael, C.B.E., D.Sc.,
M.B., F.R.C.P.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.5
Professional:	1.0
Others:	.5

Project Description:

Objectives: By perfusing the cerebral ventricles of anaesthetized cats with radio-active tubocurarine, it was hoped by subsequent autoradiography to demonstrate where the tubocurarine had penetrated the brain substance; by limiting the perfusions of the radio-active tubocurarine to one or other compartment of the ventricular system it was further hoped that the gray masses which have to be penetrated by the tubocurarine for the production of one or other of the signs, such as myoclonus, would be demonstrable. It was considered possible that the site of action of the tubocurarine at the cellular level might be determined, namely had it to enter the neuron or was it sufficient to "coat" the "cell body"?

Methods Employed: The accepted cerebral intraventricular perfusion techniques were used on cats anaesthetized with pentobarbitone or chloralose. Changes in the EEG, respiration, blood pressure and cardiac rate were continuously recorded and observations made on pupillary size, onset of salivation and myoclonus as well as other abnormal movements. Tubocurarine dimethyl chloride with a carbon-14 isotope or d-tubocurarine chloride which had been tritiated were used dissolved in artificial C.S.F. for perfusions. Dr. Trams kindly assayed and helped to

purify the various "fractions" following tritiation and these were tested by Dr. Irwin for their capacity as blocking agents. Dr. Isaacs kindly advised regarding autoradiographic techniques and Dr. Klatzo very kindly arranged for the freeze drying of tissue to be used after sectioning for dipping in photographic emulsion.

Major Findings: The tubocurarine with the carbon-14 molecule and that which had been tritiated gave at comparable duration of perfusion and in comparable dilutions similar changes in the EEG etc. as did d-tubocurarine chloride. The autoradiograms using carbon-14 showed that tubocurarine had to penetrate the periventricular masses for two to three millimeters before physical signs developed. In the region of the hippocampus, the penetration was such that the pyramidal and dentate layers of cells were readily recognized, suggesting that the "penetration" by the tubocurarine was "held up" at these cell layers. The nuclear masses of the anterior commissure appeared to be involved in myoclonus developed.

The autoradiographs with the tritiated tubocurarine are not yet extensive enough to allow definitive deductions. It looks as if this method may offer a means of demonstrating where the tubocurarine is in relation to the cell body--such as the neurons.

Significance to Bio-Medical Research and the Program of the Institute: A small piece of additional evidence indicating the penetration of brain substance by tubocurarine when introduced intraventricularly, thereby bypassing the blood-brain barrier.

Proposed Course of Project: This project will be terminated within the next few months.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-61 MN/NR 806(c)

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Axial Transverse Encephalography

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro

Other Investigators: None

Cooperating Units: Diagnostic X-Ray Department, Clinical Center,
NIH

Man Years

Total:	.1
Professional:	.1
Others:	0

Project Description:

Objectives: With the conventional pneumoencephalography we "see" the brain radiographically from side to side, from the front, or from the back. Axial Transverse Encephalography is a refinement of pneumoencephalography which allows the demonstration of the brain in a new plane, "as looking down from above".

Methods Employed: A special X-ray equipment (Massiot-Philips Radiotome), on which several original modifications have been made, is used. The patient sits on a stool, air is injected by lumbar puncture, and then radiograms are taken. The X-ray beam hits at a particular angle a pre-chosen area of the patient's head. By spinning simultaneously patient and X-ray film, only one thin horizontal layer (cut, tomogram) of the head structures is visualized. Parts above and below this layer are "blurred out" and will not appear in the radiogram. By cranking the stool, various levels of the head's structures may be demonstrated.

Significance to Bio-Medical Research and the Program of the Institute: With the adjunction of Axial Transverse Encephalography, the radiographic outlining of the brain and the other intracranial structures is for the first time obtained in a real three-dimensional fashion. The pneumoencephalographic localization and size--shape--extent appraisal of the space-occupying and other lesions is greatly enhanced.

Proposed Course of Project: This project has been completed.

Honors and Awards: None

Publications: Di Chiro, G. Axial Transverse Encephalography
with the Radiotome. Medica Mundi 10: 92-96, 1965.

Serial No. NDB(I)-62 MN/NR 922(c)

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: An Atlas of Pathologic Pneumoencephalographic Anatomy

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Mannie M. Schechter
Ingmar Wickbom

Other Investigators: None

Cooperating Units: Albert Einstein College of Medicine, Yeshiva University, New York, New York

Sahlgrenska Sjukhuset, Gothenburg, Sweden

Man Years

Total:	.6
Professional:	.6
Others:	0

Project Description:

Objectives: A reference textbook in which the great majority of pathologic pneumoencephalographic syndromes will be presented.

Methods Employed: Surgical and autopsy specimens are extensively used to illustrate and better explain the pneumoencephalographic findings. Each pneumoencephalogram is accompanied by an explanatory sketch.

Significance to Bio-Medical Research and the Program of the Institute: This will be an encyclopedic reference textbook of all the pneumoencephalographic syndromes.

Proposed Course of Project: This atlas is now in press and this project is closed.

Honors and Awards: None

Publications: Di Chiro, G. An Atlas of Pathologic Pneumoencephalographic Anatomy. Springfield, Ill., Charles C. Thomas (in press).

Serial No. NDB(I)-62 MN/NR 925(c)

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Small Sella Turcica

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Thomas Aceto, Jr.
Mary Parker
Alvin Hayles
Colin B. Holman

Other Investigators: None

Cooperating Units: The National Pituitary Agency, Baltimore,
Maryland

The Children's Hospital, Buffalo, New York

Washington University, St. Louis, Missouri

The Mayo Clinic, Rochester, Minnesota

Man Years:

Total:	.1
Professional:	.1
Others:	0

Project Description:

Objectives: An anatomo-radiographic study to evaluate the diagnostic significance, if any, of the small sella turcica.

Methods Employed: The size of the sella turcica has been studied volumetrically, with emphasis on the small sellas, according to the previously reported (Di Chiro) radiographic method. Limits have been established concerning minimum sella volumes in normal individuals of different ages. Particular attention has been devoted to the younger age group--3 to 16 years--where alterations of the normal physical growth may often be accompanied by abnormal sellar sizes. In this younger age group, autopsy studies were carried out in 22 cadavers to check the radiographic results.

Major Findings: Previous and new data under evaluation.

Significance to Bio-Medical Research and the Program of the Institute: The rarity of the small sella turcica in the average hospital population and in non-hypopituitary dwarfs and the frequency of this radiographic finding in hypopituitary dwarfs make this x-ray sign a valuable diagnostic element. The diagnosis of idiopathic hypopituitary dwarfism in pre-pubertal children is difficult and dependent to a large degree upon indirect chemical estimations of various trophic hormone deficiencies of the pituitary. The finding of a sella volume below the normal range for the patient's age should suggest and should be considered as confirmatory evidence of hypopituitarism in the doubtful cases.

Proposed Course of Project: A collaborative study on the effects of growth hormone in dwarfs is being carried out under the auspices of the National Pituitary Agency. The skull x-rays of the hypopituitary dwarfs included in this project - in whom, according to Di Chiro and Fisher findings, a small sella is frequently found - are being evaluated by Di Chiro. Emphasis is being placed upon the comparison of repeated sella turcica measurements taken during the follow-up of the patients treated with growth hormone.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-63 MN/NR 1047(c)

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Isotope-Ventriculography and Isotope-Cisternography

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro

Other Investigators: None

Cooperating Units: Radiation Safety Department, Clinical Center,
NIH

Radiopharmaceutical Service, Pharmacy
Department, Clinical Center, NIH

Man Years

Total:	.1
Professional:	.1
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.

Methods Employed: In earlier experiments the cerebrospinal fluid of each patient was tagged with ^{131}I and reinjected in the same patient: autologous ^{131}I ventriculography or cisternography. The reinjection of autologous proteins was considered, on a theoretical basis, safer than the injection of homologous albumin. However, we were haunted by the risk of contamination. For this reason, we shifted to the injection of commercial human radioiodinated serum albumin (RISA) already extensively used for isotope-myelography and other purposes. Fifty microcuries of RISA have been injected within the ventricular system of various patients. The injection was done in most cases at the same time

as the intraventricular injection of air. For RISA-cisternography, 100 microcuries of RISA diluted in 5-10 cc. of Elliot's "B" solution were injected by lumbar puncture. Immediate or one hour head scans were carried out in ventriculography and serial 1-48 hours (at various intervals according to the circumstances and the diagnostic problems) in cisternography.

Major Findings: Extensive additional experience has been gathered. The techniques of isotope-ventriculography and isotope-cisternography are now considered useful routine procedures by the members of the staff of the Neurosurgical Unit of the NIH.

Significance to Bio-Medical Research and the Program of the Institute: Legions of authors are studying this remarkable fluid (C.S.F.) which still remains uncomprehended since Cotugno first described it in 1764. Isotope-ventriculography and isotope-cisternography offer a straight-forward method for learning, in the living human, more about this elusive fluid.

Other conditions, apart from the ones already investigated, in which we expect isotope-ventriculography to be a helpful diagnostic tool are: 1) spontaneous ventriculostomy; 2) ventricular system--porencephalic cysts communication; 3) patency evaluation of neurosurgical shunts; and 4) para-normal cavities (cavum of septum pellucidum, cavum Vergae and cavum of velum interpositum).

Isotope-cisternography may, in addition to the conditions in which we have already tested it, be helpful in: 1) "internal" cerebrospinal fluid leaks; 2) leptomenigeal cysts; 3) meningocele; 4) basal and convexity arachnoiditis; 5) masses (tumoral and others) impinging on subarachnoid space; 6) subarachnoid bleedings; and 7) obstructions of draining dural sinuses and convexity veins.

Proposed Course of Project: With the help of the Tetrascanner and with the experience already acquired, the mapping and timing of the cerebrospinal fluid flow will be subjected to further detailed investigation. Particular emphasis will be put upon the pathological aspects.

Honors and Awards: None

Publications: None

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Prognostic Significance of Parasellar Carotid Calcifications

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Leslie Libow

Other Investigators: None

Cooperating Units: Section on Cerebral Metabolism, Laboratory of Clinical Science, National Institute of Mental Health, NIH

Man Years

Total:	0
Professional:	0
Others:	0

Project Description:

Objectives: There is a wide discrepancy of opinions on whether or not the carotid parasellar calcifications have any diagnostic significance. It is hoped that the present study, based on normal individuals in the older age group, will offer some clue about their diagnostic-prognostic meaning.

Methods Employed: Skull x-rays of a large group of healthy aged males which have been closely followed up since 1956 in another Institute (National Institute of Mental Health) are being reviewed. The clinical and other laboratory findings which are systematically and periodically obtained in these carefully followed-up senior citizens are correlated with the radiographic findings. Many of these patients have received skull x-rays twice with an interval of several years. This allows, in certain instances, an evaluation of the time of appearance and change in pattern of the carotid calcifications. Emphasis is being put on the cases which have died since 1956 and, of course, the cause of death is being investigated.

Major Findings: Our observations are still very preliminary. It would appear, however, that the carotid calcifications should be distinguished in various types: unilateral, bilateral, probably intimal, probably located within the media, fleck-like, cuff-like, and so on. The patients who have died since 1956 all presented, in the first baseline skull radiographic study, extensive, in most cases bilateral, carotid siphon calcifications.

Significance to Bio-Medical Research and the Program of the Institute: It is probable that with this method a relatively simple additional criterion for the prognostic appraisal of the possible life expectancy of the older people may be established.

Proposed Course of Project: The follow-up of the particular group of patients used for this project will be completed at the end of 1966 - ten years after the beginning of clinical evaluation. At that time it is hoped that the prognostic appraisal of the parasellar carotid calcifications will be established.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 MN/NR 1195(c)

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Selective Arteriography of Spinal Cord Arterio-
venous Aneurysms

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
John L. Doppman
Ayub K. Ommaya

Other Investigators: None

Cooperating Units: Diagnostic X-Ray Department, Clinical Center,
NIH

Surgical Neurology Branch, National Institute
of Neurological Diseases and Blindness, NIH

Man Years

Total:	.1
Professional:	.1
Others:	0

Project Description:

Objectives: Diagnosis of the nature of space-occupying lesions within the spinal canal is often difficult. In most instances with myelography, we are only able to put in evidence a total or partial subarachnoidal block. Myelograms, on the other hand, are seldom helpful in establishing the type of lesion which has caused the block. With recent advances in the field of arteriography - safer radiographic contrast media, better needles and better catheters, and the revival of interest in subtraction - we feel that a systematic angiographic evaluation of the intra-spinal mass lesions would be very worthwhile. Angiography is of particular importance, as has already been proven by French authors, in spinal cord vascular malformations. Significant information might, however, be obtained in other types of lesions, especially spinal cord tumors. It is conceivable that tumor stains, and pathologic vessels may be demonstrated in cases of spinal cord tumors in analogy to similar angiographic findings in brain tumors.

Methods Employed: Selective arteriograms with the use of modern catheter techniques are carried out in patients in whom intraspinal space-occupying lesions are suspected. The subtraction technique of Ziedses des Plantes is used to better visualize the injected vessels. Recently, however, we have put much less emphasis on subtraction because we are now convinced that the crucial aspect of this technique is the selectivity of the injection. In addition, surgical ligation of angiographically demonstrated feeding vessels of spinal cord arteriovenous aneurysms has been accomplished in four cases.

Major Findings: Angiographic visualization of feeding arteries of spinal cord arteriovenous aneurysms in five cases has represented the basis for a new type of surgical approach to this important group of lesions. The main feeding artery of these malformations has been ligated intradurally, after laminectomy, in four paraplegic patients. This surgery has been tolerated without complications and all four patients have already improved soon after surgery, one dramatically and three slightly. The improvement is continuing. Selective and midstream arteriography after surgery has shown in three out of four cases that, at least for the time being, the vascular malformation does not fill. Probably it has, at least partially, collapsed.

It should be noted that while a systematic angiographic study of the spinal cord arteriovenous aneurysms was originated by French authors, we have been the first ones to use selective arteriography, that is injection of dye directly within the main feeding segmental artery. This represents an important modification because with the so-called "midstream" aortic injection, demonstration of these lesions may be (and it has been in one case by us and in several cases by others) missed.

Attempts to visualize angiographically abnormal vessels in several cases of spinal gliomatous tumors have not proven successful.

Significance to Bio-Medical Research and the Program of the Institute: We have now a method which will allow us to treat rationally, and judging from our early successes, effectively, this important group of spinal cord lesions responsible for crippling hundreds of patients.

Proposed Course of Project: Long-term clinical and angiographic follow-up of patients operated upon. To increase our clinical-angiographic experience in additional patients. To carry out angiographies of spinal cord vessels in intact cadavers. Studies of spinal cord vascularization with intravascular injection of new types of plastics, followed by corrosion of the specimen (so that the vessels will stand out in all their detail) are also planned.

Honors and Awards: None

Publications: Doppman, J.L. and Di Chiro, G. Subtraction-Angiography of Spinal Cord Vascular Malformations: Report of a Case. J. Neurosurg. 23: 440-443, 1965.

Serial No. NDB(I)-65 MN/NR 1196(c)

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Clinical Comparison of Radioactive Tracers Used
for Brain Scanning

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Arthur S. Grove, Jr.

Other Investigators: William Ashburn
Irving Spar
William Bale

Cooperating Units: Department of Diagnostic Radiology, Clinical
Center, NIH

Radiation Safety Department, Clinical Center,
NIH

Radiopharmaceutical Service, Pharmacy
Department, Clinical Center, NIH

Department of Radiation Biology, University
of Rochester School of Medicine, Rochester,
New York

Man Years

Total: .4
Professional: .3
Other: .1

Project Description:

Objectives: A variety of isotopes are now being used for brain scanning. Multiple scans with multiple isotopes carried out in the same patients provide the best method for comparatively appraising the various scanning agents. Few studies of this type have been reported, and we hope that by scanning a large number of patients we can provide information about selective uptake of tracers which has been previously unavailable.

Methods Employed: The dose of ^{99m}Tc pertechnetate has been increased from one to two millicuries per patient. In addition to ^{131}I -RIHSA and technetium, we have resumed the use of ^{131}I -RIAF in some patients and have added ^{197}Hg Neohydrin to the isotopes which we are presently employing. More than 150 patients have been scanned using two, three or four of these tracers. Varying amounts of the different tracers are intravenously injected on separate days. Scans are carried out 18 or more hours after albumin and antifibrinogen injections and approximately one hour after Neohydrin and pertechnetate injections. The NIH "Tetra-scanner" is used for most of these studies.

Major Findings: a) Most space-occupying intracranial lesions can be diagnosed by several isotopes, but in some cases a diagnosis can be reached only or much better with one tracer; b) RIHSA has a "relative" specificity for metastatic lesions; c) Hg Neohydrin has a "relative" specificity for glial tumors; d) RIAF may have specificity for sarcomas and certain large clots; and e) ^{99m}Tc pertechnetate is probably most useful as a quick screening tracer and it can be most effectively employed with an Anger-type camera.

Significance to Bio-Medical Research and the Program of the Institute: This study represents a contribution to the problem of which radioactive tracer is better for brain scanning. In addition, with this study, some information will be obtained on the problem of selective uptake of specific radioactive drugs by the various types of tumors.

Proposed Course of Project: To gather and appraise further clinical material in which the multiple isotope-multiple scan technique may be used.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 MN/NR 1197(c)

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Radio-Isotope Brain Scanning for Evaluating the Function of Cerebrospinal Fluid Shunts

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Arthur S. Grove, Jr.

Other Investigators: None

Cooperating Units: Department of Neurosurgery, George Washington University Hospital, Washington, D.C.

Department of Neurosurgery, Walter Reed Medical Center, Washington, D.C.

Man Years

Total:	.2
Professional:	.2
Others:	0

Project Description:

Objectives: A wide variety of procedures have been used for shunting cerebrospinal fluid in the treatment of hydrocephalus and increased intracranial pressure. Following the establishment of a cerebrospinal fluid shunt, the problem frequently arises of whether the tube is patent and is functioning properly. Several tests are available to detect and to locate a block if it exists. Most of these tests, however, are clinically inadequate. The object of this study is to develop a new procedure for evaluating the function of cerebrospinal fluid shunts using radioactive isotopes and brain scanning.

Methods Employed: A small volume of either I¹³¹-labeled serum albumin or technetium 99m pertechnetate is injected into the shunt tubing using a small gauge needle, provided that the tubing is made of a suitable material. Soon after injection, four-view brain scans are carried out using our Tetrascanner [see Project NDB(I)-63 MN/NR 1047(c)]. The shunt tubing itself

is frequently outlined by this technique. If the shunt is patent and is functioning properly, the ventricles and cisterna magna are also visualized (in the case of ventriculo-cisternal shunts). In ventriculo-jugular shunts, patency of the distal end is proven by rapid uptake of technetium by the salivary glands, for which technetium pertechnetate has a marked affinity.

Major Findings: It has been shown that the function and patency of cerebrospinal fluid shunts can be evaluated by using radio-isotopes and brain scanning.

Significance to Bio-Medical Research and the Program of the Institute: This technique quickly and safely demonstrates the patency and evaluates the function of cerebrospinal fluid shunts. It is the first technique which permits the study of the gross morphology of these shunts and the communicating cerebrospinal fluid system.

Proposed Course of Project: This project has been concluded.

Honors and Awards: None

Publications: Di Chiro, G. and Grove, A.S. Evaluation of Surgical and Spontaneous Cerebrospinal Fluid Shunts by Isotope Scanning. J. Neurosurg. 24: 743-748, 1966

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Brain Scanning (Monograph)

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Arthur S. Grove, Jr.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	.1
Professional:	.1
Others:	0

Project Description:

Objectives: Continuing progress in radioisotope brain scanning is rapidly making this technique the most frequently used diagnostic tool for demonstrating intracranial lesions. A monograph embracing the various and newest aspects of brain scanning, with an evaluation of the many used and usable tracers, is lacking. The decision to prepare such a monograph was therefore reached.

Methods Employed: Critical bibliographic evaluation and appraisal of our own experience and material.

Major Findings: Organization of the monograph as a whole has been undertaken and a few chapters have been prepared.

Significance to Bio-Medical Research and the Program of the Institute: This monograph will be useful for students and physicians who require an introduction to the subject of brain scanning. In addition, it will be a comprehensive reference work for the investigators using neuroradiologic techniques involving radioisotopes.

Proposed Course of Project: The preparation of this monograph is being continued.

Honors and Awards: None

Publications: None

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Virus Induced Experimental Brain Tumors in Dogs

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Arthur S. Grove, Jr.
Giancarlo F. Rabotti

Other Investigators: Wirtley R. Anderson
Richard Sellers

Cooperating Units: Laboratory of Viral Carcinogenesis, National
Cancer Institute, NIH

Section on Primate Neurology, Surgical
Neurology Branch, NINDB, NIH

Man Years

Total:	.6
Professional:	.4
Others:	.2

Project Description:

Objectives: Reproducible experimental brain tumors with characteristics similar to those of spontaneous human tumors would be very useful in neurological research. Rous sarcoma virus (RSV) injected intracerebrally in small animals has produced tumors which resemble primary human gliomas and sarcomas. The aim of this project was to produce similar tumors in large animals (dogs), with which tracers used for brain scanning could be evaluated.

Methods Employed: Intracranial gliomas and sarcomas have been induced in dogs by intracerebral injections of RSV.

Major Findings: Primary brain tumors have been induced in large animals (dogs). Approximately 75% of the newborn dogs injected intracerebrally developed tumors with the Schmidt-Ruppin strain of RSV. Other strains of virus appear to be less virulent.

Significance to Bio-Medical Research and the Program of the Institute: Virus induced brain tumors in dogs will provide a biological model which should be useful for many types of neurological research. Through these experiments we hope to provide an objective technique for evaluating the usefulness of many brain scanning tracers which are now being used or new tracers which might otherwise not be tested.

Proposed Course of Project: Investigators in the National Cancer Institute are now planning to use similar tumors for a variety of experiments. We are contemplating a large scale evaluation of isotopic uptake by these experimental tumors.

Honors and Awards: None

Publications: Rabotti, G.F., Grove, Jr., A.S., Sellers, R.L. and Anderson, W.R. Induction of Multiple Brain Tumors (Gliomata and Leptomeningeal Sarcomata) in Dogs by Rous Sarcoma Virus. Nature 209: 884, February 26, 1966.

Serial No. NDB(I)-65 MN/NR 1200

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Secondary Radiation from Irradiated Residual
X-ray Opaque Material in the Spinal Canal

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Arthur S. Grove, Jr.

Other Investigators: Norman Modine
Emmett Murphy
David Janes
John Howley
Charles Sondhaus

Cooperating Units: Division of Radiological Health, Rockville,
Maryland

Radiation Safety Department, Isotope
Laboratory, NIH

Department of Radiology, Los Angeles County
General Hospital (Unit II), Los Angeles,
California

Man Years

Total:	.1
Professional:	.1
Others:	0

Project Description:

Objectives: Secondary radiation is known to be produced when any material is irradiated with X- or gamma rays. The nature of this secondary radiation is dependent upon the energy of the primary radiation and upon the atomic number of the material irradiated. Previous studies have suggested that beta rays are the most significant secondary radiations produced by irradiation of relatively high atomic number materials such as iodine.

Following myelography, x-ray opaque materials are frequently left behind in the subarachnoid space of the spinal canal. Adhesions

may be caused by surgery or by inflammatory response to the contrast materials. The radio opaque media frequently loculate within these adhesions. Therapeutic or repeated diagnostic x-ray studies in this area may produce secondary radiation which would penetrate that segment of the spinal cord lying beneath the contrast material. It is important that we study the nature and amount of this secondary radiation in order to prevent inadvertent damage to the underlying neural tissue.

Methods Employed: Lithium fluoride (LiF) thermoluminescent dosimeters have recently been introduced for measuring beta and gamma radiation. By irradiating these dosimeters with and without an adjacent layer of contrast media, the secondary radiation produced at the contrast media-dosimeter interface is studied.

Major Findings: Our early experiments using LiF crystals have been disappointing. We have not been able to obtain accurate reproducible results after irradiating these crystals.

Significance to Bio-Medical Research and the Program of the Institute: Since many patients with retained contrast media require radiation therapy and are exposed to the hazards of secondary radiation, these studies may bring about a new awareness of this problem to many physicians. We believe that the secondary radiation provided inadvertently may be significant and should be calculated for these patients.

Proposed Course of Project: The introduction of LiF-Teflon dosimeters gives us new hope that possibly we will be able to pursue this project with some success after all. Dr. Charles Sondhaus has shown great interest in this project of ours and in cooperation with him we hope to obtain in the coming years satisfactory results.

Honors and Awards: None

Publications: None

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A Repeating Surgical Monostapler

Previous Serial Number: Same

Principal Investigator: Arthur S. Grove, Jr.

Other Investigator: George Lawrence

Cooperating Units: Instrument Engineering and Development Branch,
Division of Research Services, NIH

Section on Primate Neurology, Surgical
Neurology Branch, NINDB, NIH

Man Years

Total:	.1
Professional:	0
Others:	.1

Project Description:

Objectives: Stapling devices have been available for years as aids in surgery. Their common use, however, has never been frequent or widespread. An instrument which would repeatedly feed small staples would be especially useful in vascular and neuro-surgery.

Methods Employed: Design, construction and testing of a new surgical instrument (the repeating monostapler).

Major Findings: A simple and efficient repeating surgical monostapler has been constructed. Preliminary tests indicated that modifications in the original design were necessary for dependable use. Modifications and further tests are contemplated.

Significance to Bio-Medical Research and the Program of the Institute: It is hoped that this stapler will provide an efficient device for assisting in and shortening of a variety of surgical procedures such as closing vascular defects, forming vascular grafts and closing dural incisions or defects.

Proposed Course of Project: Further experiments will be carried out to determine the usefulness of the stapler in vascular and neuro-surgery. Interest in the instrument has been expressed by the Surgical Neurology Branch, NINDB.

Honors and Awards: None

Publications: None

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Salivary Gland Scanning with Technetium 99m
(^{99m}Tc) Pertechnetate

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Arthur S. Grove, Jr.

Other Investigators: None

Cooperating Units: Naval Medical Center, Bethesda, Maryland
George Washington University Hospital,
Washington, D.C.

Man Years:

Total:	.3
Professional:	.3
Others:	0

Project Description:

Objectives: Technetium 99m pertechnetate is known to be taken up rapidly and highly specifically by salivary gland tissue. The glands may, therefore, be visualized and their morphology studied by external scanning after intravenous injection of technetium pertechnetate. The aim of this project is to evaluate salivary gland scanning with technetium 99m pertechnetate as a diagnostic technique.

Methods Employed: Patients with normal salivary glands (mostly brain tumor suspects) and patients with salivary tumors and other salivary gland pathology have been studied by external scanning.

Major Findings: Salivary tumors can be differentiated by technetium scanning into "isotope-concentrating" neoplasms (Warthin's tumors) and "non-concentrating" tumors (mixed tumors, carcinomas and a variety of tumors arising from structures adjacent to the salivary glands). Some tumors of neighboring structures can be outlined by displacement of normal salivary glands. Atrophy

or decreased functional activity of the salivary glands (Sjögren's disease, post-radiation damage, etc.) can be evaluated.

Significance to Bio-Medical Research and the Program of the Institute: It is hoped that technetium 99m pertechnetate scanning will provide a simple technique for evaluating the presence and nature of lesions in the salivary glands.

Proposed Course of Project: More patients with a greater variety of lesions within or in the vicinity of the salivary glands are being studied. Results of technetium scans will be compared with sialographic and clinical pathological findings.

Honors and Awards: None

Publications: None

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A Bilateral Angiographic Computer Evaluation of
the Superficial Veins and Sinuses of the Brain

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Norman E. Chase
Irvin I. Kricheff

Other Investigators: None

Cooperating Units: New York University Medical Center, University
Hospital, New York, New York

Man Years

Total:	.1
Professional:	.1
Others:	0

Project Description:

Objectives: It has been previously found (Di Chiro) that the angiographic superficial venous hemispheric patterns are often different in the two hemispheres of the same patient. A tentative correlation of these differences was established with functional lateralized cerebral dominance. More specifically, it has been found that the vein of Labbe "predominates," i.e., is larger than other superficial veins in the dominant hemisphere, and the vein of Trolard "predominates" in the nondominant hemisphere with a statistically significant incidence. Considering the implications of these findings it has been decided to check them on a much larger material in order to ameliorate their statistical value.

Methods Employed: The superficial venous drainage is evaluated in the venous phase of cerebral angiography and the "predominant" superficial vein of each hemisphere (Labbe, Trolard or superficial sylvian) is noted. Cases in which bilateral carotid angiography has been carried out are preferred. The handedness and the result of the Wada's test (if available) are also noted. These data are then fed into a computer for further evaluation.

Major Findings: Previous and new data under evaluation.

Significance to Bio-Medical Research and the Program of the Institute: The different angiographic pattern of the superficial discharging veins of the brain and its relationship to the cerebral dominance will, if confirmed, be extremely important from a theoretical point of view. Definite evidence of morphological differences between the two hemispheres would then be available. From a practical point of view, if a good statistical significance of the differences between the two hemispheres (dominant and non-dominant) is found, a new diagnostic method to establish the cerebral functional dominance, would be available.

Proposed Course of Project: To collect further data for computer evaluation.

Honors and Awards: None

Publications: None

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Empty Sella Turcica

Previous Serial Number: None

Principal Investigator: Giovanni Di Chiro

Other Investigator: Joseph Morel

Cooperating Units: Diagnostic X-Ray Department, Clinical Center,
NIH

Surgical Neurology Branch, National Institute
of Neurological Diseases and Blindness, NIH

Endocrinology Branch, National Cancer
Institute, NIH

Pathologic Anatomy Branch, National Cancer
Institute, NIH

Man Years

Total:	.1
Professional:	.1
Others:	0

Project Description:

Objectives: During previous anatomic work on the sella turcica, "empty" sellae, i.e. instances in which the pituitary may so far fail to fill the sella that the anatomic specimen appears empty, were observed in several cases. The empty sella is usually larger than normal: in fact, in a series of 66 postmortem anatomic observations of the sella turcica and its content the largest sella was an "empty" one. A selective hydrocephalus of the intrasellar subarachnoid space is considered to be the cause of the large empty sella.

The existence of a large empty sella has pragmatic importance. A large empty sella may mimic an intrasellar tumor and a differential diagnosis has to be made. In addition, the abnormal dilatation of the intrasellar subarachnoid space may cause,

probably with a pressure mechanism, a hypopituitary syndrome (as in several of our cases) or the development of an intrasellar tension cyst. These cysts may have eroding ability resulting in a break of the sellar floor and an ensuing cerebrospinal fluid rhinorrhea. The empty sella acquires, therefore, significance as a specific pathologic entity. Finally, the knowledge of the existence of a large intrasellar subarachnoid space is important if therapy of the pituitary gland (whether trans-sphenoidal hypophysectomy, trans-sphenoidal ⁹⁰Y interstitial therapy, or external irradiation) is contemplated.

Methods Employed: Suitable views (hanging-head lateral with autotomography or zonography) are taken at the end of most pneumoencephalographies. Prior to taking these special views the patient is kept in the hanging-head position for at least five minutes, the intent being to fill with air the intrasellar subarachnoid space if present. Particular attention is paid to patients with large sellas and with "dis-pituitarism". Occasionally surgical or autopsy follow-up is obtained.

Major Findings: Moderately large to large or very large intrasellar subarachnoid spaces have been observed in an unexpectedly high number of patients with normal, borderline big, or big sellas. The intrasellar subarachnoid space is located in the anterior part of the sella, with the pituitary gland occupying the infero-posterior part of the sellar cavity. In certain instances the compressed pituitary gland appears to be reduced to a shell-like structure filling only the postero-inferior section of the sella.

Significance to Bio-Medical Research and the Program of the Institute: The knowledge of the large empty sella is important for: a) the differential diagnosis of intrasellar tumor; b) the explanation of certain hypopituitary syndromes and certain types of spontaneous cerebrospinal fluid rhinorrhea; and c) to avoid mistakes when contemplating sphenoidal (surgical and ⁹⁰Y) or external radiation pituitary treatment.

Proposed Course of Project: To gather further clinico-radiographic experience, obtain new surgical and pathological correlations, and study the intrasellar subarachnoid space in a large number of "normal" corpses for a better understanding of this particular section of the cerebrospinal fluid cavities.

Honors and Awards: None

Publications: None

1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Tetrascanner

Previous Serial Number: None

Principal Investigators: Giovanni Di Chiro
William B. Matthews

Other Investigators: William L. Ashburn
Grant C. Riggle
Frank O. Anderson
Homer D. Chalifoux

Cooperating Units: Diagnostic X-Ray Department, Clinical Center,
NIH

Biomedical Engineering and Instrumentation
Branch, Division of Research Services, NIH

Man Years

Total:	.1
Professional:	.1
Others:	0

Project Description:

Objectives: We have reached the crossroads in the choice of equipment for in vivo isotope localization: moving-detector (scanners) versus stationary-detector devices (cameras). Four views are necessary for reliable localization of brain lesions: antero-posterior, postero-anterior, right and left lateral. This is because regardless of the isotope's energy and type of detector (moving or fixed, with or without focused collimation), useful geometric information is obtained only within a limited space range from the detecting probe. This range may vary depending upon different factors but it will never exceed the thickness of an hemicranium. Signals originating beyond the useful range are not sharply resolved and the ensuing record is distorted (smeared, magnified). Four-view brain scanning is actually used in only a few centers. Time requirement for four views is prohibitive with certain types of equipment. Another hindrance may be the necessity of positioning the probe or

patient four times when using a single detector. Time-lapse scans taken simultaneously in two planes are necessary in isotopic studies of the cerebrospinal fluid dynamics. If too much time lapses between lateral and sagittal views in isotope-cisternography and isotope-ventriculography, the records obtained will not be comparable due to intervening flow of cerebrospinal fluid.

The purpose of this project is to evaluate a new device for rapid three-dimensional brain scanning, the Tetrascanner, which has now been in operation for over one year and has proven itself to be a remarkably useful piece of equipment.

Methods Employed: Our Tetrascanner has four separate detection and recording systems, each consisting of scintillation detector with focusing gold collimator, spectrometer, binary scaler, photorecorder and solenoid tapper. The four probes are arranged in opposite pairs in planes at right angles. Four simultaneous views, each recorded both as dot- and photo-scan, are obtained in from 14 to 34 minutes depending on count-rate and size of patient's head. Resolution is excellent, good depth response allows detection of deep-seated lesions, and the patient, positioned only once for four views, lies comfortably supine. Three-dimensional time-lapse motion pictures may be made of dynamic isotope studies of cerebrospinal fluid movement with the Tetrascanner.

A comparison is being made between the Tetrascanner and other commonly used rectilinear scanners and stationary devices (cameras). This comparison is being carried out (insofar as the other compared devices) from data reported in the literature. Emphasis is being placed on the following parameters: sensitivity, resolution, speed, and versatility. The speed and versatility of the Tetrascanner has permitted up to 23 four-views scans in one working week, the equipment being operated by a single technician.

Major Findings: Although the Tetrascanner is not as rapid as some of the other instruments, this scanner has some advantages which are ideal for the work being done at the N.I.H.

1. Excellent resolution for isotopes from the ^{197}Hg energy range through the ^{198}Au range.
2. Excellent depth response allows detection of deep-seated lesions.
3. Four simultaneous views results in three-dimensional scans.

4. The patient is positioned only once, which means that very sick or hard to handle patients (and children) may be quickly scanned with a minimum of discomfort.

5. "Time-lapse motion pictures" may be made of dynamic isotope studies of cerebrospinal fluid movement.

6. There is minimal scalloping on the photostan because information is not "dumped" in the wrong place.

7. In the event that an electronic or mechanical problem develops, the patient can be scanned with the operational pair of detectors and the usual four projections obtained by turning the patient on his side.

Significance to Bio-Medical Research and the Program of the Institute: To establish the relative merits of two general classes of detecting machines used for localization of radio-activity in vivo: multiple-head moving-detector scanners versus stationary-detecting devices (cameras).

Proposed Course of Project: It is hoped that we will soon add at least one type of camera to our isotopic equipment. Then the comparison of the relative merits of our Tetrascanner and the cameras will be carried out in a rigid fashion using the two types of devices on the same groups of patients and thus obtaining reliable correlative data.

Honors and Awards: None

Publications: None

1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A Comparative Pharmacological Study of Fast and Slow Muscles

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin

Other Investigators: Jay B. Wells

Cooperating Units: None

Man Years:

Total:	.1
Professional:	.1
Other:	0

Project Description:

Objectives: Why some muscles contract faster than others is not well understood. Also, little is known concerning the differential response of fast and slow contracting muscles to pathological conditions or drug response. The object of the present study is to determine the differential response of fast and slow muscles in several different species to drugs which produce increased or decreased contraction. This study has the further objective of determining how the condition parameters of length-tension, fatigue and rate of stimuli, both direct and indirect, are related to drug induced muscle potentiation.

Methods Employed: Direct comparisons between slow and fast muscles will be made in vivo in the same animal to eliminate variation between animals. This design eliminates the need for large group comparisons. Stimulation parameters are electronically programmed and controlled. Data evaluation of these type of experiments are slow procedures. Thus the NIH Instrument Section was requested in January, 1961 to assist in developing electronic data collection system for this project. It is surmized that their progress has been finitely above zero since the problem has been incorporated into a larger and more grandiose

plan to use computers for data evaluation at NIH. Meanwhile back in the laboratory I am still gathering and evaluating the data in the same laborious slow way. Progress with the Instrument Section is hoped for during the coming year.

Major Findings: The necessary instrumentation has been completed and many of the technical problems of the instrumentation system have been solved.

Significance to Bio-Medical Research and the Program of the Institute: The present study is one of a fundamental nature to further elucidate the function of muscles during altered states. It thus may lead to increased understanding of muscle function in skeletal muscle disease.

Proposed Course of Project: The reliability of the instrument system will be tested. The recorded parameters of muscle function will be correlated and drug studies involving potentiation of muscle tension will then be continued.

Honors and Awards: None

Publications: None

1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Electromechanical Coupling in Muscle and Drug Activity

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin

Other Investigators: Manfred M. Hein

Cooperating Units: None

Man Years:

Total:	1.8
Professional:	1.8
Other:	0

Project Description:

Objectives: How the electrical events of muscle membranes by movement of ions induce protein molecules to slide or coil and thus produce shortening or tension has not been experimentally explained. The object of the present study is to explain the ionic basis for the electromechanical coupling of skeletal muscle.

Methods Employed: The general methods used are described in detail in the project reports of 1964 and 1965. During 1966 a variety of buffers have been used as ion substitutes within the framework of the previously used methods. The development of this substitution method necessitated the testing of many buffers to find a buffer with an appropriate pKa to permit nearly complete ionization of the buffer at a physiological pH.

Major Findings: Slow type muscle offers unique experimental advantages for the study of excitation contracture coupling process. During the past year we have been able to clarify the ionic events which lead to contracture of slow muscle. Myogenic contracture can be initiated in slow muscle by the withdrawal of external Ca^{++} . Present evidence indicates that loss of membrane Ca^{++} is the initial ionic event. This is followed by

increased permeability to Na^{++} which leads to internal sodium accumulation. Contracture and an inflow of Na occur concurrently. Smooth muscle develops Ca deprivation contractures without depolarization. We have been able to develop muscle models relating ionic movement to tension development without consideration of depolarization. Further experimental verification of such models are needed. The present studies have furnished evidence that (1) Ca^{++} has a dual role in the function of slow skeletal muscle; (2) there exists a Na^+ current mechanism different from the one in fast muscle or nerves which is responsible for the action potential because (a) it occurs in slow muscle that is not capable of a propagated potential and, (b) it is not affected by procaine which inactivates the Na^+ current responsible for the action potential; (3) there is a K^+ requirement for complete relaxation in slow muscle. This requirement indicates that part of the active transport of Na in slow muscle is K^+ dependent.

A manuscript has been submitted to the American Journal of Physiology in relation to "the ionic requirements for relaxation of slow striated muscle." Another dealing with "Ca and Mg^{++} in the excitation-contraction coupling of slow muscle" is nearly complete. A third dealing with the substitution of Na by impermeant charged ions is in an early stage of preparation.

Significance to Bio-Medical Research and the Program of the Institute: The present study relates to physiological events in slow type skeletal muscle which are not well understood. Slow type skeletal muscle is now known to occur in several species including man. A greater knowledge of the slow muscle systems may therefore lead to an increased understanding of muscle function in a variety of human muscle diseases.

Proposed Course of Project: The project will continue to furnish data in order to establish the ionic basis for electromechanical coupling in skeletal muscle. Drug induced contractures in relation to ion environment will be studied.

Honors and Awards: None

Publications: None

1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

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

Previous Serial Number: Same

Principal Investigator: Jay B. Wells

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.5
Professional:	.5
Other	0

Project Description:

Objectives: Establishment of the mechanical properties of active skeletal muscle as a prerequisite for evaluation of drug effects on the tissue response. Specific goal: Analysis of the energy exchanged by the series elastic component of rat limb muscle to further characterize mechanical response during normal contractile activity.

Methods Employed: Appropriate concepts and procedures employed in the mechanical analysis of elastic material were used. Establishment of the best fit mathematical expression of the series elastic stress-strain character was achieved by regression analysis of load-extension data. This provided an estimate of potential elastic energy stored in the muscle during isometric contraction which, when compared with the mechanical energy recovered during elastic retraction of the muscle, permitted interpretation of the damping and energy exchanging properties of the muscle.

Major Findings: The stretch or strain of the series elastic component of active rat muscle was found to vary exponentially with stress. Integration of this expression between appropriate limits of stretch (or release) gave the potential energy stored

(or available) for elastic work. The amount of elastic work i.e., mechanical work and kinetic energy recovered during the elastic recoil of the active muscle following quick release, when compared to the available potential energy did not support the prevailing concept of a passive undamped elastic body. The interpretation depends on the assumption that the method used for determination of the stress-strain relation (originally described in amphibian muscle) is valid for mammalian skeletal muscle.

Significance to Bio-Medical Research and the Program of the Institute: Quantitative description of the mechanical properties of active muscle is necessary for full understanding of the physiological responses of normal and diseased muscle. The present study contributes toward that end and in addition establishes normal values upon which to assess the action of drugs which influence muscular contraction.

Proposed Course of Project: A test of the validity of the previously mentioned assumption is proposed by using an alternate more direct means of obtaining stress-strain data. Instrumentation toward this end is nearly complete. Measurement and analysis of elastic and contractile responses obtained from muscles subjected to the influence of drugs believed to act on the cell membrane are planned.

Honors and Awards: None

Publications: Wells, Jay B. Comparison of Mechanical Properties Between Slow and Fast Mammalian Muscles. J. Physiol. 178, 252-269, 1965.

1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Relationship Between the Functional Activity
and the Mechanical Properties of Normal and
Modified Striated Muscle

Previous Serial Number: Same

Principal Investigator: Jay B. Wells

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.5
Professional:	.5
Other:	0

Project Description:

Objectives: Definition and characterization of the adaptive mechanisms which control the functionally different contractile activity observed among the several skeletal muscle types. The specific goal was to determine the effect of modifying the mechanical requirements of the limb muscle on normal contractile mechanisms.

Methods Employed: Disuse atrophy was produced in rat limb muscles maintained at physiological lengths as follows: A small metal appliance, developed in cooperation with the instrument development section, when aseptically installed in rats immobilized the ankle joint. Length changes were thus prevented in the triceps surae and anterior tibialis muscles. Two to four weeks after application the animals were subjected to the appropriate mechanical procedures leading to the objectives stated above. The method of modification produces changes in the muscle environment while leaving the nerve supply intact. Thus trophic influences due to nerve injury or section were not present.

Major Findings: The amount of energy liberated during active contraction, inferred from mechanical measurements, is decreased in muscles made atrophic by disuse. This occurs without loss of the capacity for tension development in the case of anterior tibialis, (a rapid contracting muscle), but with a concomitant decrease in the maximally developed tension by the slower soleus muscle. Similarly, altered anterior tibialis showed a decrease in the rate of energy expenditure, which is normally several times that observed in soleus while the corresponding rate estimate from altered soleus muscle increased although not by a considered significant amount. Such changes in energy liberation, induced by disuse, resulted in a significant increase in the "intrinsic" speed of the slower muscle while no change was observed in the more rapid one. Functional modification of the rapid muscle significantly decreased the elastic retraction rate of the series elastic component while the corresponding rate from fixed soleus muscle was only insignificantly decreased. These changes were sufficient to alter the series elastic properties of the soleus muscles so that they more resembled those of the faster muscle. Alternation of the response of the muscle elastic element by disuse does not support previous interpretations of a "passive" series elastic element and suggests further definition.

Thus, disuse appears in general to have a deleterious effect on both the contractile and elastic responses of the more rapid acting skeletal muscle while changing the corresponding responses from the slower muscle to appear as those from normal rapid muscle.

Significance to Bio-Medical Research and the Program of the Institute: An understanding of the functional changes produced in muscle by various environmental requirements will lead to more effective clinical programs for treatment of abnormal and maintenance of normal muscle function.

Proposed Course of Project: Similar measurements are planned for muscle made hypertrophic by excess exercise. A literature search is proposed for a possible experimental animal subject whose natural habits lead to seasonal variations in muscular activity, for example certain hibernating mammals, for confirmation and expansion of the above findings.

Honors and Awards: None

Publications: None

1. Medical Neurology
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Genetic Studies of Abnormal Plasma Cholinesterase
in Relation to Drug Metabolism

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin

Other Investigators: Manfred M. Hein
Jerome S. Resnick
Gerald N. Gold

Cooperating Units: None

Man Years:

Total: No time assigned to this project this year.

Project Description:

Objectives: Soon after the neuromuscular blocking agent succinylcholine began to be used during anesthesia it was observed that many individuals failed to recover promptly from its paralytic effects. The prolongation of paralysis is related to low cholinesterase activity in the plasma which fails to metabolize the succinylcholine to a non-paralytic level. It is known that certain aspects of cholinesterase activity is heritable. The objective of this project is to further define the genetic basis of the occurrence of abnormal cholinesterase in relation to drug metabolism and action.

Methods Employed: Cholinesterase levels, inhibition and substrate specificity of appropriate subjects are studied using titrametric and spectrophotometric techniques. Genetic genotypes are determined by dibucaine and fluoride inhibition tests.

Major Findings: Abnormal plasma esterase activity was found in a family of four in which the mother had previously responded to succinylcholine with a prolonged paralysis. Her plasma failed to metabolize succinylcholine and thus a correla-

tion was established between lack of esterase activity and drug response.

Each of the 4 family members had a unique substrate activity pattern for plasma esterase but only 3 phenotypes were identified. The two siblings had identical phenotypes but markedly different substrate specificity for cholinesterase. The father and son which had the most nearly alike cholinesterase substrate specificity had distinctly different phenotypes. The study indicates that the genetic pattern of inheritance of cholinesterase is not always paralleled by the ability to metabolize drugs or enzyme substrates.

Significance to Bio-Medical Research and the Program of the Institute: The inability of an individual to metabolize succinylcholine endangers life and imposes rigorous procedures upon medical and nursing staffs following surgical procedures upon such individuals. A more complete definition of the problem is therefore desirable in relation to drug use.

Proposed Course of Project: Project is inactive but will be reactivated provided suitable chemical material can be obtained.

Honors and Awards: None

Publications: Irwin, Richard L. and Hein, Manfred M. The Substrate Specificity of Atypical Cholinesterase in Relation to Phenotypes. Biochemical Pharmacology, 15, 145-154, 1965.

ANNUAL REPORT
July 1, 1965 through June 30, 1966
Branch of Surgical Neurology, IR
National Institute of Neurological Diseases and Blindness

Maitland Baldwin, M. D., Chief

Since the last report, this Branch has conducted investigations under the following categorical titles: developmental defects, epilepsy, involuntary movements, brain tumor, cerebral edema, cerebral trauma, language and memory, effects of low temperatures, microbial analysis of neurosurgical environments, and neurosurgical monitoring. Twenty-nine reports were prepared for publication in appropriate journals.

During the period 16 April 1965 through 15 April 1966, 214 persons participated in the clinical investigations as inpatients, while 593 were examined as outpatients in a total of 754 visits. There were 154 major operative procedures in the surgical suite.

In its various investigations, the Branch has actively collaborated with the following organizations: Branch of Electroencephalography, NINDB; Laboratory of Neurophysiology, NINDB; Laboratory of Neurochemistry, NINDB; Section on Animal Behavior, NIMH; Section on Pharmacology, NCI; Clinical Center Departments of Clinical Pathology and Diagnostic X-ray; Biomedical Engineering and Instrumentation Branch, DRS, NIH; Computer Facilities, DRS, NIH; Walter Reed Army Medical Research Institute; Personnel Protection, David Taylor Model Basin, Department of the Navy; Berlitz School of Languages.

Developmental Defects

X-irradiation of freshly drawn blood lends to the perpetuation of a chromosome-type lesion in the lymphocytes which are in the pre-DNA synthesis phase (G1). Radiation of the proliferating lymphocytes, post-DNA synthesis phase (G2) lends to chromatid-type lesions which are not transmitted to the daughter cells, being generally sublethal to the affected cell. This study suggests that persons who have been exposed to radiation in the past (as long as five to twelve years) have lymphocytes with a significantly greater sensitivity to chromosome damage than persons who have not suffered similar exposures.

Chromosome studies in a female who had repeated therapeutic x-irradiations during the past twelve years revealed persisting chromosomal aberrations, and also a clone of cells with a deficient chromosome in group C. About 20 per cent of all her lymphocytes in repeated cultures over two years showed this abnormality. Recently, she gave birth to a malformed child. Although the gross appearance of the chromosomes in this offspring was normal, undetectable maternally transmitted inversion may be present.

A serine amino aciduria concomitant with amelioration of this acid level in the plasma has been demonstrated in patients with Hurler's syndrome. In similar patients, the ratio of chondroitin-sulfate-B to heparitin-sulfate was

changed by administration of hormonal compounds. This finding has led to a reinterpretation of the syndrome itself and may prove of some therapeutic value. In one such case, after complete pathological examination of brain and internal organs, two major types of monopolysaccharides in various tissues were demonstrated. It was found that the brain contained an excess of acid mucopolysaccharide as well as a lipid-like substance which resisted extraction. The latter contained a small amount of sialic acid.

Epilepsy

As in the past, epileptic mechanisms in children and adults have been variously investigated in the clinic, while some electrophysiological characteristics of epilepsy have been studied in the laboratory as well.

A total of 18 children suffering from frequent epileptic attacks was investigated and treated. Seven of these received a high-fat diet for therapeutic purposes and this treatment afforded the unique opportunity to study partitioned plasma lipids and their relationship to the control of seizures. When this diet was followed by satisfactory control of attacks, there was a marked change in the value of the partitioned plasma lipids. In another group of children, the cause of seizures was identified as hypoglycemia. This hypoglycemia was further subdivided into leucine sensitive and leucine insensitive types. Then a special dietary regimen was devised for control of the leucine sensitive hypoglycemia. Once this was achieved, the seizures stopped. It should be noted that seizures resultant from hypoglycemia in early life can be followed by severe mental retardation and paralysis unless the attacks are adequately controlled.

Seventy-one patients with cerebral seizures were studied in the operating theaters and on the wards during the period of this report. The consistency, longevity, and some clinical relationships of focal cortical discharge (ECG) have been studied in the operating theaters. It appears that focal cortical discharge (in motor cortex) is unaffected by analogous postcentral excision, but remains consistent for some time thereafter. Conversely, relatively normal electrocorticograms have been obtained over precentral cortex during clinically evident and relevant focal motor seizures. The obvious presumption is that these are due to subcortical mechanisms which do not project to somatomotor cortex, but there is no way the clinical observer can differentiate them from those of surface origin. Finally, the 'focal' cortical discharge which does not disappear after relevant surface excision seems explicable in terms of the experimental lesions in monkey thalamus which were followed by persistent 'focal' cortical discharges.

A group of 18 centrencephalic patients was examined by tests designed to measure the effects of spike-and-wave type of EEG activity on sustained attention, sensory input, memory, and simple motor behavior. In addition, measurements were made simultaneously of the following autonomic variables: blood pressure, heart rate, finger volume, skin resistance, esophageal and gastric motility, and respiration. A total of 1,267 bursts of spike-and-wave was recorded in conjunction with the several tests. The behavioral correlates of the bursts were analyzed from the point of view of character (form), organization, maximal discharge, voltage, frequency, length, the background activity, and several time-related variables. All of these were

shown to be related to behavior on one or more of the tests. Bursts which were symmetrical, regular, and bilaterally synchronous tended to produce more behavioral deficit than other bursts; tests which required the complete attentive act were more impaired during bursts than those which required only a part of this sequence; the motor task tended to be affected least. In addition, there was a retrograde amnesic effect. The most frequent autonomic changes were arrest of respiration, finger vasoconstriction, fall in skin resistance, and increase in esophageal motility. These were not related in a one-to-one fashion to the behavioral modifications.

These patients were impaired on the attention test in the absence of observable bursts. The behavior loss tended to lead the electrographic symptom in time; in some patients, lack of behavioral change was observed even in the presence of well-organized symmetric bursts.

Two epileptic patients who underwent temporal lobectomy revealed a transitory drop in urinary 17-OHCS, epinephrine, and norepinephrine levels one month postoperatively. However, these findings did not persist at the end of one year's study. Similarly, amygdaloid and hippocampal stimulation in epileptic patients revealed slight plasma 17-OHCS changes associated with a particular type of electrical stimulation.

In the laboratory, propagation of afterdischarge from mesial and basal amygdala to contralateral homologue has been studied. Such propagation is of considerable interest to the clinical analysis of temporal lobe seizures, particularly those arising from mesial temporal structures.

Involuntary Movements

Respiratory and heart rates, and GSR responses from the human diencephalon have been recorded in 24 consecutive patients with electrodes implanted for treatment of motor disorders. Respiratory depression followed stimulation of these electrodes in the medial frontal white matter, genu of the internal capsule, and medial portions of the ventral-lateral thalamic nuclei. The second area of respiratory depression lay on the posterior-superior border of the lateral thalamus in the vicinity of the fornix and the stria terminalis. Respiratory depression from stimulation of electrodes located in or on the border of the ventral and oral portion of the thalamus had a significantly lower threshold on the left than on the right brain. This was not true of respiratory depression evoked elsewhere. Bradycardia followed stimulation of electrodes in the posterior border of the pulvinar. Tachycardia was evoked from the superior-lateral edge of the hypothalamus. Changes of skin resistance seemed to be related to the presence of subjective sensations during stimulations.

A method of relating commissural landmarks to the skull (Hassler & Riechert) has been tested in cases when two separate pneumoencephalograms were done. The anterior and posterior commissures in the midline third ventricle of the first pneumogram were reconstructed on the second. These were then compared with the position of the actual commissures on the second pneumogram. The mean variations and the standard deviations seemed acceptable in the vicinity of the A-C P-C line and the third ventricle, but three to

four centimeters away, the variations possible with additive errors assumed sufficient size so that the use of a ventricular landmark closer to the site to be localized would seem advisable.

The human stereotaxic instrument which has been in use since 1960 has been reevaluated and found to have the following features:

1. Localization is achieved by fractional pneumography through routine demonstration of both the anterior and posterior commissures without the use of radiopaque oils.
2. The instrument permits use with standard x-ray equipment.
3. It allows full surgical draping.
4. It may be aligned with the intracerebral axis.
5. Movement is full in three graduated planes.
6. It permits entry into the skull at any point (apart from the central area at the vertex which underlies the apparatus), without the use of phantom target points or recomputation.
7. Precise realignment for stage procedures is within its ordinary capabilities.

Single cell discharges are being recorded from cerebral cortex and thalamus of patients with motor disorders. Data have been accumulated for statistical computation which is now underway.

In the laboratory, using extracellular and intracellular microelectrodes, the spike activity from the motor cortex in response to stimulation of various subcortical and cerebellar structures has been recorded on motion picture film as well as on magnetic tape and electronic digital recorders. It was observed that "the midbrain reticular formation" may play a role in the transmission of impulses from cerebellum to the cerebral cortex. Transmission mechanism through this subcortical structure was found to be unaffected by barbituate anesthesia. Other observations suggest inhibition of facilitation of cortical motor neurons in response to transcallosal stimulation.

Brain Tumor

Three patients with meningeal leukemia have received approximately four perfusions each with methotrexate. Two of these have been in remission for two years after perfusion. Twelve patients with glioma have been subject to perfusion with methotrexate. 8-Azo-guanine is also being tested for its destructive effect on the gliomas.

Cerebral Edema

Selectively differential behavior of the blood-brain barrier in rabbits was studied in chemical injuries produced by intracerebral injections of

various compounds. Unilateral blood-brain barrier injury was produced by intracarotid injection of mercuric chloride, penicillin, or sodium acetrizoate. These regional injections were followed by systemic administration of combinations of two different fluorescent and radioactive tracers. The brain tissue was studied by fluorescent microscopy and radioautography.

Combinations of red and green fluorescent albumin showed no separation in the distribution pattern on the damaged side. Simultaneous administration of red fluorescent albumin and green fluorescent globulins revealed in numerous instances a distinct separation in distribution of protein tracers on the damaged side. In slightly or moderately damaged areas, numerous blood vessels were surrounded by only red fluorescence. The perivascular exudates and globules showed a range of color depending on relative concentration of respective tracers. Differential passage from the injured vessels was also observed in combinations of fluorescent and radioactive tracers. C^{14} inulin appeared to penetrate in damaged areas more extensively than sodium fluorescein or fluorescein labeled albumin. An interesting result was obtained in sodium fluorescein- C^{14} methyl-o-glucose combination. Whereas in moderate or severe blood-brain barrier damage, both sodium fluorescein and C^{14} methyl-o-glucose spread intensely from the injured vessels, in very slight blood-brain barrier damage in which no abnormal passage of sodium fluorescein could be detected, there was a distinct inhibition of the normal transport of methyl-o-glucose from the blood into the brain tissue. This inhibition of normal glucose transfer from blood to brain in very slight blood-brain injuries, which are undetectable by visual tracers, may be of considerable clinical significance.

The blood pressure in cats was altered by means of hyper- and hypotensive drugs administered prior to the production of brain edema. Then edema was produced by application of a cooled metal plate to the exposed cerebral cortex. Changes of the systolic blood pressure significantly affected the rate of progression of the edema through the white matter of the injured gyrus. It was shown that the lowering of the blood pressure can almost completely prevent development of edema.

By means of these standardized methods for intracarotid injection of injurious solutes, the blood-brain barrier in rabbits was studied so that one hemisphere was subjected to the injections, whereas the other was not. Comparative assay of the chloride-in-water content in damaged and relatively undamaged hemispheres of the same brain was performed, varying the type and concentration of the injuring solute and the time interval between injury and the termination of the experiment. In most experiments, the injected damaging agent was $HC\text{Cl}_2$, while Penicillin-C was administered in a smaller group of animals. Slight or moderate damage was directly related to decreased chloride content in the hemisphere showing extravascular passage of tracer dyes. This suggests a functional disturbance in exchange mechanisms operating at the blood-brain interphase and not a mere leakage through ruptured blood-brain barrier structures. On the other hand, more severe mercurial injury was followed by the development of edema which was evident grossly as well as microscopically, and as suspected was directly correlated with an increase in both chloride and water content. The effect of penicillin is of a more complex nature. This substance, after passing through the blood vessels, appeared to penetrate quickly to the neurons as evident by the development of convulsive phenomena predominantly on the side contralateral to the injection.

The enzymatic changes occurring during the development of brain edema were studied in cerebral tissue and in CSF at various stages of experimental cold injury edema. Thus, five days after production of cold injury, a six to eight-fold increase in the level of lactic dehydrogenase (LDH) was noted. At seven days, this increased activity persisted. There was no concomitant serum increase in these animals and normal animals did not show this level of activity in CSF. Similarly, brain slices from these preparations which were stained histochemically for LDH showed enlargement of astrocytes and intense staining from five to seven days after injury. Such changes did not appear either in the controlled side where one animal served as its own comparison, or in control animals. Similarly, several enzymes of CSF and sera as well as proteins in the elasmobranch species have been studied. The proteins of brain and CSF were compared by electrophoretic techniques to analogous proteins in mammalian specimens. Thus several elasmobranch enzymes were compared to mammalian enzymes, both in total activity and in isozyme patterns. These studies indicate that, in the shark, CSF protein content and the CSF levels of LDH activity are significantly higher than those in mammalian CSF.

In a further diversification of species studied, the non-neuronal elements of the elasmobranch brain have been analyzed in the laboratory during the past year. In the specimens obtained, astrocytes showed a striking radiating arrangement around blood vessels and neurons. No vascular sucker-foot which are characteristically seen in the mammalian brain could be noted. Otherwise, the blood vessels of the forebrain were densely surrounded by the glial cells. A system of ependyma-lined canals was described in the cerebellum. It is assumed that these channels, as well as sacculus vasculosis, play an important role in the circulation of the CSF. Elasmobranch studies such as this are of considerable importance in broader perspectives on mechanisms of cerebral circulation and edema because elasmobranch has, as previously noted, a unique blood-brain barrier which is singularly resistant to agents which clearly damage these transfer mechanisms in the mammalian species.

Cerebral Trauma

Sufficient data on occipital blows has accumulated to establish 10, 50, and 90 per cent "dosage" curves for concussion in terms of impulse of blow and linear acceleration of head. These curves arrived at by probit transformation enable rigorous statistical comparison of blows to frontal and temporal positions, and are also serving as a baseline on which tests for various hypotheses of the mechanics of cerebral concussion are being made. These include the effect of a cervical collar, head fixation, increasing the head mass, shifting the center of gravity of the head, and the protective effect of various devices. The most important observation to date is the finding that a cervical collar protects against the concussive effects of impact to the head in monkeys.

Cardiovascular effects after head injury have been found to be of considerable significance. A period of arterial hypotension invariably follows a concussive blow and this is associated with a widening of the pulse pressure, a moderate increase in the central venous pressure, bradycardia, and other electrocardiographic abnormalities. The latter, particularly, seem

to be significantly related to the prognosis following the blow. Persisting EKG abnormalities of either rate or pattern were invariably associated with a fatal outcome after a concussive blow. The slowing of the dye-circulation noted by cerebral angiography has been further studied and appears to be biphasic in nature. Slowing is noted within the period 10 - 30 seconds after the blow; this is followed by a return to normal circulation after approximately five minutes, and then 15 - 30 minutes later a second slowing occurs, persisting for a few hours. It is our intention to pursue this aspect further with studies of flow at the times of impact.

The study of blood and C.S.F. gases and pH has revealed a considerable lag between the two compartments, particularly for pO_2 . Rapid alterations in the C.S.F. compartment can be made with little or no effect on the blood. The changes in these factors following various degrees of head injury are now being pursued.

Studies on spreading depression, D.C. potential, cortical electrical impedance, and response to pCO_2 alterations after experimental head injury in collaboration with Dr. Wade Marshall are in progress. To date, it does not appear that spreading depression is produced by the blow. However, there is some indication that the effect of increased pCO_2 on the D.C. potential is reversed immediately after the blow.

Language and Memory

A recent finding that certain sets of scores from the Wechsler-Bellevue Intelligence Scale correlated inversely with the extent of neurosurgery in some of our cases encouraged the development of computer programs for investigation of the efficiency of factor-weighted scores. Three factors were derived from the standardization data by Maxwell's method. The scores from Atwell and Wells' vocabulary test and the first WB factor (Verbal Comprehension) correlated with the amount of left temporal removals. The scores from Mooney's Closure Faces test and the two minor factors of the WB ("Perceptual Organization" and "Freedom from Distractibility") were affected by right temporal removals, and these impairments differed for the two sexes. These results discouraged the view that intelligence or some highest integrative process is a function only of the brain as a whole.

During performance of a standardized object-naming task, stimulation of the left superior thalamus was associated with a significant degree of anomia. In contrast, stimulation in a homologous region in the right thalamus failed to produce a similar disruption.

As previously noted, right and left temporal lobectomized patients are being subjected to a standardized language learning system which presents a maximum of 2,500 words in a foreign language over a 60-hour period. To date, four right temporal lobectomized and one left temporal lobectomized patients, all of whom are left dominant by WADA test and all of whom have undergone operation at least five years prior to this test series, have been studied. These patients are relatively well matched for age, sex, full scale performance scores, and all are incorporate in an experimental protocol which is composed of psychological tests, physiological evaluations, and which ends with examination by the Princeton Modern Language Test Service. Three right temporal

lobectomized left dominant patients achieved a first year college rating on the Princeton Modern Language Series. One right temporal lobectomized left dominant and one left temporal lobectomized left dominant failed to make first year college level, but scored comparably at about a 500 word performance level. The latter finding suggests that conventional teaching with regard to effects of left temporal lobectomy in a left dominant person is not valid since such a removal is thought to affect language learning capabilities adversely and permanently. Conversely, a right temporal lobectomy in a left dominant person is thought to have little or no effect on language learning capabilities.

Frontal opercular cortex is being investigated for language representations with reference to the area called Broca. In a correlative study, electrocorticographic recording, electrical stimulation points, amplitude of speech responses, and a tape delivered sub-verbal syllable test series are correlated with recordings from two electromyographic points on right upper and lower lips in the conscious, alert patient. Myographic activity, frequency in amplitude of electrocorticographic recording and amplitude of expressed speech correlate well in this matrix.

Effects of Low Temperatures

Two separate cold perfusion systems have been developed for the purpose of creating hypothermic conditions in the brain. The first of these is dependent upon the finding that high catheterization of the brachiocephalic arch in the dog as an end point to an extracorporeal perfusion system would provide for relatively selective or regional cooling of the carotid circulation to the brain. In such a preparation, it was possible to provide hypothermic temperatures of choice in the brain, while the body of the experimental animal remained relatively warmer and the heart continued to beat and thus provide systemic circulation. The exclusion of the cardia from hypothermic effect is of considerable importance in clinical applications of hypothermia and is of fundamental interest as well. In the second system, unilateral cooling of one hemisphere of the cat was achieved and used as a basis for electrophysiological studies both of single units and by means of macro-electrode recording. It seemed desirable to explore the prior findings which relate a critical reduction in amplitude of the surface ECG to a temperature frontier in the brain of approximately 25° C. The unilateral cooling of one hemisphere provides a potential clinical model and experimentally is being used as a means of studying effects of low temperatures on blood-brain barrier and cerebral permeability since the opposite warmer hemisphere can serve as a relative control.

Cerebral vasospasm in the experimental animal has been the subject of some study within these projects. Obviously, vasospasm or dilatation may affect the course of heat exchange procedures in the brain and have some particular importance in brain surgery at ordinary temperatures as well. Present findings indicate that gentle mechanical stimulation of the middle cerebral artery in a plane parallel to its long axis can produce a gelatinous clot which rapidly becomes adherent to the intima. This intravascular clotting is preceded by a vasodilatation and pallor of the vessel. If a similar vessel is mechanically stimulated, using microdissection instruments,

in a plane transverse to its long axis, vasospasm usually results. These changes in caliber of the vessels are comparable to those seen by topical application of papaverine, which produces vasodilatation, and serotonin which produces constriction. Interestingly enough, papaverine will relieve the vasostriction produced by transverse mechanical stimulation, but serotonin cannot or does not alter the vasodilatation produced by longitudinal mechanical stimulation.

In another study, the effect of urea on the type of cerebral edema produced by freezing cold was pursued. However, these experiments yielded equivocal results and it was concluded that urea did not have a predictable or consistent effect on this particular type of local hypothermia.

Neurosurgical Monitoring

Earlier preliminary analyses indicated a correlation between alpha frequency and skin temperature. This is currently being examined more thoroughly using techniques of time series analysis (auto- and cross-correlation spectral analysis) to determine the nature and validity of this correlation within finite time spans over the entire course of the experiment, and to compare the strengths of this relationship among groups of subjects undergoing different degrees of psychological testing.

An asymmetry in evoked respiratory depression between right and left thalamus has been observed, as has a difference in latency of visual evoked response between patients with adrenalin insufficiency, and either adrenalin insufficient patients under treatment or normal controls.

Microbial Analysis of the Neurosurgical Environment

The neurosurgical environment has been sampled through air-collection plates, skin culture, wound site collection methods, and collection from drapes, instruments, and various geographic locations in the system. Despite a known defect in the plenum ventilation system, counts continued to range in the order of one or less organisms per 4 cu. ft. of air per 2-hr. sample period. These results are being compared to those obtained in an experimentally designed laminar flow ventilation system as part of an investigative protocol. It is possible that laminar flow ventilation will provide for reduction and simplification of aseptic techniques with particular emphasis on those related to personnel, clothing, and patient draping.

Summary

This report marks the thirteenth year of Surgical Neurology, a Branch which was named and founded by the present Chief in 1953. Originally designed as an aggregate of physicians and surgeons with special training in anatomy, physiology, pathology, and behavioral sciences, its name was selected from the writings of Wilfred Trotter as indication of purpose. The once and future intent is contribution to knowledge of functional anatomy of the human brain, with particular emphasis on behavioral correlations. In its practical attempts to reach these idealized goals, the Branch has published 244 papers and authored or co-authored 17 books. The categories of the

present report represent evidence of the contemporary effort, as well as communities of interest among the members. Thus the categorical work in cerebral edema is headed by the pathologist and joined by several surgeons. The anatomist (also a surgeon) heads the projects on involuntary movements which contribute to information on basal ganglia function as well as relevant disease states. Under the Branch Chief, all collaborate in studies on epilepsy which provide physiological, pathological, and clinical information. The physician is responsible for the programs in developmental defects and makes a considerable contribution to the epilepsy studies, as well as a unique addition through various investigations in child neurology. A psychologist heads the behavioral sciences effort in which a part-time psychiatrist plays an essential role, but many other members belong to this particular community of achievement. Last but not least, the new head injury projects are directed by a surgeon with special training in chemistry and physiology.

Like the head injury projects, all categories of achievement are disease oriented and clinically structured, although based in fundamental science. Thus laboratory and ward space is required for each goal-directed program. But all are severely limited in modular allotment and bed space. For example, the head injury program is based in two beds and one and one-half modules. Moreover, the severe limitations of bed space constrain the use of the new neurosurgical suite and prevent maximum exploitation of these excellent facilities. Finally, the lengthening lists of patients seeking admission to the Branch cannot be served with desirable dispatch because of these stringent spatial restrictions.

Despite these restrictions, the present and past productivity level has been high, consistent, and generally rewarding. The Branch looks to the future with an appetite for challenge, keen interest in achievement, and a sincere hope for help.

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

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

Previous Serial Number: Same

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: J. Van Buren, M.D., A. Ommaya, F.R.C.S., I. Klatzo, M.D.,
R. Farrier, M.D., C. Ajmone-Marsan, M.D., D. Sadowsky,
F. Lamberti, R.N.

Cooperating Units: CC-CP; N-B

Man Years:

Total:	2.3
Professional:	1.3
Other:	1

Project Description:

Objectives:

- a. To study causal mechanisms of epileptic seizures in man and other primates.
- b. To study the electrographic characteristics of epileptogenic activity in the brain of man and other primates.
- c. To study the approved methods of surgical therapy for these lesions and develop new therapeutic methods.

Methods Employed:

- a. Clinical neurological examination.
- b. Special radiographic and other contrast examinations.
- c. Electrographic, including electrocorticographic, examination.
- d. Electrophysiological techniques as indicated.
- e. Experimental epileptogenic lesions.
- f. Hypothermia.
- g. Cerebral drug deposition and scintillation counting of tagged drugs.
- h. Histological and chemical examinations as required.

Major Findings: During the period of this report, 71 patients with cerebral seizures were studied in the operating theaters and on the ward. (Report period 16 April 1965 through 15 April 1966.)

As previously noted, these patients were admitted for surgical therapy and received the now well-established evaluation, after which their suitability for corticography and focal excision was determined. They were studied by psychometric tests, psychiatric examination, depth electrode studies and, where appropriate, by electrocorticography. Illustrative electrocorticographic, therapeutic and psychiatric findings, as well as some of the information from depth electrode studies, are presented with this section of the annual report. The reader is referred to other sections within the Branch of Surgical Neurology for extension of the depth electrode work, including microelectrode studies, and for psychometric and psychological results as well as studies of epilepsy in childhood particularly including those related to diet.

A group of 18 centrencephalic patients were examined on tests designed to measure the effects of spike and wave type of EEG activity on sustained attention, sensory input, memory and simple motor behavior. In addition, measurements were made simultaneously of the following autonomic variables: blood pressure, heart rate, finger plethysmogram, skin resistance, esophageal and gastric motility and respiration. A total 1,267 bursts of spike and wave were recorded in conjunction with the several tests. The behavioral correlates of the bursts were analyzed from the point of view of character (form), organization, maximal discharge, voltage, frequency, length, the background activity and several time-related variables. All of these were shown to be related to behavior on one or more of the tests. Bursts which are symmetrical, regular and bilaterally synchronous tend to produce more behavioral deficit than other bursts; tests requiring the complete attentive act are more impaired during bursts than those which require only a part of this sequence; the motor task tended to be affected least. In addition, there is a retrograde amnesic effect. The most frequent autonomic changes were arrest of respiration, finger vasoconstriction, fall in skin resistance and increase in esophageal motility.

These patients were impaired on the attention test in the absence of observable bursts. The behavior loss tends to lead the electrographic symptom in time; in some patients, lack of behavioral change was observed even in the presence of well-organized symmetric bursts.

The persistence of focal discharge recorded from the precentral cortex after excision of analogous postcentral cortex has been studied, as have the functional representations from precentral cortex after postcentral excision. For example, a patient from whom postcentral gyrus representing tongue, lips, thumb and fingers was removed six years ago, has recently been subjected to electrocorticography as part of re-operation for focal seizures. Electrical stimulation of precentral cortex elicited a movement pattern similar to or identical with that evoked at operation six years ago despite the fact that concomitant postcentral gyrus had been removed. Similarly, focal discharges

arising from and around motor representation for thumb and fingers were recorded and were quite comparable to records obtained at the first operation. Neither the functional loss resultant from that ablation or the subsequent cicatrix had affected either normal or abnormal function in neighboring precentral gyrus. Originally, focal epileptogenic abnormality had been recorded from both post- and precentral gyri. The longevity and apparent consistency of the focal abnormality is of interest, as is the stability of motor responses from this gyrus.

In therapy, conventional subpial excision work has continued and the new therapeutic combination of head cooling and intravenous dilantin has been reapplied in several cases. One of these, a 2½ year old infant with massive cerebral damage presumed due to birth injury, was observed to have at least 20 seizures per day. Neurological examination revealed relative smallness of one hemisphere, contralateral hemiparesis and bilaterally abnormal reflexes. The electroencephalographic tracing showed diffuse abnormality, maximal over the left hemisphere, with some indication of subcortical or atypical centrencephalic discharges as well. Because of the bilateral reflex changes and the diffuseness of the EEG as a whole, focal excision and hemispherectomy were rejected as methods of treatment. The frequency and severity of attacks (sometimes leading into status) required treatment beyond that provided by referring physicians which had included numerous variations of accepted anticonvulsant medication as well as dietary regimes. After suitable preparation, the child was taken to the operating room where the head was cooled by direct application of ice after suitable protection of eyes and skin of face. Brain temperature was monitored by a thermister introduced through a twist-drill hole in the parietal region. The brain was cooled to 20°C and maintained there for 30 minutes, after which intravenous dilantin was given in the body-weight ratio as prescribed by the Schwab technique. Thereafter, the patient was returned to the ward in good condition. In the immediate post-therapeutic course, there was change in the electrographic tracing and considerable reduction in seizure activity. The tracing was considered less abnormal throughout, with particular reduction of left hemispherical epileptogenic activity and no further evidence of atypical centrencephalic abnormalities. The seizure frequency as observed on the ward was reduced to a few each day. This child has been followed through the referring physician over a period of seven months and has not had any further seizures since returning home. An adolescent of 17 years was similarly treated ten months ago and has not experienced any attacks since. This young person also had a hemispherical predominance of epileptogenic activity with a diffusely abnormal record. One young adult patient so treated has been free of attacks for 24 months. However, two patients subjected to focal cooling in the course of craniotomy and after electrocorticography did not respond to intravenous dilantin in combination with this regional heat exchange as evidenced by observed reduction of seizures and change in the postoperative electroencephalography. At this time it is assumed that the entire head must be cooled and, as previously noted, the brain temperatures recorded must be at least as low as 25°C for a minimum period of 30 minutes before the injection of dilantin.

In one patient with intractable focal motor seizures, diagnostic depth electrodes were placed in caudate and thalamus. Stimulation of caudate appeared to decrease frequency of abnormal discharges as recorded from scalp and depth EEG, that is to say, prior to caudate stimulation. 17 discharges in 12 seconds were recorded, while 5 were recorded during a 12 second stimulation and 15 in the 12 seconds immediately following the period of stimulation. No other electrode pairs in this patient produced decrease in the number of seizure discharges during their stimulation nor was there any particular correlation between the apparent reduction in abnormal discharges as observed, and the clinically evident motor phenomena. This may be a hint that some areas of striatum have an inhibitory function with regard to development and propagation of epileptiform discharges.

Two patients with temporal lobe seizures characterized by autonomic concomitants were subjected to the physiological monitoring parameters but, strikingly and surprisingly, no changes were noted at the time of recording despite the fact that clinical observation indicated various autonomic features of the pre-ictal and ictal periods.

In the laboratory, the propagation of afterdischarges from one mesial temporal region to the other is being studied through the means of stereotaxically placed electrodes within the mesial and basal components of amygdaloid nucleus in the macaque. This is being done with the use of the Fry apparatus and the concomitant x-ray controls of the placements. 5 ma. trains of stimuli from mesial amygdala on one side propagate afterdischarge to the homologous area of the opposite side. Electrolytic lesions of the anterior commissure will interfere with this propagation and its successful recording on the other side. The effects of amygdaloid and hippocampal stimulation in epileptic patients reveals slight, if any, plasma-17-OHCS changes which are associated with the particular type of electrical stimulation given.

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

These observations have contributed to the knowledge of epilepsy and its treatment.

Proposed Course of Project: The categorical nature of the patient material will be modified so that the primary selection criteria are etiological. Thus, post-traumatic epilepsy will be selected as a principal etiological category. In the past selection criteria have been derived from functional anatomy related to the origin of the attacks. It is anticipated that the effects of anticonvulsants at low temperatures will be observed in the future, and the micro- and depth electrode studies will be continued.

Honors and Awards: None

Publications:

Milhorat, T.H. and Baldwin, M.: A technique for surgical exposure of the cerebral midline. Transcallosal microdissection. J. Neurosurg. 24: 595-611, March, 1966.

Publications, continued:

Milhorat, T.H., Hautman, D.E. and Baldwin, M.: Epilepsy after rostral reticular formation excision. J. Neurosurg. 24: 687-691, March, 1966.

Mirsky, A.F. and Van Buren, J.: On the nature of the "absence" in centrencephalic epilepsy: A study of some behavioral electroencephalographic and autonomic factors. Electroenceph. clin. Neurophysiol. 18: 334-348, Feb., 1965.

Serial No. NDB(I)-54 SN/OC 101(c)

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Functional Representation in the Temporal Lobe of Man
and Higher Primates

Previous Serial Number: SAME

Principal Investigators: Maitland Baldwin, M.D. and A.K. Ommaya, F.R.C.S.

Other Investigators: J. Van Buren, M.D., R. Farrier, M.D., H. Lansdell, Ph.D.
G. Ojemann, M.D., F. Lamberti, R.N., R. Wadson, M.D.,
J.W. Mason, M.D., Richard Chase, M.D., Paul MacLean, M.D.

Cooperating Units: M-LP; N-B; M-LNP, Dept. Neuroendocrinology, WRAIR, WRAMC;
Berlitz School of Language; Neurocommunication Lab., Dept.
Psychiatry, Johns Hopkins Medical School.

Man Years:

Total:	2.3
Professional:	1.3
Other:	1

Project Description:

Objectives: Further understanding of a significant functional representation within the temporal lobe and related structures in man and other primates.

Methods Employed:

- a. Electrical stimulation and recording from human, chimpanzee and monkey temporal lobes; directly, after operative exposure; indirectly, by depth electrodes and scalp recordings.
- b. Ablation of all or parts of the temporal lobes and limbic systems.
- c. Surgical interruptions of connecting pathways.
- d. Use of drugs for stimulation and suppression, or other effects, on these systems.
- e. Test situation, utilizing machine or automatic programing.
- f. Other psychological and physiological examinations.

Major Findings: The clinical parts of this project have concentrated on language representations during the period of this report. Thus, a protocol has been developed for use in the operating room so that speech representations in the area of Broca and those in the posterior temporal and inferior parietal

cortices can be evaluated as part of a program series of data. After temporoparietal craniotomy performed for surgical treatment of epilepsy under local anesthesia, these speech representations are usually exposed. After customary stimulation of pre- and postcentral cortex, the patient is subjected to an object-naming test in which he sees a series of pictures representing familiar objects. Before each object he is required to say, "This is a ___." Using this test and a 5 ma. monopolar stimulating current applied to areas of cortex expected to represent speech, the language representations are demarcated with the usual sterile numbered tickets and the patient's response is taken down in shorthand and on the program tape. Thereafter electrocorticographic electrodes are placed in two rows of six across the speech representation so that the electrodes are separated by a distance of 5 mm. The patient's upper and lower lips are then connected to an electromyographic recording device through suction cups and while the electrocorticogram is running, the electromyogram recording, he hears a programmed test tape divisible into six parts of five minutes duration each. This test material is designed so that in its separate parts it presents non-verbal phonemic stimuli which the subject will repeat after hearing, will listen to but not repeat, will listen to and identify by the word yes, or deny by the word no, etc. The language test tape, the amplitude of patient's speech, the ECG, the EMG and the electrode arrangement on the cortex, as well as the electrical stimulation points related to speech on the cortex, are correlated in a program matrix. Thus, location of speech arrest and Broca, amplitude or frequency change in ECG, positive response in the EMG, test material and speech amplitude are correlated. At the time of this report, only Broca has been studied but this protocol will be extended to posterior temporal and inferior parietal regions as well. At that time, the test material will largely consist of verbal and/or semantic stimuli. Each patient subjected to this test series has had his lateralization for speech confirmed by the WADA test and has undergone considerable conventional psychometric and traditional language competence testing before operation. Patients with ictal arrest of speech or with demonstrable and particularly prolonged postictal aphasia are excluded from this study.

Five patients who have undergone right or left temporal lobectomy more than five years ago have been subjected individually to intensive language training. Each has received an average mean of 60 hours concentrated instruction in French. This instruction was part of an investigative protocol established with the use of normals and developed through instructional experience in Spanish and Vietnamese. The protocol has been described in a prior report and essentially consists of an 8-parameter physiological monitoring data set, eosinophil counts, urinary ketosteroid estimations, as well as a psychometric test series which includes Minnesota Modern Language Evaluation and examination through the test material of the Princeton Modern Language Series. Each patient was tested before and after the instructional period and subjected to physiological monitoring at the beginning and end of each day's instruction, with daily estimation of ketosteroid and four-hourly estimation of eosinophil counts. These data have been subjected to a program developed by the Computer Branch, NIH. The results of this off-line computation are not available at the time of the present report. However, overall performance

evaluation of these patients is available and is of some interest. All were right handed and all were left dominant. Four had undergone a right temporal lobectomy and one, left temporal lobectomy. Their individual characteristics are a part of a relatively well matched series of ten and so these five do not completely match. However, the left temporal lobectomized person and one right temporal lobectomized were closely matched for age, sex, full-scale performance, as well as neurological and social histories. Each was in the relatively low I.Q. range, averaging approximately 95 full-scale. These two were the poorest students and averaged less than 500 new French words. The other three (all right temporal lobectomized persons) were well matched by I.Q. and full-scale performance and were in the high full-scale range averaging approximately 120. These persons acquired more than 1,950 words after instruction. Since it is usually accepted that left temporal cortical removal in a left dominant person contributes to or produces a language and intellectual deficit, one might expect that the left temporal lobectomized person would perform poorly or be completely incompetent in this instructional situation. However, this patient performed poorly, but at the same level as a matched counterpart who had undergone right temporal lobectomy and who was also left dominant. One of the other patients who had undergone right temporal lobectomy and who had a relatively high I.Q. had suffered from a deficit in recent memory (which was demonstrable by some of the test series) after a severe head injury which occurred some time before his temporal lobectomy, ten years ago. The severity of this deficit prevents a gainful employment as an attorney and is subjectively described as an inability to remember words. Yet, this person completed his post-instructional examination at a first year college level and acquired approximately 2,000 words. None of these patients had had any satisfactorily completed instruction in French before this experience. Two had failed French in high school and one had dropped out of a first year college French course because of failure after one month. There was no particular indication from either the physiological monitoring, the eosinophil count, or the ketosteroid levels that these patients showed greater stress or responded more stressfully to the instructional situation than the normals which preceded them.

EEG tracings from the mesial temporal lobe of patients have been recorded during a paired associate learning situation structured so that the EEG can be recorded at the time a patient first makes the association and this compared to the EEG of pairs not yet associated, as well as those previously learned. EEG analysis has been by power spectrum on the Linc computer. The initial patient appeared to show a power spectrum pattern of a unique nature, from a left mesial temporal lobe structure during these occasions when an association was first learned compared to the unlearned or previously learned. Data on three other patients is now being analyzed.

In the laboratory, attempts are being made to excise the accessible parts of the limbic system in the macaque. As of this report, a surgical exposure which permits suction removal of pes, hippocampal gyrus, and cingulate gyrus on one side at one operation, has been designed. This has been applied on right or on left in a series of ten monkeys. These animals do not show any particular neurological or performance deficits after this extensive uni-

lateral removal. It is planned that the other side will be removed in the near future in each case.

Significance to Bio-medical Research and Program of the Institute: This project provides opportunity for increased understanding of functional representations related to language, memory and limbic counterparts of the temporal lobe systems. In its present form it is providing information concerning communication in the human and behavior in the monkey. The communications studies represent an attempt at further exploration of language representation in the temporal regions.

Proposed Course of Project: The protocol for analysis and correlation of language data derived from the temporal cortices will be continued in the operating room. Similarly, the results of foreign language instruction after temporal lobectomy will be gathered, with particular bias toward the acquisition of persons with right temporal lobectomy and right dominance, while the systematic matched patients series is followed through.

Honors and Awards: None

Publications:

Wadson, R.W., Jr.: Anxiety in the dreams of a neurosurgical patient.
Arch. Gen. Psychiat. 14: 249-252, March, 1966.

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Involuntary Movements

Previous Serial Number: Same

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

Other Investigators: C. Ajmone-Marsan, M. D., Herbert Lansdell, Ph.D.,
Choh-luh Li, M. D., G. A. Ojemann, M. D., E. S. Flamm,
M. D., Ayub K. Ommaya, F.R.C.S., H. E. Rosvold, M. D.

Cooperating Units: Branch of EEG, NINDB; Surgical Neurology, NINDB; Sec-
tion on Neuropsychology, NIMH

Man Years

Total:	3.0
Professional:	1.9
Other:	1.1

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 offered 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 which is presently not readily enlisted. Studies of motor pathophysiology are technically very difficult due to the necessity of quantitating human movement. Preliminary work, however, in this field is being undertaken.

The main objective in the present study has been to take advantage of the opportunities offered by the stereotaxic surgical treatment of the dyskinesias by means of recording and stimulation in depth in the human subject.

Methods Employed: As will be noted below, the variety of studies has entailed a wide variety of techniques. The studies of motor pathophysiology entail computer generated signals, and computer analysis is required for evaluation of results. Depth electrode stimulation employs specially built electrodes and a current monitored stimulator. Surface and depth microelectrode recording of the human brain makes use of specially built high impedance pre-amplifiers, provisions to reduce the effective capacitance coupling at the

input, tape recording of signals and eventual use of computer for averaging and cross correlation of cellular activity and other functions such as muscle activity and brain potential variations from larger electrodes. Autonomic recording makes use of a variety of input terminals predominantly of types presently commercially available. EMG and EEG recording is done with commercial instruments.

Major Findings: Sub-projects presently under consideration:

- (1) Alterations in the H reflex with thalamo tegmental stimulation and coagulation were sought after the receipt of the biphasic square wave monitored constant-current stimulator. The project was not found to be practical due to the instability of the h reflex and the great difficulty in obtaining even relatively stable baseline observations. At present this project is not being pursued.
- (2) Evaluation of motor function in dyskinesias by means of a visual rotor tracking task. In the past year some progress has been made in collaboration with the Section on Technical Development, NIMH/NINDB. At the present time two programs are in use employing the Linc computer for analysis. One is essentially an evaluation of reaction time and the other is a frequency analysis of the patient's responses to the visual signal. It has been found that the original test employed by the MIT Unit in which a patient followed a pseudo-random wave and the difference between his response and the original input analyzed in the form of Bode plots, was probably a fallacious test. This is due to the fact that no correction was made for distinguishing tremor or the voluntary introduction of movements in the higher frequencies. In essence, the problem at present is deciding exactly what function should be tested.
- (3) During the past year there was a period of six months in which essentially no satisfactory extracellular thalamic recordings were made due to technical difficulties. These have been largely circumvented by the fabrication of a recording unit which can be moved close to the patient thereby eliminating many of the difficulties inherent in long distance cabling. A variety of new equipment designed and fabricated by Mr. William Schuette has also materially improved the consistency of depth recording in man. Recording of cortical potentials is still a considerable problem due to pulsation artifacts, and no units have been held for any extended periods. Primary attention will be focused upon the periodicity of unit firing and the relationships between unit activity in the depth and on the surface and the electroencephalogram recorded in the close vicinity of the microelectrode at both sites. Relationships between the EMG and the unit activity will also be investigated. (See further details in Dr. Choh-luh Li's annual report)
- (4) EEG studies employing depth electrodes: Collection of material continues document:

1. The effect of lesions in the basal ganglia and thalamus upon the EEG

ii. Comparison of depth and scalp activity during normal and induced sleep (with Dr. Ajmone-Marsan).

(5) Study of evoked activity from peripheral stimuli has been temporarily laid aside due to insufficient personnel.

(6) The effect of thalamic lesions upon psychological tests immediately after coagulation and after one-year follow-up. Collection of data in this group continues (with Dr. Lansdell).

(7) Respiratory, heart rate and GSR responses from the human diencephalon. The above functions have been studied in twenty-four consecutive patients with implanted electrodes for treatment of motion disorders. Respiratory depression followed stimulation of electrodes in the medial frontal white matter, genu of the internal capsule and medial portions of the ventral-lateral thalamic nuclei. The second area of respiratory depression lay along the posterior-superior border of the lateral thalamus in the vicinity of the fornix and the stria terminalis. Respiratory depression from stimulation of electrodes located in or on the border of the ventral and oral portion of the thalamus had a significantly lower threshold on the left than in the right brain. This was not true of respiratory depression evoked elsewhere. Bradycardia followed stimulation of electrodes in the posterior border of the pulvinar. Tachycardia was evoked from the superio-lateral edge of the hypothalamus. Changes in skin resistance seemed to be related to the presence of subjective sensations during stimulation. (Collaborative studies by Dr. G. A. Ojemann)

(8) Aphasic responses from depth stimulation. Electrodes directed to the therapeutic target in the base of the nucleus ventralis lateralis have been placed through both frontal and parietal burr holes on both sides of the brain in man. Stimulation in the fronto-striatal area on either side has produced the "arrest response" which was discussed in more detail in the previous annual report. The study of the effects of depth stimulation from the posteriorly placed electrodes with the use of a special test requiring the subject to name an object flashed on the screen, then to read the word "and" before another object is shown to him. It has been demonstrated that stimulation in the dominant thalamus (pulvinar region) and in the deep parietal radiations of both sides may produce inability to name simple objects. This has not been seen from stimulation at similar thresholds in the nondominant thalamus. When stimulation of sufficient intensity to produce aphasia is carried out during a continuous performance task, the patient shows no impairment. This is opposed to the marked impairment in this test seen with the fronto-striatal arrest response (Collaborative study with Dr. G. A. Ojemann, Dr. Paul Fedio and Dr. H. E. Rosvold).

(9) A method of relating commissural landmarks to the skull (Hassler and Riechert) has been tested in cases when two separate pneumoencephalograms were done. The anterior and posterior commissures and the midline third ventricle of the first pneumogram were reconstructed on the second. These were then compared with the position of the actual commissures on the second pneumogram. The mean variations and the standard deviation seemed acceptable in the vicinity of the AC-PC line and the third ventricle, but 3 to 4 cm.

away the variations possible with additive errors assumed sufficient size so that use of a ventricular landmark closer to the site to be localized would seem advisable (Collaborative study with Dr. E. S. Flamm).

(10) i. The lateral gastrocnemius-soleus nucleus in the cat has been investigated by retrograde cell change and found to lie predominantly within the L7 segment of the spinal cord and to measure at a maximum about $10.0 \times 0.6 \times 0.3$ mm. In the cell group occupied by the nucleus, two predominant sizes of neurones were found having peak incidences of minimum soma diameter at 20μ and $40-50\mu$. The density of the small cells was about 399/cu. mm. and of the larger about 630/cu. mm. Within this group the maximal density of larger neurones projecting to the 1. gastrocnemius-soleus nerve was about 160/cu. mm.

ii. An indirect method of estimation of the population of the 1. gastrocnemius-soleus nucleus suggested that the smaller cells in the nucleus (in ratio of about 2 small to 3 large cells) must project to the periphery in order to obtain results comparable to counts made directly of the cells showing retrograde chromatolysis. It is suggested that this is the motor supply to the intrafusal fibres.

iii. Golgi studies of the cells in the region of the 1. gastrocnemius-soleus showed that they had a diffusely radiating dendritic pattern in the transverse plane (up to $1500-2000\mu$ in diameter) but when viewed in horizontal section the dendrites tended to show a transverse orientation.

iv. The antidromic response to stimulation of the 1. gastrocnemius-soleus nerve showed variation in the number of participating elements but the magnitude of the potential field was not greatly different from that of known single neurone fields. The fields approximated the size of the dendritic arborization of the larger cells.

v. The gradient of the initial positive deflection with distance (ascribed to activity within the myelinated segment of the axon) was less than that of the major negative deflection (AD spike) of the antidromic action potential.

vi. The gradient of the AD spike when recorded with a $2-4\mu$ pipette in its "normal" negative going form was quite similar to its "injured" positive form recorded with large electrodes (Collaborative study with Dr. Karl Frank).

(11) A human stereotaxic instrument has been in use since 1960 which embodies the following features: 1) Localization is achieved by fractional pneumography by routine demonstration of both the anterior and posterior commissures without the use of radiopaque oils; 2) Permits use with standard x-ray equipment; 3) Permits full surgical draping; 4) Instrument may be aligned with the intracerebral axes; 5) Full three plane graduated movement; 6) Permits skull entry at any point (apart from the central area at the vertex which underlies the apparatus) without the use of phantom target points or recomputation; 7) Precise realignment for staged procedures.

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

Recording and stimulating deep structures in the brain by implanted electrodes and electrodes introduced at the time of surgery, allows a variety of studies not heretofore possible. The use of a human subject who is cooperative and unanesthetized easily permits many tests of psychological function as opposed to the much more time consuming conditioning experiments and cruder tests which must be applied in lower animals. Quantitative work on the pathophysiology of the actual motor disorder remains an exceedingly difficult problem and it is still too early to assess the value of the present efforts.

Proposed Course of the Project: Plans for this work entail further experience in depth recording and stimulation. The technical feasibility of chronic recording with microelectrodes has been demonstrated recently along with use of averaging techniques and the value of these capabilities will have to be further assessed. Earlier reports from other sources have indicated characteristic unit activity in various thalamic structures. This is being evaluated and more experience with individual patients is required. In the coming year, particular attention will be paid to the effects of depth stimulation upon special psychological tests such as tests of speech and speech recognition. Attempts at further objective evaluation of motor function, in terms of power spectrum analysis and reaction times, will continue.

Honors and Awards: None

Publications:

Van Buren, J. M.: Pathophysiological and neurosurgical aspects. In Spiegel, E. A., Barbeau, A. and Doshay, L. J. (Eds.): Parkinson's Disease, Trends in Research and Treatment. New York, Grune & Stratton, Inc., 1965, p. 113.

Van Buren, J. M.: Incremental coagulation. In Spiegel, E. A., Barbeau, A. and Doshay, L. J. (Eds.): Parkinson's Disease, Trends in Research and Treatment. New York, Grune & Stratton, Inc., 1965, pp. 155-156.

Van Buren, J. M.: Evidence regarding a more precise localization of the posterior frontal-caudate arrest response in man. J. Neurosurg. 24, Part II (Suppl): 416-417, Jan. 1966.

Van Buren, J. M.: Incremental coagulation in stereotactic surgery. J. Neurosurg. 24, Part II (Suppl): 458-459, Jan. 1966.

Van Buren, J. M., and Frank, K.: Correlation between the morphology and potential field of a spinal motor nucleus in the cat. Electroenceph. clin. Neurophysiol. 19: 112-126, Jan. 1965.

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Study of the Functional Anatomy and Pathology of the Human Visual System

Previous Serial Number: Same

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

Other Investigators: Peter Gouras, M. D.

Cooperating Units: **Ophthalmology Branch, NINDB**

Man Years

Total: (See Dr. Gouras report)

Professional:

Others:

Project Description:

Objectives: The major objectives of this project, which were originally to relate the subjective aspects of vision to anatomy of the human visual pathways, were largely fulfilled in the publishing of the volume, "The Retinal Ganglion Cell Layer". Further material remained, however, for reconstruction studies in the retina. A collaborative study proposed by Dr. Gouras offered the opportunity to access the stages of retinal degeneration in a dynamic fashion.

Methods Employed: An attempt has been made to add a quantitative aspect to anatomy by reconstruction techniques and these are again proposed for the studies of the retina cited below. Microelectrode recordings from retinal ganglion cells in the intact monkey eye are now feasible without entailing the destruction of the animal after the experiment.

Major Findings:

(1) The material collected (14 pairs of retinae in serial section) for evaluation of the effects of papilledema upon the ganglion cell population, remains on file. Evaluation awaits personnel.

(2) Two macaques have had the optic chiasm transected in the midline. Dr. Gouras is making microelectrode recordings of ganglion cell population at monthly intervals by scleral punctures. The location of the recording sites is being recorded with retinal photographs. Recordings are made both on the degenerating retinal half and the lateral retinal half. At approximately a year from original

operation (Fall of 1966) the animals will be killed and later reconstructions carried out. These will form the anatomical control for the retinal unit activity.

In the first months of recording, remarkably little change was seen in the retina and units continued to be driven on occasion by flash. Interest has centered upon the possibility of demonstrating electrophysiological deterioration as the ganglion cells and bipolar layer deteriorate.

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

Correlation of form and function has always been a prime object in all clinical neuro-ophthalmology and the present studies attempt to perpetuate this tradition. The new techniques of transcleral recording of ganglion cell activity in chronic preparations have permitted the Neurophysiologist to contribute a dynamic aspect to what was previously a static anatomical approach.

Proposed Course of the Project: Due to lack of space for collaborative workers, much of this project remains static. The work, however, has a considerable potential value and will be pursued as time permits.

Honors and Awards: None

Publications: None

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

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

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

Previous Serial Number: Same

Principal Investigator: Dr. J. M. Van Buren

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	.3
Others:	.7

Project Description:

Objective: The investigation centers upon study of the topography of the main nuclear masses in the human thalamus with collaborative work in higher primates. The orientation of this anatomical work is to provide an anatomical substratum for the human electrophysiological study.

Methods Employed:

- (1) Embedding of gross formalin fixed human brains in jeltrate for macro-sectioning then superimposition of drawings for the variation atlas.
- (2) Serial section of human and animal brains in celloidin for myelin and Nissl series.
- (3) Section and staining of primate brains with Nauta technique for demonstration of degenerating pathways.

Major Findings:

- (1) The gross variations of 16 hemispheres prepared in the sagittal plane were reported some time ago. In the past year, 24 brains were prepared in both horizontal and transverse planes and superimposition drawings of all this material made at 5 mm. intervals with a relation to both the anterior and posterior commissures. Additional drawings were made of each superimposition diagram demonstrating the maximum, minimum and average outlines of basal ganglia structures at all levels.

(2) Four to five human brains have now been prepared in myelin and Nissl series in serial section in each of the three cardinal planes related to the anterior-posterior commissure line and the midline of the third ventricle. Material is also available (some 30 brains) for establishment of thalamo-cortical degenerative relationships in man.

(3) During the past year, three macaque brains and one chimpanzee brain with lesions of the superior cerebellar peduncle have been prepared with the Nauta technique to define the areas of projection of these fibers in the thalamus. It has been found that the incoming thalamic fibers pass well above the region of V. o. p. (Hassler) pass into the upper third of the thalamus. This renders at least one of Hassler's major divisions untenable and may indeed end by discrediting at least a part of this system of thalamic division.

Proposed Course of Project:

(1) The variation atlas is now complete for the gross sections.

(2) Work is now starting on the variation of the major architectonic subdivisions in the human thalamus reconstructed in all three planes. After achieving a reasonable thalamic division which can be reliably seen in all three planes, reconstruction of the cortical thalamic degeneration will proceed.

(3) One of the difficult remaining problems is the transition between lateralis posterior and ventralis lateralis. There seems little to demarcate this border on a cytoarchitectonic basis. In order to elucidate this, lesions have been made in the area behind the postcentral gyrus extending backward to the parietal occipital fissure and then laterally to the angular gyrus in the chimpanzee. This material is still being processed as well as that of a second chimpanzee with lesions of the superior cerebellar peduncle. Decision as to the separation between the ventralis lateralis and lateralis posterior in man may have to await completion of these preliminary anatomical investigations in the chimpanzee.

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

Knowledge of the variations and the anatomy of the human basal ganglia and thalamus is essential for plotting of human subcortical electrodes and lesions. Since material of this sort is not presently available, the present atlas is required.

It has been pointed out for several years that this project is well formulated and ready for intensive work. Because of the lack of space precluding a local collaborator at a professional level, the work progresses at a much slower level than is desirable. The meaningful interpretation of all human depth studies is entirely dependent upon these basic anatomical studies.

Honors and Awards: None

Publications: None

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

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Single Cell Discharges in Motor Cortex in Response to Stimulation of Basal Ganglia and Cerebellum

Previous Serial Number: SAME

Principal Investigators: Choh-luh Li, M. D., Ph.D.
Maitland Baldwin, M. D.

Other Investigators: None

Cooperating Unit: None

Man Years

Total:	2.0
Professional:	1.5
Other:	0.5

Project Description:

Objective: This is a continuation of the investigation reported last year. The purpose of this investigation is to gain more information about the relation between the extrapyramidal and pyramidal systems, first in laboratory animals, then in human patients.

Methods Employed: The spike activity from the motor cortex in response to stimulation of various subcortical and cerebellar structures is recorded on motion picture film as well as on magnetic tapes and electronic digital recorders. Cats either subjected to barbiturate or mid-brain transection are used.

Major Findings: Aside from those reported last year, it was further observed that the "mid-brain reticular formation" may play a role in the transmission of impulses from the cerebellum to the cerebral motor cortex. The transmission mechanism through this subcortical structure was found to be unaffected by barbiturate anesthesia. Finally, preliminary observations showed inhibition and facilitation of cortical motor neurons in response to transcallosal stimulation.

Significance to Bio-Medical Research: The recent advance in surgical treatment of patients with Parkinson's disease and those with motor dysfunction demands an understanding of the interaction of the pyramidal and so-called extrapyramidal system. It is known that a surgical lesion in the thalamus or in the basal ganglia may alleviate the symptoms of these diseases

but the rationale of this operation is not completely known. This investigation, which is carried out in laboratory animals, could allow thorough exploration of all the neuronal structures related to motor function and may eventually uncover the physiological basis of these diseases.

Proposed Course of Project: This investigation is to be continued and will further establish the role of association fibers, especially the trans-callosal fibers, upon the activity of cortical motor neurons.

Honors and Awards: None

Publications:

Li, C-L., and Tew, J. M., Jr.: The effect of cerebellar stimulation on neuronal activity in the motor cortex. Exp. Neurol. 14: 317-327, March 1966.

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Effects of Cold and the Relation of Temperature to
Functions of the Central Nervous System

Previous Serial Number: Same

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

Other Investigators: Philip Yarnell, M.D., Fonda Ghiardi

Cooperating Units: None

Man Years:

Total: 2.2

Professional: 1.5

Other: .7

Project Description:

Objective:

a. A study of comparative temperature gradients in nervous and other tissues under various normal and abnormal conditions and in response to heating and cooling of small areas (in cats).

b. A study of the effect of freezing temperature on blood vessels of varying calibre in animals (cats and monkeys).

c. A study of the effect of moderate hypothermia (28-32° C.) on the viscosity of blood in man and animal.

Methods Employed: The following instrumentation and techniques are being used or developed:

a. A 24-point multiple-pattern thermocouple array for temperature gradient recording. This array consists of micro-thermocouples arranged around a central probe shaft which can be used for heating, cooling, or biopsy. The data is to be recorded through a Dymec data acquisition system on punched tape, thus facilitating analysis.

b. A differential temperature thermocouple to study related problems in situations where the 24-probe array cannot be used.

c. Angiographic X-ray demonstration of zones of freezing block and other changes in blood vessels of cats.

d. Construction of a new system for focal heating and cooling within the nervous system.

e. A series of temperature gradient measurements is being made in water, gelatin and other gels, and fluids of known viscoelastic properties.

Major Findings: Confirmation of findings in 1964-1965. The study of thermal gradient has been further delayed by instrumental difficulties but is now re-established. However, the results are insufficient to warrant any conclusions.

The study of the effect of urea on the type of cerebral edema produced by freezing cold was pursued. This series of experiments revealed equivocal results and has been discontinued.

Significance to Biomedical Research and the Program of the Institute: The direct applications of the findings on freezing lesions are obvious. The observation of ease of reversibility of freezing block of blood flow in larger vessels suggests the use of a freezing probe in the management of the more difficult cerebral aneurysms and other vascular malformations. Thus the definitive treatment of occlusion by clipping or coating with plastics, etc. could be done without fear of premature rupture by freezing the parent artery, or aneurysm itself. In the frozen state, the lesion is easily dissected and since the freezing block is reversible, there is no danger of unwanted ischemic complications.

The implications of the viscosity study would suggest that the increased viscosity of blood in moderate hypothermia is part of some general effect and that the high incidence of postoperative thrombosis after hypothermia is related to this rather than to direct handling of blood vessels.

The temperature gradient study will provide basic data on the comparative thermal properties of brain and other tissue which will allow direct estimation of thermodynamic aspects of their function. From points of difference and/or similarity, considerable insight could be obtained into the nature of these functions in vivo.

Proposed Course of Project: The analysis of normal and abnormal temperature gradients and thermal properties of tissues will continue. With the system for local cooling, we propose to investigate the effects of prolonged moderate cooling and freezing on gliomas in situ as a method of treatment. This will be coordinated with the new project on gliomas of the nervous system.

Honors and Awards: None

Publications:

Ommaya, A.K. and Coe, J.: An experimental appraisal of cryogenic brain lesions in the cat. Confin. Neurol. 26: 10-13, Jan. 1965.

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Trauma to the Nervous System. Experimental and Clinical.

Previous Serial Number: Same

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

Other Investigators: A. Hirsch, R. Mahone, F. Faas, M.D., E.S. Flamm, M.D.,
D. Goldman, P. Yarnell, M.D., H. Richter, M.D.,
F. Ghiardi, W.H. Marshall, M.D. and D.B. Tower, M.D.

Cooperating Units: Personnel Protection Branch, David Taylor Model Basin;
Laboratory of Biophysics, NMRI; Laboratory of Neuro-
chemistry, NINDB; Laboratory of Neurophysiology,
NINDB-NIMH.

Project Description:

Objective: A clinical and experimental study of the biophysics, physiology and pathology of trauma and its effects on the central nervous system in order to enable prediction of the results of such trauma in man and to establish its management on a rational basis.

Methods Employed:

a. The Field Emission type of ultra-high-speed x-ray apparatus is now in use and preliminary experiments with carotid angiography and radio-opaque markers are in progress.

b. Lucite calvarium and lucite "window" monkeys are being prepared and studied by high-speed cinematography during impact for measuring actual brain displacements.

c. The mechanical impedance of the living monkey head and its structural components is being investigated with apparatus permitting direct recording of the mechanical impedance and phase angle changes, in both translatory and rotatory movement.

d. The electrical impedance, EEG, and D.C. potential changes of the monkey cortex are being recorded under supervision of and in the laboratory of Dr. Wade Marshall. The response of these observations to various concentrations of CO₂ before and after experimental concussion is being pursued in collaboration with Dr. Marshall.

e. The arterial and C.S.F., pO_2 , pCO_2 , and pH are being measured simultaneously in patients after severe head injuries and in monkeys before, during and after experimental concussion.

Major Findings:

a. Sufficient data on occipital blows has accumulated to establish 10, 50, and 90 per cent "dosage" curves for concussion in terms of impulse of blow and linear acceleration of head. These curves arrived at by probit transformation enable rigorous statistical comparison of blows to frontal and temporal positions and are also serving as a baseline on which tests for various hypotheses of the mechanics of cerebral concussion are being made. These include the effect of a cervical collar, head fixation, increasing the head mass, shifting the center of gravity of the head, and the protective effect of various devices. The most important observation to date is the finding that a cervical collar protects against the concussive effects of impact to the head in monkeys.

b. Cardiovascular effects after head injury have been found to be of considerable significance. A period of arterial hypotension invariably follows a concussive blow and this is associated with a widening of the pulse pressure, a moderate increase in the central venous pressure, bradycardia and other electrocardiographic abnormalities. The latter particularly seem to be significantly related to the prognosis following the blow. Persisting EKG abnormalities of either rate or pattern were invariably associated with a fatal outcome after a concussive blow. The slowing of the dye-circulation noted by cerebral angiography has been further studied and appears to be biphasic in nature. Slowing is noted within the period 10-30 seconds after the blow; this is followed by a return to normal circulation after approximately 5 minutes, and then 15-30 minutes later a second slowing occurs, persisting for a few hours. It is our intention to pursue this aspect further with blow flow studies.

c. The theoretical study is in abeyance due to delay in assigning the funds allotted by the Bureau of Weapons to this aspect of the program. Thus the services of a consultant mathematician have not been available.

d. The study on electrophysiology of peripheral nerve under shear and tensile stress is also in abeyance until further technical help is available.

e. The study of blood and C.S.F. gases and pH has revealed to date a considerable lag between the two compartments, particularly for pO_2 . Rapid alterations in the C.S.F. compartment can be made with little or no effect on the blood. The changes in these factors following various degrees of head injury is now being pursued.

f. The biochemical studies have been re-established with the help of Dr. F. Faas and Dr. D. Tower.

g. Studies on spreading depression, D.C. potential, cortical electrical impedance and response to pCO₂ alterations after experimental head injury in collaboration with Dr. Wade Marshall are in progress. To date, it does not appear that spreading depression is produced by the blow. However, there is some indication that the effect of increased pCO₂ on the D.C. potential is reversed immediately after the blow.

h. Histological studies including routine stains and Marchi techniques by Dr. Sabina Strich at the Maudsley Hospital on 12 monkey brains have not shown significantly constant microscopic lesions at any site. These specimens included 2 normal control brains, 4 brains after non-concussive trauma, and 6 brains after concussive trauma.

Significance to Biomedical Research and the Program of the Institute:

The findings so far are changing our previous concepts of the mechanics of brain injury and in addition to improving the treatment of head injured patients, provide increasing insight into basic neural functions.

Proposed Course of Project:

a. The high-speed x-ray and cinematographic methods will be pursued to decide finally the question of degree and nature of brain distortion and movement after head injury.

b. Rheological studies will be made on fresh in vitro and in vivo samples of nervous tissues to establish fundamental data on the viscoelastic properties of these tissues and their behavior under stress.

c. Input from the above and from the ongoing mechanical, biochemical, and physiological studies will be used to develop the theoretical analysis and produce a theoretical model for the biochemical behavior of the brain in response to trauma.

d. Controlled "whiplash" and whole-body acceleration trauma experiments in monkeys will be conducted to round out other aspects of the mechanics of injury.

e. Prophylactic and therapeutic measures aimed at reducing the noxious and enhancing the beneficial responses after trauma will be studied experimentally in the monkey.

f. Autoradiographic and electromicroscopic studies will be pursued to determine if some morphological correlate of concussion can be vigorously established.

Honors and Awards: None

Publications:

Ommaya, A. K.: Trauma to the nervous system. Clinical and experimental studies. (In press) Annals of the Royal College of Surgeons of England

Ommaya, A. K.: Experimental head injury in the monkey. In Caveness, W. F. and Walker, A. E. (Eds.): Head Injuries. J. B. Lippincott, Philadelphia. (In press)

Mahone, R. M., Hirsch, A., Flamm, E. S. and Ommaya, A. K.: Production of brain concussion in the Rhesus monkey. (In press) J. Aviation Med.

Serial No. NDB(I)-62 SN/CC 913(c)

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

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Single Cell Discharges from Cerebral Cortex of Man

Previous Serial Number: SAME

Principal Investigators: Choh-uh Li, M. D., Ph.D.
Maitland Baldwin, M. D.
John Van Buren, M. D.

Other Investigators: Eugene S. Flamm, M. D.
Howard Richter, M. D.

Cooperating Unit: Instrument Engineering & Development Branch, NIH, DRS

Man Years

Total:	3.0
Professional	2.5
Other:	0.5

Project Description:

Objective: To continue the investigation reported last year in the study of single cell discharges recorded from the cerebral cortex and thalamus of patients with epileptic and motor disorders. The purpose of this study is to gain insight into the physiological mechanisms by which these diseases are produced.

Methods Employed: The instrumentation for this study has been markedly improved during the past year. At present, unitary action potentials from the cortex, thalamus and muscles in the patient are recorded on magnetic tapes. Attempts are also made to disclose changes of activity in these structures in response to tactile and photic stimulation.

Major Findings: Data has been accumulated for statistical computation. At present, no definitive statement could be made before this computation is completed.

Significance to Bio-Medical Research: The present investigation will lead to a new understanding in the sensory and motor function of the brain in man.

Proposed Course of Project: This study is to be continued and similar studies will be conducted in chimpanzees so that the progress will not be limited by human factors.

Honors and Awards: None

Publications: None

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

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Tumors of the Nervous System

Previous Serial Number: Same

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

Other Investigators: David Rall, M.D., M. Baldwin, M.D., M. Walker, M.D.

Cooperating Units: Section of Pharmacology, NCI

Man Years

Total: .8

Professional: .4

Other: .4

Project Description:

Objective: To understand how gliomas develop and grow and how best they can be treated in man.

Methods Employed: A special silicone device described as a C.S.F. reservoir has been designed by the principal investigator. This allows sterile access to the ventricular C.S.F. by subcutaneous puncture. This device allows perfusion of the C.S.F. pathways with fluids containing cytotoxic agents e.g. Methotrexate.

Major Findings: To date, twelve patients with glioma and three with meningeal leukemia have received approximately four perfusions each with Methotrexate. Much larger doses have been given in the last four patients, and in two a significant remission is apparent. However, it has become evident that there is no adequate method available to decide whether the tumor has indeed been destroyed. Further attempts are being made to obtain a suitable animal model. Currently, only two beds are available for these patients, and it is hoped that more will be forthcoming. A new drug being tested now (in one patient) is 8-Azo-guanine.

Significance to Biomedical Research and the Program of the Institute: It is hoped to improve the presently inadequate treatment of glial tumors of the nervous system.

Proposed Course of Project: In addition to C.S.F. perfusion treatment with various cytotoxic agents, it is proposed to conduct some tissue culture and animal glioma experiments with the ultimate view of establishing a reliable and practical method of curing these lesions. An attempt will be made to coordinate our techniques and results with those of other workers in this field in order to determine the following: (1) The most suitable drug; (2) The most suitable patient; (3) The most suitable route; (4) The most suitable program of drug administration; (5) A method for testing for tumor response.

Honors and Awards: None

Publications:

Rubin, R.C., Henderson, E.S., Ommaya, A.K., Walker, J.T., Jr. and Rall, D.P.: Cerebrospinal fluid production, bulk flow and modification by acetazolamide in the human. (In press) J. Neurosurg.

Rubin, R.C., Ommaya, A.K., Henderson, E.S., Bering, E.A., Jr., and Rall, D.P.: Cerebrospinal fluid perfusion therapy for the treatment of central nervous system neoplasm. (In press) Neurology.

Witorsch, P., Williams, T.W., Jr., Ommaya, A.K., and Utz, J.P.: Intraventricular administration of amphotericin B. Use of subcutaneous reservoir in four patients with mycotic meningitis. JAMA 194: 699-702, Nov. 1965.

Ommaya, A.K., Hirsch, A.E., Flamm, E.S., and Mahone, R.M.: An experimental model for cerebral concussion in the primate (Malaca Mulatta). (In press) Science.

Serial No. NDB(I)-65 SN/OC 1205(c)

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

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Neurosurgical Monitoring

Previous Serial Number: Same

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: Eugene Harris, M.D., Robert Farrier, M.D., George Ojemann, M.D., Ayub Ommaya, F.R.C.S., John Van Buren, M.D., Gerald Cohen, B.E., Paul Fedio, Ph.D., M. Haddock, R.N.

Cooperating Units: R-BEI; CR-DSA

Man Years

Total:	3.2
Professional:	1.7
Other:	1.5

Project Description:

Objectives:

1. Physiological parameters of neurosurgical operations.
2. Processing of data thus derived.
3. Estimation of clinical and physiological significance of correlations obtained from such data.
4. Physiological effects of cortical and subcortical electrical stimulation.

Methods Employed:

1. Application of the Sanborn Physiologic Data Monitoring System.
2. Recording from patients during operation.
3. Recording from non-operated patients.
4. Recording from normal controls.
5. Data programming by the Computer Branch in accordance with a collaborative plan.

Major Findings: Earlier preliminary analysis has indicated a correlation between alpha frequency and skin temperatures. This is currently being examined more thoroughly using techniques of time series analysis (auto- and cross-correlation, spectral analysis) to determine the nature and validity of

this correlation within finite time spans over the entire course of the experiment and to compare the strengths of this relationship among groups of subjects undergoing different degrees of psychological testing.

Changes in heart rate, respiratory rate and galvanic skin resistance during thalamic stimulation have been recorded and noted. Apparently there is an assymetry in evoked respiratory depression between right and left thalamus.

Visual and auditory evoked responses from scalp of patients with adrenalin insufficiency, on and off treatment, were studied. Evoked responses were obtained by a summing technique on the Linc computer. The latency of the visual evoked responses was found to be 10-20 msec. longer in those patients off treatment compared to those in a treated condition.

Visual evoked responses have also been measured from two thalamic electrodes that were unusually deep. The most distal of this pair was close to the optic tract, while the most proximal lay in posterior pulvinar and parietal white matter, thus bridging the relay in the visual pathway across the lateral geniculate. In the first of these patients there was an increase in latency and change in wave form of the sum visual evoked responses across several of the thalamic electrodes and between the most proximal thalamic electrodes and scalp.

Neurosurgical monitoring concomitant with language studies, memory testing, studies in relation to epilepsy and Parkinson's syndrome are reported elsewhere in the contents of the Branch report.

Significance to Bio-medical Research and Program of the Institute: This project has scientific as well as clinical significance. It is designed to provide physiological recording of various functions following electrical stimulation of the human nervous system. It now provides the neurosurgeon with a prototype system for a sophisticated recording of a patient's operative course. Through data processing it is hoped that it may provide a measure of predictability (for the surgeon) of the patient's future course in the operating room as well as significant physiological correlations.

Proposed Course of Project: It is proposed that further baseline data be obtained from normal subjects and that the patient material evaluated by the monitoring system will be diversified and enlarged. A plan has been formulated for correlation of data obtained from these systems with biochemical profile data obtained in clinical pathology. It is hoped that this combination and correlation will provide further significant relationships.

Honors and Awards: None

Publications: None

Serial No. NDB(1)-65 SN/OC 1206(c)

1. Surgical Neurology Branch

2. Office of Chief

3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Microbial Characteristics in a Neurosurgical Environment

Previous Serial Number: Same

Principal Investigator: Maitland Baldwin, M.D.

Other Investigators: R. J. Weatherby, F. Lamberti, R. N.

Cooperating Units: R-ES, CC-EHS

Man Years:

Total:	1
Professional:	.7
Other:	.3

Project Description:

Objectives: Analysis of salient microbial features in a neurosurgical room environment.

Methods Employed:

- a. Personnel and environmental microbial sampling.
- b. Analysis of physical environment.
- c. Analysis of personnel structure.

Major Findings: During the period of this report, the microbial characteristics of the new surgical wing facilities have been studied extensively in order to provide practical controls for the various surgical techniques employed in that environment and also to provide a data basis for comparison with microbial sampling being undertaken in a laminar air flow system. The major findings listed in the new surgical suite during the period of this report are given below:

PRELIMINARY RESULTS ON NEUROSURGERY SURVEYS, 1965

<u>All scrubbed personnel</u>		<u>Number</u>	<u>Number per Rodac (mean) (4 sq. in.)</u>	
Cap	before	33	1.1	
	after	32	1.1	
Mask	before	33	4.8	
	after	32	38.4	
Shoe sole	before	31	96.0	
	after	29	69.2	
<u>a. Surgeons</u>				<u>% with HSA</u>
Cap	before	8	1.3	12.5
	after	8	1.4	0
Mask	before	8	2.1	0
	after	8	1.8	12.5
Shoe sole	before	5	145.6	0
	after	8	93.6	20
<u>b. Asst. Surgeons</u>				
Cap	before	15	0.5	0
	after	15	1.3	7
Mask	before	15	0.3	0
	after	15	6.5	13
Shoe sole	before	15	116.4	13
	after	15	50.0	13
<u>c. Scrub nurses</u>				
Cap	before	10	2.2	0
	after	9	0.3	0
Mask	before	10	13.8	0
	after	9	1.3	0
Shoe sole	before	8	86.7	12.5
	after	6	85.0	0

<u>d. All circulating personnel</u>			Total count per Rodac (mean)	% with HSA
		<u>Number</u>		
Cap	before	30	5.8	7
	after	19	10.8	5
Mask	before	30	4.6	3
	after	18	4.6	0
Shoe sole	before	25	117.8	8
	after	17	86.8	12

e. All anesthesia personnel

Cap	before	16	18.9	6
	after	10	27.6	10
Mask	before	16	17.0	19
	after	10	55.7	50
Shoe sole	before	10	140.2	0
	after	6	145.7	33

<u>Surgical instruments</u>	<u>Number</u>	No growth	<u>Growth</u>	% with HSA
before use	22	16	6 (27%)	0
after use	15	8	7 (47%)	7

Mean/glove% with HSAGlove cultures

Surgeons	96.7	9
Asst. surgeons	244.0	5
Scrub nurses	97.7	0
All persons	116.5	

<u>Holes in gloves</u>	<u>Number</u>	<u>% with holes</u>
Surgeons	34	26
Asst. surgeon	41	29
Scrub nurses	29	21

Number of Organisms on 5 Finger Tips, All Cases

	<u>No.</u>	<u>0</u>	<u>1-50</u>	<u>51-100</u>	<u>101-150</u>	<u>% w/HSA</u>
Surgeons						
Before scrub	16	0	81	19	0	44
After scrub	16	50	44	6	0	0
Gloves off	16	56	44	0	0	0
Asst. surgeons						
Before scrub	22	5	77	14	4	55
After scrub	22	50	50	0	0	0
Gloves off	22	59	41	0	0	0
Scrub nurses						
Before scrub	18	0	72	28	0	0
After scrub	18	11	83	6	0	0
Gloves off	20	25	60	15	0	0

	<u>No.</u>	<u>% Positive</u>	<u>#/ml. when positive</u>
Wound washings	12	25	10, 125, 140
Irrigation solution	20	5	1

	<u>No.</u>	<u>% Positive</u>
Scrub sink faucet heads		
Before use	14	93
After use	16	12.5
	<u>No.</u>	<u>Organisms per Plate</u> <u>(2.4 sq. in.)</u>
Patient skin preparation		
Before preparation	10	22.2
After preparation	10	0.2
Sutures in	10	19.0

Summary of Floor Cultures as # Mean/Rodac (4 sq. in.)

	<u>Before</u>	<u>% w/HSA</u>	<u>After</u>	<u>% w/HSA</u>
Anesthesia room	14.5	0	30.1	0
Corridor by O.R.	5.4	14	10.3	14
Scrub room	12.3	0	20.0	0
Side of op. table by inst. table	0.4	0	56.8	0
Side of op. table by anesth.	0.0	0	7.0	0
Head op. table	0.7	0	55.1	25
Foot op. table	0.6	0	6.9	0
O.R. near work room door	1.4	0	4.4	0

Locker Room Floor Cultures, total count per square inch

	<u>Before</u>	<u>% w/HSA</u>	<u>After</u>	<u>% w/HSA</u>
Doctors				
Entrance vestibule	25.0	25	157.5	25
Front of clothing cabinet	104.5	25	782.0	25
Front of lockers	251.2	25	635.5	50
Nurses				
Entrance vestibule	139.5	25	30.6*	0
Front of lockers	2212.0	0	180.0	25
Exit to corridor	217.5	25	824.0	0

* Does not include one sample with 1 million per square inch.

Airborne Organisms (Volumetric Sampling)

<u>Case #</u>	<u>Date</u>	<u>Number Samples</u>	<u>Organisms per cu. ft.</u>	<u>Plates w/HSA</u>
13	3-2-65			
Wound site		1	0.16	1
Foot op. table		1	1.2	1
14	3-3-65			
Wound site		2	0.12	1
Foot op. table		3	1.0	2
15	3-4-65			
Wound site		1	0.12	0
Foot op. table		3	1.4	1
Anesthesia room		1	2.9	0
16	3-10-65			
Wound site		2	0.6	0
Foot op. table		3	1.1	1
Head by inst. table		2	2.5	0
Anesthesia room		1	3.1	1

Airborne Organisms (Volumetric Sampling)

<u>Case #</u>	<u>Date</u>	<u>Number Samples</u>	<u>Organisms per cu. ft.</u>	<u>Plates w/HSA</u>
17	3-23-65			
Wound site		3	0.07	0
Foot op. table		5	0.6	1
Head by anesth.		4	0.6	1
Head by inst. table		4	0.5	0
Anesthesia room		1	1.1	1
18	3-24-65			
Wound site		2	0.03	0
Foot op. table		3	1.0	1
Head by anesth.		3	1.2	2
Head by inst. table		2	1.3	2
Anesthesia room		2	3.4	2
19	3-25-65			
Wound site		4	0.01	0
Foot op. table		5	1.2	0
Head by anesth.		5	1.1	2
Head by inst. table		4	0.75	3
Anesthesia room		1	4.8	0
20	12-14-65			
Wound site		4	1.0	0
Foot op. table		5	1.5	0
Head by anesth.		4	1.9	0
Head by Inst. table		3	0.6	0
Northeast Corner		4	0.6	0
Anesthesia room		1	0.5	0

Airborne organisms
(Fallout or Settling Plates)

<u>Case #</u>	<u>Date</u>	<u>Number Samples</u>	<u>Organisms per sq.ft. per hr.</u>	<u>% w/HSA</u>
20	12-14-65			
Instr. table		3	115	0
Stereo table		2	307	0
Large Mayo		1	0	0

Airborne Organisms
(Fallout or Settling Plates)

<u>Case #</u>	<u>Date</u>	<u>Number Samples</u>	<u>Organisms per sq. ft. per hr.</u>	<u>% w/HSA</u>
21	12-21-65			
	Inst. table	5	78	0
	Stereo. table	5	78	1
	Large Mayo	5	30	0
	Lewis tray rack	4	60	0
22	12-23-65			
	Inst. table	2	70	0
	Large Mayo	1	15	0
	Lewis tray rack	1	0	0
	Prep. table	1	26	0

Summary - Volumetric Sampling

	<u>Number</u>	<u>Number w/HSA</u>	<u>% w/HSA</u>
Wound site	19	2	11
Foot op. table	28	7	25
Head by anesth.	16	5	31
Head by inst. table	15	5	33
Anesth. room	8	4	50

These findings indicate a remarkably low number of organisms in the air or on personnel, linen or instrumentation. Through microbial monitoring of the wound site area and through particular monitoring of the personnel, we have revealed characteristics of the present plenum air flow system as well as provided for disclosure of two otherwise unknown sets of contaminants introduced into the environment. These data will be used as a means of comparing the plenum air system and other characteristics of the new neurosurgical suite ambient to a laminar flow system which has been constructed and is being studied elsewhere on the reservation.

Significance to Bio-medical Research and Program of the Institute: This methodology provides for continued sophistication in the development of modern neurosurgical operating room environments.

Proposed Course of the Project: As the laminar air flow system develops to an adequate degree of sophistication and as microbial data is acquired in sufficient amounts through the use of that system, these data will be compared with those demonstrated above. This will provide for a critical analysis of two types of air flow systems, one of which (laminar flow) has never been used

in surgery before.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-60 SN/NA 702(c)
1. Surgical Neurology Branch
2. Section on Neuroanesthesiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Experimental Hypothermia

Previous Serial Number: Same

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: T. Ohta, M.D., A. ~~Oomaya~~, F.R.C.S., R. Farrier, M.D.,
F. Lamberti, R.N., Choh-luh Li, M.D., J. Sagarminaga, M.D.

Cooperating Units: R-BEI; Dept. of Anesthesiology, Georgetown University

Man Years:

Total: 4.6
Professional: 3
Other: 1.6

Project Description:

Objectives:

- a. To study the effects of low temperature on the brain.
- b. To study various related physiological parameters.
- c. The development of topical and selective cooling techniques potentially available for clinical application.

Methods Employed: The methods employed are body surface cooling, topical cooling of the exposed brain, and/or spinal cord, intravascular perfusion, and combinations of these.

Major Findings: Investigations of the hemodynamics of cerebral circulation in the cat were made. Through roentgenographic studies, it was found that in the cat the internal carotid arteries are not significant in cerebral circulation and that almost all the blood supply to the brain is from the external carotids through the rete externum and large anastomotic branches to the distal portion of the internal carotids. The anterior communicating artery is extremely small and, in most cases, invisible in roentgenography. The rest of the blood supply is from the vertebral system through the considerable sized posterior communicating arteries. When the common carotid arteries are occluded, the posterior communicating arteries become larger and the vertebral system is solely responsible for the blood supply of the brain and, clinically, there is no neurological deficit discernible in the cat.

With this information in mind, the following experiments were designed.

The blood in the common carotid arteries was separately exteriorized through polyethylene tubings, propelled at a predetermined speed by a roller type pump, cooled to various temperatures by means of two Creech type heat exchangers equipped with 'bubble traps' and, finally, returned to the brain via the distal portion of the common carotid arteries. With this arrangement, the temperature of the two hemispheres of the brain can be separately controlled, dependent on the temperatures of the heat exchangers and the speed of the roller pump. It was found that the perfusion pressure, which was measured by a pressure transducer close to the distal portion of the common carotid arteries, varies with, among many factors, the temperature of the brain. With the other factors remaining constant, the brain temperature at a given time in turn depends on the arterial pressure of the cat. Thus the rate and the extent of cooling of the hemisphere can be regulated by adjusting the level of the anesthesia, the rate of the roller pump and the temperature of the heat exchanger. In this study, a temperature difference of 12° C. between the two hemispheres of the brain can be readily achieved.

In the physiological study of the cooled brain, selective cooling of one cerebral hemisphere allowed an opportunity of investigating the electrophysiological changes of the nervous tissue. The investigation was carried out by inserting four microelectrodes, two on each side, into the cortex of the warm and cold cerebral hemisphere of the cat. On the side of the recording microelectrodes were placed the bipolar stimulating electrodes. Current electrodes were also placed on the soft palate and the posterior fossa. The temperature of the cortex on both sides and of the corpus callosum was measured with thermisters. This arrangement permitted the study of single cell discharges, EEG, direct cortical responses, transcallosal responses, and the conductivity of the cold as well as the warm hemisphere, in relation to changes in brain temperatures. All the measurements were recorded on magnetic tapes.

The data obtained from these experiments are to be subjected to statistical treatments. At present, no definitive statement can be made. However, it is clear that at a temperature of 24° C., the hemisphere had failed to produce electrical activity and evoked responses recorded from the surface of the cortex; yet, single cell discharges continuously occur until the brain temperature reaches 16° C. The value of the impedance measurements of the hemisphere also changes with the temperature.

Vasospasm has considerable importance on both heat exchange in the neurological patient and in the experimental animal. It also has a considerable importance in the after-effects of heat exchange experimentally and clinically. Therefore an investigation was undertaken to determine the effects of mechanical stimuli and two drugs, serotonin and papaverine, on the middle cerebral artery of the cat. This vessel was exposed at its origin from the circle by microdissection techniques and thereafter, its manipulation was undertaken with microdissection instruments using the 50 power magnification in the Zeiss scope. Photographs were obtained by the appropriate attachments to this surgical dissecting microscope. It seemed clear that when the artery was touched lightly in a plane parallel to, and coincident with, its longitudinal axis, dilatation was evident under the microscope and prompt in

occurrence after the mechanical stimulus occurred. This dilatation was observed for periods as long as four hours and it was not reversible. Section of the vessel thus dilated showed a jelly-like clot which was adherent to the end of several points. Stimulation of the vessel in a transverse plane did not produce dilatation but rather produced some degree of constriction which was short lived and reversible. It was not coincident with an internal clot or coagulum. Serotonin produced constriction and papaverine dilatation as expected. Papaverine completely reversed the constriction from mechanical stimulation but serotonin did not affect the dilatation produced by longitudinal stroking of the vessel. These findings are of some significance to the neurological surgeon providing interspecie similarities are present. He must manipulate middle cerebral and other vessels close to the circle of Willis and, if these observations can be translated to the clinical operating room, should avoid longitudinal stimulation of the vessel. The finding of the coagulum within following such stimulation is itself of considerable interest.

A technique for selective brain cooling using intra-aortic catheterization was developed in the dog. The catheter tip was placed in the brachiocephalic arch and cooled blood from the perfusion system reservoir introduced as close to the carotid take-offs as possible. Using this technique, it was practicable and simple to cool and maintain brain temperatures of any desired level from 30°C. to 7°C. while coincidentally the heart continued to pump without significant dysrhythmia. This means that selective cerebral perfusion without dependence on extracorporeal circulation as a heart substitute is possible. With this new system, an experimental animal or patient can undergo relatively selective cerebral perfusion with or without heat exchange while heart action continues to provide somatic support. This system might be used equally well as a means of drug perfusion of carotid circulation or of heat exchange, that is cooling of the same pathways in order to provide hypothermic conditions for brain operations.

Macaque monkeys were given RISA in the same manner as applied to a patient and scanned by the scintillation counter in the AP and lateral plane. This is a brain scan comparable in technique and duration to that applied to a patient for diagnostic purposes. When the head of the monkey was cooled following injection of the RISA so that the brain temperature, monitored by intracerebral thermister, reached 20°C. and maintained at that level for 30 minutes, the scan showed a greater intracerebral deposition than similar records at normal temperatures. Similarly, autoradiographic studies of cooled brains showed deposition whereas the autoradiographic studies of brains at normal temperatures did not. This study suggests that there is a relationship between brain cooling and permeability as previously noted and that hypothermic patients may have abnormal scans if cooled to sufficiently low levels.

The drug deposition studies with curare have been reconfirmed in a succession of cats during the period of this report.

Significance to Bio-medical Research and Program of the Institute: The change in impedance of brain tissue is known to be related to the conductivity of the nerve cell membrane and neuroglial membrane and the volume of the inter-cellular space. It is also closely related to the excitability or activity of the nerve cells. The evoked potentials, produced in a manner designed in this investigation, provided information about presynaptic and postsynaptic activity of the nerve cells in the brain. Thus, the present study will ultimately add to our knowledge of brain function at low temperatures.

The studies on cerebral vasospasm are of importance to the neurosurgeon in his technical approaches to the circle of Willis. If these are born out by more extensive observations which are being pursued, we may have a better idea of the causation of vasospasm during operation, at least insofar as instrumentation stimulation is concerned. Studies using the clinical brain scan system add to the prior observations which suggests that there is a clear relationship between brain temperature and permeability. Such observations constitute an admonition to the clinical surgeon to avoid these levels of brain temperature so as to avoid conditions promoting edema and also provide some opportunity for introduction of drugs across the blood-brain barrier.

Proposed Course of Project: The studies on electrophysiological characteristics of hypothermia will be continued and extended. The clinical application of the perfusion results will begin after further testing in the chimpanzee.

Honors and Awards: None

Publications:

Ohta, T., Sagarminaga, J. and Baldwin, M.: Profound hypothermia with differential cooling of the brain in dogs. J. Neurosurg. (In press).

1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965, through June 30, 1966

Project Title: Clinical, Biochemical and Genetical Studies of Mental Retardation, Progressive Cerebral Degeneration and Cerebral Palsy in Children

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Owen M. Rennert, M.D. and Diane Mizel

Cooperative Units: None

Man Years (July 1, 1965, through June 30, 1966)

Total:	1.8
Professional:	0.9
Other:	0.9

Project Description:

Objectives: Actually this is a large permanent program. Chronic neurological disorders affecting children constitute one of the major medical and social problems of our times. According to conservative estimates for this country, about four million children are currently and permanently handicapped by mental retardation, cerebral palsy, epilepsy or progressive cerebral degeneration. Many of them have to be cared for to the end of their life by the joined services of the society and government. Until recently these conditions did not receive sufficient attention in the field of medical research. Our main objectives are:

1. To subdivide the large number of studied patients with mental retardation and cerebral palsy and epilepsy into distinct clinical categories using developmental and neurological examinations along with laboratory procedures.
2. To establish types of cerebral lesion characteristic of each category whenever possible.
3. To apply basic biochemical and genetic methods to selected diseases or syndromes within each category with the aim of advancing the underlying pathogenesis and etiology.
4. To advance when possible the therapy and prevention of these chronic diseases.

Methods Employed:

1. Detailed general medical and neurological studies.
2. Developmental and psychological examinations.
3. Modified electroencephalographic studies.
4. Biochemical assays of protein, lipid and carbohydrate metabolism.
5. Cytogenetic and pedigree studies.
6. Employment of special procedures such as pneumoencephalography and analysis of brain biopsies whenever these are performed for diagnostic purposes.

Patient Material: 34 inpatients and 39 outpatients including consultations.

Clinical Project

Major Findings:

1. A new syndrome consisting of congenital amaurosis of retinal origin associated with various abnormalities of the central nervous system has been investigated. Analysis of the pedigrees of four families studied indicates recessive autosomal transmission of the trait. The blindness dates since birth or shortly thereafter. The fundoscopic examination may be normal initially; in later stages a variety of retinal lesions can be encountered. Electroretinogram is of considerable diagnostic help especially in early cases and in young infants: it shows either absence of electrical potentials or greatly diminished ERG response. Large proportion of the affected children have mental retardation, epilepsy and focal neurological signs; these are generally of a non-progressive type. No autopsy material has been available as yet but it is postulated that at least in a proportion of these children congenital anomalies of the brain will be present. Further studies are needed to characterize clinically and pathologically the type of retinal and cerebral abnormalities and elucidate the pathogenesis.
2. We have demonstrated in a study of 12 patients with Hunter-Hurler syndrome, excessive excretion in their urine of tryptophane and serine. Plasma levels of serine are also elevated but not of the tryptophane. Hydrolysis of urinary acid mucopolysaccharides performed in normal subjects and in patients with Hurler's syndrome showed deficiency in amino acids in the "peptide" backbone in the patients; serine, however, was better represented than other amino acids (Table I).

	Control				
	Mean	K.J.	T.E.	M.K.	M.G.
Aspartic Acid	.038	0	.014	.035	.062
Threonine	.028	0	.006	0	0
Serine	.103	.076	.009	.052	.042
Glutamic Acid	.018	0	0	.094	.022
Glycine	.140	0	.059	.077	0
Alanine	.014	0	.015	.043	0
Tryptophane	0	0	0	0	0
Tyrosine	0	0	0	0	0
Phenylalanine	0	0	0	0	0
O-Phosphoserine	0	0		.034	.010

TABLE I

In overall interpretation of our findings we believe that support has been rendered to the hypothesis that excessive accumulation of AMPS in organs and their excessive excretion in the urine in this syndrome is related to abnormal bonding between mucopolysaccharides and protein. In this connection, serine may be playing a key role. While doing validation of plasma amino acids we have provided normal control values in children for 19 amino acids, adding 4 more to the tables existing in the literature.

3. A rare syndrome known as leprechaunism has been studied clinically, biochemically and from genetic viewpoint. The major clinical abnormality consists of a persisting failure to gain weight (our patient weighed at birth 3.6 kg., at 4 months 3.5 kg. and at 16 months 4.8 kg.). He exhibited marked hypertelorism, low set ears and developmental retardation. Plasma lipids, proteins and glucose were all abnormally low. His mother received repeated x-ray treatments during 10 years preceding his birth. She showed abnormality in chromosomes compatible with radiation damage and also a persisting clone of cells with a deficient chromosome in Group C. The patient's chromosomes were normal but grossly undetectable inversion is a distinct possibility. Eventually we were able to afford a slow but steady increase in this patient's weight by periodic administration of anabolic hormones.

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

The described findings constitute contributions to the research on metabolic and chronic neurological disorders in children.

Proposed Course of the Project: This field is enormous and the present program will continue for many years to come.

Honors and Awards:

1. Clinical Assistant Professor of Neurology, George Washington University Medical School.
2. Consultant, District of Columbia Children's Hospital.

Publications:

Dekaban, Anatole: Leprechaunism - A syndrome. Trans. Amer. Neurol. Assoc. 90: 60-62, 1965.

Dekaban, Anatole and Carr, Ronald: Congenital amaurosis of retinal origin. Frequent association with neurological disorders. Arch. Neurol. 14: 294-301, March, 1966.

Rennert, O. M. and Dekaban, A. S.: Amino acid metabolism in patients with Hurler's syndrome. Metabolism, May, 1966 (In press).

Serial No. NDB(I)-60 SN/CN 707(c)

1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965, through June 30, 1966

Project Title: Study of Pathological Lesions in the Central Nervous System
Occurring During Prenatal, Intranatal and Early Postnatal Life

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Rosemarie Thron and Jan K. Steusing

Cooperating Units: None

Man Years

Total:	1.0
Professional:	0.3
Other:	0.7

Project Description:

Objectives: In the majority of patients the causation and underlying pathology of mental deficiency and cerebral palsy are largely unknown. Detailed histological and histochemical examination of the brains of such children are conducted. The main purpose is to correlate the encountered lesions with the clinical data; accumulation of larger body of data is expected to provide valuable information for etiological clues. Limited, experimental approach in animals is used whenever indicated.

Methods Employed:

1. Detailed evaluation of the clinical and laboratory data of the children who suffered from chronic neurological disorders.
2. Gross examination of brains after their demise.
3. Microscopical study of large histological sections stained by a variety of chromatic, myelin and silver methods as well as by special histochemical procedures.
4. Reconstruction of the brain and spinal cord in young human embryos.
5. Histochemical studies of brains and organs of patients who died of progressive cerebral degeneration and certain metabolic disorders.
6. Tissue culture of affected organs.

Material: Brains of patients who died with a diagnosis of birth injury, cerebral palsy, epilepsy, mental deficiency or progressive cerebral degeneration.

Major Findings:

1. We have performed complete autopsy and brought to our laboratory eight brains from patients who died with a clinical diagnosis of cerebral palsy, mental retardation or progressive cerebral degeneration. The brains were processed for large paraffin and celloidin section and for frozen section stained by the histochemical methods. The neuro-pathological diagnoses were as follows: chronic stage of cerebral birth injury in three; congenital malformation of the brain in two; and inflammatory, metabolic or postepileptic lesion in each one of the remaining three patients. These eight brains increase our material for comprehensive evaluation of chronic brain syndromes.
2. The brain and internal organs of one of our patients who died from diffuse mucopolysaccharidosis were examined in detail. Using histochemical procedures on cryostat cut frozen section, two major types of mucopolysaccharides in various tissues were demonstrated: chondroitin sulfate B and heparitin sulfate. Chemical extraction from the gray and white matter of the brain, liver, spleen and skin supplied the quantitative data. It was found that the brain contains excess of acid mucopolysaccharide as well as a resistant to extraction lipid substance. The latter showed presence of a small amount of sialic acid. More detail biochemical characterization study is in progress.
3. Chromosome studies in a woman who had repeated therapeutic x-radiations during the past 12 years revealed presence of persisting chromosomal aberrations and also a presence of a clone of cells with a deficient chromosome in Group C. About 20 percent of all her lymphocytes in repeated cultures over two years showed this abnormality. Recently she gave birth to a malformed child. Although the gross appearance of the chromosomes of this offspring is normal, it is postulated that undetectable (with present methods) inversion in one of his chromosomes may be present; the inversion would have been transmitted from his mother.

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

Knowledge of detailed pathology and pathogenesis of numerous conditions which underlie cerebral palsy, mental retardation and progressive cerebral degeneration is of basic importance for eventual therapeutic and preventive measures. Some of our studies in this field are of applied type and some as the above quoted have basic implication of inheritable abnormalities. The disorders listed are clearly the major responsibility of our institute.

Proposed Course of the Project: This project has a wide scope and pertains to one of our major interests. It supplements also our principal clinical project, NINDB-74(c); therefore it is our permanent project.

Honors and Awards: None

Publications:

Dekaban, Anatole: Persisting clone of cells with an abnormal chromosome in a woman previously irradiated. J. Nuclear Med. 6: 740-746, Oct. 1965.

Serial No. NDB(I)-60 SN/CN 708(c)

1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965, through June 30, 1966

Project Title: Cytogenetical Study of Human Chromosomes Especially in Patients with Congenital Malformations and Mongoloids and Their Families

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Rosemarie Thron and Jan K. Steusing

Man Years

Total:	1.0
Professional:	0.3
Other:	0.7

Project Description:

Objectives: Recently it has been found that a proportion of patients with severe congenital malformations and also mongoloids have abnormal chromosomal constitution. Since chromosomes contain hereditary material responsible for expression of all traits, the abnormality in the number of chromosomes or their morphology may be associated with or even be a cause of malformation. This approach is opening a new horizon in the research on congenital malformations and certain familial disorders.

Methods Employed:

1. Comprehensive clinical and laboratory investigation of selected patients to establish diagnosis and extent of abnormality.
2. Tissue culture of lymphocytes and fibroblasts from the peripheral blood. Bacto-Phytohemagglutinin is added in order to stimulate lymphocyte proliferation. Six hours prior to sacrificing the culture colchicine is added in a final concentration of 0.5×10^{-6} . Fibroblast cultures are similarly processed when indicated.
3. Through a number of steps the white blood cell suspension is centrifuged and washed, then exposed to hypotonic salt solution and fixed.
4. Concentrated suspension of cells are stained on the slide with orcein, Giemsa or Feulgen methods.

5. Under high power resolution in light and contrast phase microscope the chromosomes are counted, their morphology studied and karyotype figures made for final analysis.
6. In special cases, DNA duplication sequence is studied in the chromosomes during the late phase of lymphocyte culture using tritium labelled thymidine.

Material: Patients with congenital malformations, mentally retarded and mongoloids as well as their families are the subject of this study. Experimental approach on human tissue cultures is also used.

Major Findings:

1. Karyotypic examinations were carried out on 28 patients and on 41 persons from their immediate families. Also, chromosome service was provided to the investigators in other institutes. Out of 28 patients tested, 17 showed chromosomal abnormalities in a form of trisomy, monosomy, translocation or morphological aberrations. These findings provided important diagnostic information; they also availed us a unique opportunity to apply experimental techniques to the blood cultures (see 2) in order to assess radiation effect on human chromosomes.
2. Aliquots of freshly drawn blood from 5 patients with various chromosomal abnormalities and from 3 normal controls were divided into four samples each. The first set of blood samples were immediately cultured and then processed for chromosomes; they constituted controls. The second set of blood samples were first irradiated and then cultured. The third and fourth sets of blood samples were first cultured and then irradiated 4 hours prior to harvesting. In all instances 100 rads were given at the rate of 52.1 R/min. (See tables on the following 2 pages).

It was found (Table I) that the rate of chromosome-type aberrations induced by irradiation of blood (G1 stage) was significantly higher in the woman who was previously exposed to irradiation and whose control blood cultures had about 20% of "spontaneous" breaks. This was less striking in a mongoloid patient with fewer chromosomal aberrations in the control blood cultures. In the remainder of the mongoloids and in the patient with treated leukemia the rates of chromosome-type aberrations in the irradiated blood were not significantly different from those in the normal controls. The rates of chromatid-type aberrations (Table II) induced by irradiation of blood cultures 4 hours prior to harvesting (G2) were not significantly different in the patients tested as compared to the normal controls. The relation of the number of acentric fragments to di- and tri-centric chromosomes in the first cell division was investigated.

Irradiated Blood	No. cells scored (Hypo/hyper modal)	Chromosome Type Aberrations				Total Chromos. breaks
		Dicentric	Rings	Deletion		
Case 1 Normal male	50 (3/1)	4 (1D \bar{c} 1F 3D no F)	-	1	9	(.18)
Case 2 Normal male	50 (2/0)	1 (1D \bar{c} 1F)	-	4	6	(.12)
Case 3 Normal male	50 (3/1)	3 (1D \bar{c} 1F 2D no F)	-	2	8	(.16)
Case 4 Trisomy G	50 (2/2)	3 (1D \bar{c} 1F 2D no F)	1 (1R no F)	4	12	(.24)
Case 5 Mongoloid 48 Chromosomes	50 (2/1)	7 (3D \bar{c} 2F 2D \bar{c} 1F 1D \bar{c} 5F 1D no F)	1 (1R \bar{c} 1F)	1	17	(.34)
Case 6 Trisomy G Chromos. lesion	50 (2/1)	6 (1D \bar{c} 2F 3D \bar{c} 1F 2D no F)	2 (1R \bar{c} 1F 1R \bar{c} 2F)	7	23	(0.32)
Case 7, 20 yrs. Treated leukemia	50 (2/0)	4 (2D \bar{c} 2F 2D \bar{c} 1F)	2 (1R \bar{c} 2F 1R \bar{c} 1F)	4	16	(0.3)
Case 8 Female with chromos. breaks	50 (2/1)	9 (6D \bar{c} 1F 3D no F)	1 (1R \bar{c} 1F)	15	35	(.5)

RESULTS OF CHROMOSOME ANALYSIS IN FIFTY CELLS OF CULTURES
ESTABLISHED FROM IRRADIATED BLOODS IN CASES 1 TO 8

TABLE I

Radiated Cultures	No. cells scored (Hypo/hyper modal)	Chromosome type aberrations		Chromatid type aberrations				
		Dicentric and Rings	Deletions	Chromat. break	Chromat. Exchanges	Isochr. gap	Chromat. gap	Total Chromat. Aberr.
Case 1 Normal male	50 (6/1)	-	-	31	3 (2)	6	8	59 (1.18)
Case 2 Normal male	50 (4/1)	-	-	38	4 (3)	2	6	62 (1.24)
Case 4 Mongoloid Trisomy G	50 (2/1)	-	-	31	1 (1)	2	9	46 (0.92)
Case 5 Mongoloid 48 chromosomes	50 (6/2)	-	-	30	3 (2)	6	21	74 (1.48)
Case 6 Trisomy G Chromos. lesion	50 (4/2)	1 (1D no F)	3	49	1 (1)	8	9	72 (1.44)
Case 7, 20 yrs. Treated leukemia	50 (1/4)	-	1	46	2 (2)	4	17	74 (1.48)
Case 8 Female with chromos. breaks	50 (3/2)	1 (1D no F)	10	30	1 (1)	3	9	48 (0.96)

RESULTS OF CHROMOSOME ANALYSIS IN FIFTY CELLS OF BLOOD
CULTURES IRRADIATED 4 HOURS PRIOR TO THEIR HARVESTING AT 72 HOURS

TABLE II

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

The etiology and pathogenesis of a vast number of conditions associated with congenital malformation and mental deficiency are largely unknown. Demonstration of chromosomal aberration in some of these patients or their parents is a great step forward in our understanding of these conditions and it may even suggest in the distant future certain preventive measures. Clinical and experimental study of the effect of ionizing radiation on the offspring and their chromosome constitution are of great importance in the present era.

Proposed Course of the Project: This project will continue. The findings are clearly applicable to our principal projects relating to the broad field of mental retardation and congenital malformations.

Honors and Awards: None

Publications:

Dekaban, A. S., Thron, R. and Steusing, J. K.: Chromosomal aberrations in irradiated blood and blood cultures of normal subjects and of selected patients with chromosomal abnormality. Radiat. Res. 27: 50-63, Jan. 1966.

1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965, through June 30, 1966

Project Title: Clinical and Biochemical Studies in Epilepsy

Previous Serial Number: Same

Principal Investigator: Anatole Dekaban, M.D., Ph.D.

Other Investigator: Diane Mizel

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.3
Other:	0.6

Project Description:

Objectives: Idiopathic epilepsy is a clinical syndrome of unknown etiologies.

In the first instance it is important to accumulate a sufficient number of such patients for the purpose of subdividing them into different categories depending on certain clinical and laboratory findings. This will permit better directed therapeutic application of certain regimens and drugs under investigation. The patient's response or its lack may provide us with some informations on the pathogenesis of the condition.

Methods Employed:

1. Necessary clinical examinations and tests.
2. Routine and special electroencephalographic studies.
3. Pneumoencephalography and arteriography when indicated.
4. Urinary and plasma amino acids.
5. Assays of carbohydrate metabolism.
6. Plasma lipid studies.
7. Endocrinological assays when indicated.
8. Application of special therapeutic procedures.

Patient Material: 18 inpatients and 11 outpatients with idiopathic epilepsy.

Clinical Project

Major Findings:

1. A total of 18 children suffering from frequent epileptic attacks were investigated and treated. Of these, 7 children required administration of high fat diet for control or amelioration of their seizures. This afforded a unique opportunity to study partitioned plasma lipids and their relationship to the control of seizures. Table I shows abbreviated data on the patient in whom complete control of seizures with high fat diet was accomplished. Note marked change in values of partitioned plasma lipids.
2. One of not too rare causes of persistent convulsions in childhood is hypoglycemia. We have studied in the past few years patients with isolated and familial type of hypoglycemia of childhood; the low blood sugar level was the cause of their seizures. Distinction between leucine sensitive and leucine insensitive type of hypoglycemia is of practical value in treatment of this disorder. It is known that severe mental retardation and paralysis result with great frequency if hypoglycemia is not controlled during early stage of life. Recently, we have devised special dietary regimen which can be used successfully in controlling leucine sensitive variety of hypoglycemia with a resultant control of seizures. Details and rationale of this regimen will be subject of a special report.

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

As it has been demonstrated by our previous findings (J. Dis. Child. 100:181-188, 1960) frequent daily attacks (usually of minor variety) if occurring during the first years of life may lead to irreversible mental retardation. So far, the broad biochemical approach to this type of epilepsy in children has been scarcely used. Some of our previous publications indicate that such studies can be fruitful. This investigation enlarges the scope of the study of epilepsy which is the major theme of the Surgical Neurology Branch, NINDB.

Proposed Course of the Project: The project will continue for years to come.

Honors and Awards: None

Publications:

Field, James B. and Dekaban, Anatole S.: Clinical and physiologic aspects of hypoglycemia. Postgrad. Med. 38: 23-30, July, 1965.

DIET DATE	REG. 6/10/63	4:1 6/26	4:1 12/17	4:1 3/11/64	3:1 11/17	2:1 11/20	2:1 11/25	2:1 4/13/65	REG. 11/12/65
TOTAL LIPIDS	652	791	1124	1014	1068	1080	1157	948	673
TOTAL CHOLESTEROL	221	300	466	405	433	420	507	408	303
PHOSPHOLIPIDS	230	282	347	331	315	321	363	320	218
TRIGLYCERIDES	232	247	248	323	363	383	336	248	152
TOTAL FATTY ACIDS	899	686	1979	1972	1950	1771	1889	1765	1450
FREE FATTY ACIDS	70	83	163	128	175	128	102	219	125
GLUCOSE	83	71	69	69	66	73	71	66	78.2
KETONES (BLOOD)	1.8/5	8/32	11/36	15/41	14/38	12/36	16/40	14/40	9/18
KETONES (URINE)	0	35/153	-	178/1260	109/474	84/328	106/460	110/435	-
CALORIES	1415	1405		1450	1370	1270	1410	-	1264
WEIGHT, KG.	12.6	12.7		13.6	13.9		14.0		16.0
SPELLS/DAY	44	12	0	0	0	1	0	0	0

TABLE I

1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965, through June 30, 1966

Project Title: Studies on the Incorporation of an Amino Acid Analogue -
TRIFLUOROLEUCINE - Into Protein of Mammals

Previous Serial Number: Same

Principal Investigators: Anatole S. Dekaban, M.D. and Owen M. Rennert, M.D.

Other Investigator: None

Cooperating Units: None

Man Years

Total: 0.3
Professional: 0.2
Other: 0.1

Project Description:

Objectives: Trifluoroleucine is an analogue amino acid which can be incorporated into new proteins under certain conditions. The changed protein has different physiological and biochemical properties. It has been shown before by one of us that the analogue administration ameliorates significantly the course of leukemia in mice. Our aim is to study the basics of protein synthesis in mammalian embryos using the analogue.

Methods Employed:

1. Standard methods of amino acid analysis.
2. Electrophoresis and diffusion studies.
3. Ultracentrifugation - density gradients.
4. Histochemical studies.

Material: Synthesized by us amino acid analogue trifluoroleucine. Mice as needed.

Major Findings:

Trifluoroleucine was injected intraperitoneally into pregnant mice at various stages of their gestation; the animals were sacrificed 24 hours later and the embryos were delivered by cesarian section. Gross and microscopic examinations were made regarding litter size, anomalous development, and histology of uterus and placenta. The amino acid composition of

amniotic fluid, fetal and amniotic proteins were performed utilizing the Technicon amino acid analyzer. The results indicated that: (1) trifluoro-leucine rapidly enters the fetal circulation as evidenced by its appearance in amniotic fluid 20 minutes following injection into the mother, (2) the analogue is incorporated into the amniotic and fetal proteins apparently replacing leucine, (3) trifluoro-leucine is still present in the fetal system when it is no longer present in maternal urine and blood, (4) maximal incorporation of the analogue occurs during the first 12 days of gestation. In these short term experiments there was no evidence of malformation of the fetus.

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

Our preliminary study indicates that the in vivo incorporation of tri-fluoro-leucine can be achieved in embryonic systems. Long term aims relate to defining in chemical terms the protein synthesizing systems of embryonic tissue, and to correlate changes in the primary structure of the proteins (and secondary) with morphogenesis.

Proposed Course of the Project:

The data described under Major Findings are prepared for publication. For the time being we do not plan further extension of this study.

Honors and Awards: None

Publications: None

Serial No. NDB (I) - 63 SN/NP 1027 (c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Changes in Chloride and Water Content of Brain Tissue
After Chemical Injury of the Blood-Brain Barrier.

Previous Serial Number: Same

Principal Investigator: Oskar Steinwall, M.D.

Other Investigators: Eugene Streicher, Ph.D., Igor Klatzo, M.D.

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.5
Other:	0.4

Project Description:

Objectives: By means of standardized method for intracarotid injection of injurious solutes in rabbits blood-brain barrier (BBB) damage, characterized by the abnormal extravasation of acid dyes was produced within one hemisphere, the other serving as a control. Comparative assay of the chloride and water content in damaged and undamaged hemisphere of the same brain was performed varying the type and concentration of the injuring solute and the time interval between injury and the termination of the experiment.

Methods Employed: Adult rabbits under urethane anesthesia were used. The unilateral BBB injury was produced by injection of chemicals with known BBB damaging properties via a catheter placed in the left common carotid artery (after ligation of its external branches) with a pressure causing the expulsion of the blood in the left hemisphere (controlled by visual inspection of the cortex through the dura via a trephine opening). The injection time was usually 30 sec. Afterward the injected hemisphere was recirculated with blood from the circle of Willis. The degree and extent of a BBB damage was assessed by intravenous injection of various tracer dyes. The rabbits were sacrificed by exsanguination at various time intervals after the carotid injection and symmetrical samples from the

injected (left) and the control (right) hemispheres were taken for chloride and for water determinations.

In most experiments the injected damaging agent was HgCl_2 (0.01 - 0.1 mM) while Penicillin G was administered in a smaller group of animals. Control experiments were carried out with intracarotid injections of Ringer's solution.

Major Findings: A comparison between the injected (barrier-damaged) and the noninjected (control) hemispheres with regard to the chloride and water content revealed the following results:

1. Control experiments (6 rabbits injected with Ringer's solution; 8 rabbits injected with chemicals but at a dose not producing any microscopic evidence of extravasation of tracer dyes) showed either no significant differences between hemispheres in chloride and water content or slightly higher values in the injected side (up to 1.5 mEq/Kgm chloride and 0.35% water).
2. Experiments with slight or moderate BBB damage induced by mercuric chloride (16 rabbits) showed consistently a lower chloride content on the barrier damaged side than in the control hemisphere. The differences exceeded 2 mEq/Kgm in 10 rabbits and 4 mEq/Kgm in 3 rabbits. The side differences in water content were on the whole not significant.
3. Experiments with slight or moderate BBB damage induced by Penicillin G (5 rabbits) showed higher chloride (0.8 - 3.0 mEq/Kgm) content on the BBB damaged hemisphere. With regard to water content there were no significant differences.
4. Experiments with strong mercurial BBB damage (7 rabbits) showed a considerable increase of both chloride (4 - 10 mEq/Kgm) and water (0.62 - 4.22%) in the damaged hemisphere.

In interpretation of these findings the most interesting observation is the effect of slight or moderate mercurial BBB damage expressed in significantly decreased chloride content in the hemisphere showing extravascular passage of tracer dyes. This suggests a functional disturbance in exchange mechanisms operating at the blood-brain interphase and not a mere leakage through ruptured BBB structures. On the other hand, stronger mercurial injury resulted in the development of edema (evident grossly and microscopically) which as usual is associated with an increase in both chloride and water content. The effect of Penicillin is of a more complex nature since this substance after passing through the blood vessels appears to penetrate quickly to the neurons as it is evidenced by development of convulsive phenomena predominantly on the side contralateral to the injection.

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

The observed differences in chloride content following the slight vascular injury is of significance for understanding the mechanisms of the blood-brain barrier, which is of great importance for the maintenance of the normal physio-chemical environment of the brain.

Proposed Course of the Project: This project has been discontinued due to the departure of the principal investigator.

Honors and Awards: None

Publications: None

Serial No. NDB (I) - 63 SN/NP 1030 (c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Observations on Differential Penetrability of Simultaneously Administered Tracers in Blood-Brain Barrier (BBB) Damage.

Previous Serial Number: Same

Principal Investigator: Oskar Steinwall, M.D.

Other Investigators: Igor Klatzo, M.D., Diane E. Smith, M.S.

Cooperating Units: None

Man Years

Total:	1.0
Professional:	0.8
Other:	0.2

Project Description:

Objectives: In view of the recent concept suggesting that the BBB phenomenon may be related to a variety of specific transfer mechanisms, a selectively differential behaviour of the BBB was studied in chemical injuries produced by intracarotid injection of various compounds.

Methods Employed: Unilateral BBB injury was produced by intracarotid injection of mercuric chloride (0.03 - 0.08 mM), Penicillin (3.5 - 5%), or sodium acetrizate (14 - 18%). This was followed by systemic administration of combinations of two different fluorescent and radioactive tracers. The combinations of tracers were as follows:

- a) red fluorescent Rhodamine B labeled albumin (FLA) with green fluorescent fluorescein labeled albumin (FLA)
- b) red fluorescent Evans blue - albumin complex (EBA) with FLA
- c) EBA with green fluorescent fluorescein labeled gamma globulin FLGG
- d) RLA with FLGG
- e) sodium fluorescein or FLA with C¹⁴ sucrose

- f) sodium fluorescein or FLA with C¹⁴ inulin
- g) sodium fluorescein with C¹⁴ methyl-O-glucose

The brain tissue was studied by fluorescence microscopy and radioautography.

Major Findings: Combinations of red and green fluorescent albumins showed no separation in the distribution pattern on the damaged side. Simultaneous administration of red fluorescent albumins and green fluorescent globulins revealed in numerous instances a distinct separation in distribution of protein tracers on the damaged side. In slightly or moderately damaged areas numerous blood vessels were surrounded by only red fluorescence. The perivascular exudates and globules showed a range of color depending on relative concentration of respective tracers. Differential passage from the injured vessels was also observed in combinations of fluorescent and radioactive tracers. C¹⁴ inulin appeared to penetrate in damaged areas more extensively than sodium fluorescein or FLA. An interesting result was obtained in sodium fluorescein - C¹⁴ methyl-O-glucose combination. Whereas in moderate or severe BBB damage both sodium fluorescein and C¹⁴ methyl-O-glucose spread intensely from the injured vessels, in very slight BBB damage in which no abnormal passage of sodium fluorescein could be detected, there was a distinct inhibition of the normal transport of methyl-O-glucose from the blood into the brain tissue.

Significance to Bio-medical Research and the Program of the Institute: The demonstrated differential vulnerability of the blood-brain barrier and especially the demonstration of selective inhibition of glucose uptake due to very slight vascular injury is of great potential significance for the interpretation of many neurological conditions in which a slight interference with the normal function of the blood-brain barrier may be involved.

Proposed Course of the Project: This project is concluded. It demonstrated conclusively the selective features in vulnerability of the BBB. The inhibition of normal glucose transfer from blood to brain in very slight BBB injuries, which are undetectable by visual tracers, may be of considerable clinical significance.

Honors and Awards: None

Publications:

Steinwall, O., and Klatzo, I.: Double tracer methods in studies on blood-brain barrier dysfunction and brain edema. Acta Neurol. Scand. 41, Suppl. 13, R31/1-4, March 1965.

Steinwall, O., and Klatzo, I.: Selective vulnerability of the blood-brain barrier in chemically induced lesions. J. Neuropath. Exp. Neurol. In Press.

Serial No. NDB (I) - 64⁵ SN/NP 1207 (c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Effect of Systolic Blood Pressure on the Dynamics of Brain Edema.

Previous Serial Number: Same

Principal Investigator: Igor Klatzo, M.D.

Other Investigators: Diane E. Smith, M.S., Ayub K. Ommaya, M.D.

Cooperating Units: None

Man Years

Total:	1.9
Professional:	1.0
Other:	0.9

Project Description:

Objectives: This investigation is concerned with the effect of systolic blood pressure on the dynamics of brain edema. The blood pressure in cats was altered by means of hyper- and hypotensive drugs administered prior to the production of brain edema. Edema was produced by the application of a cooled metal plate to the exposed cerebral cortex. Changes of the systolic blood pressure significantly affected the rate of progression of edema through the white matter of the injured gyrus. It was shown that the lowering of the blood pressure can almost completely prevent the development of edema.

Methods Employed: Blood pressure was recorded through polyethylene catheters placed in the femoral artery under nembutal anaesthesia. The elevation of the blood pressure was produced by the intravenous drip of Levophed (2 ml of a 0.2% solution added to 500 ml of 5% Dextrose). The lowering of the blood pressure was obtained by intravenous drip of Arforad (10 ml of a 0.5% solution added to 500 ml of 5% Dextrose). After stabilizing the blood pressure at a desired level, edema was produced by means of a cold plate (-56°C) applied to the previously exposed cerebral cortex for one minute. As a blood-brain barrier tracer 3 ml of 10% sodium fluorescein was injected prior to the application of the cold plate.

The animals were sacrificed at varying time intervals and the brains were sectioned for gross observations and photography under the U. V. light.

Major Findings: Elevation of the systolic blood pressure up to 200 mmHg resulted in a striking acceleration of the progression of the edema. Thus, the extent of the edematous area in cats with 200 B.P. sacrificed after 2 hours following cold lesion corresponded to that observed in animals under normal blood pressure, 10 hrs. after cold injury. Conversely, cats with systolic B.P. lowered down to 50 mmHg which were sacrificed 6 hrs. following the cold injury revealed a conspicuous inhibition in the spread of edema; the area outlined by the fluorescein tracer corresponding to that observed in normotensive cats 30 minutes after cold injury.

Significance to Bio-medical Research and the Program of the Institute: The investigation concerning the effect of systolic blood pressure on dynamics of brain edema is of potentially great significance for clinical management of cerebral edema.

Proposed Course of the Project: This project is concluded. The findings were reported at the Symposium on Brain Edema in Vienna, September 1965.

Honors and Awards: None

Publications:

Klatzo, I., Steinwall, O., Streicher, E., Wisniewski, H., and Smith, D. E.: Dynamics of the experimental cold injury edema. Proc. Workshop on Brain Edema. Vienna, Springer Verlag. In Press.

Serial No. NDB (I) - 66 SN/NP 1285 (c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Cellular Morphology of the Lemon Shark Brain.

Previous Serial Number: None

Principal Investigator: Igor Klatzo, M.D.

Other Investigators: None

Cooperating Units: Lerner Marine Laboratory, Bimini, Bahamas

Man Years

Total:	2.4
Professional:	1.5
Other:	0.9

Project Description:

Objectives: Although comparative neuroanatomy has provided a wealth of information concerning the structural aspects of Elasmobranch brain, these studies have been mostly centered on elucidation of architectual organization of neurons and their interconnections without morphological description of other nervous tissue components. The importance and role of non-neuronal elements of the nervous tissue has become increasingly apparent in the recent years. Accumulating data indicate that the function of the neurons is considerably controlled by metabolic influences transmitted to them with participation of cellular components such as glia, ependyma and especially cerebral vasculature which is interposed between the circulating blood and the special biochemical environment of the nervous parenchyma. The present studies were prompted by the meagerness of available information concerning the morphological characteristics of various cellular elements comprising the brain of Elasmobranchs. The lemon shark (*Negaprion brevirostris*) served as the subject of the investigation in view of availability of this species at the Lerner Marine Laboratory.

Methods Employed: The observations are based on the study of 14 lemon shark brains obtained at the Lerner Marine Laboratory. The sharks caught in the local waters were anesthetized on the wharf by irrigation through the gills with approximately 100 ml of 1.8% MS-222 dissolved in sea water.

The animals were placed then on the operating table in the laboratory, a rubber hose being inserted into their mouths to provide continuous irrigation with sea water through the gills. The tail was severed and a polyethylene catheter was introduced into the tail artery. Shark physiological saline was perfused under 160 mm of Hg. pressure and was followed by a fixative which consisted of 10% formalin or, in a few cases, of formalin ammonium bromide for metallic impregnations of the glia. The brains were removed and placed for further fixation into respective fixatives. The 10% formalin-fixed tissue was embedded into paraffin, whereas the formalin ammonium bromide-fixed brains were sectioned on the freezing microtome. The histological stains used in this study were as follows: Cresyl violet, Luxol blue, PAS, Masson, Van Gieson, Bodian, Bielschowsky, Cajal's gold chloride, Hortege's silver carbonate, PTAH, and Foot's modification of Hortege's stain for reticulin.

Major Findings: In the forebrain several distinct neuronal groups were described. The cerebellum revealed two striking patterns of neuronal architecture. Some of the cerebellar folia contained no Purkinje cells but a prominent granular layer. The others showed Purkinje cells in direct connection with the white matter cores without the granular layer. The giant neurons of the tectum revealed an intimate special relationship with the blood vessels.

The astrocytes showed a striking radiating arrangement around the blood vessels and neurons. No vascular sucker-feet, which are characteristically seen in the mammalian brain, could be seen. Otherwise the blood vessels in the forebrain were densely surrounded by glial cells. A system of ependyma-lined canals was described in the cerebellum. It is assumed that these channels, as well as, saccus vasculosus play an important role in the circulation of the CSF.

Significance to Bio-medical Research and the Program of the Institute: The elucidation of morphological substrate was urgently needed for our studies on behaviour of the blood-brain barrier and circulation of the cerebrospinal fluid in shark. The study of the blood-brain barrier constitutes the major research program of this section.

Proposed Course of the Project: This project has been completed. The findings were presented at the Symposium on Current Investigations on Elasmobranchs Biology, held in February 1966, in Bimini, Bahamas.

Honors and Awards: None

Publications: None

Serial No. NDB (I) - 66 SN/NP 1286 (c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Enzyme Levels in Experimental Brain Edema.

Previous Serial Number: None

Principal Investigator: Lois E. Rasmussen, Ph.D.

Other Investigators: Edward A. Carmichael, M.D., Igor Klatzo, M.D.

Cooperating Units: None

Man Years

Total:	1.9
Professional:	1.0
Other:	0.9

Project Description:

Objectives: The main purpose of this project is to evaluate the enzymatic changes occurring during the development of brain edema. This appears to be especially important in view of histochemical alterations involving primarily the astrocytes which have been demonstrated by Rubinstein, Klatzo and Miquel (J. Neuropath. & Exp. Neurol. 21, 116, 1962) and others. The nature of histochemical alterations has not been elucidated and therefore quantitative biochemical enzymatic assays are of obvious significance. The dynamic changes of lactic dehydrogenase (LDH) and other enzymes occurring in edematous brain tissue and CSF at various stages of experimental cold injury edema are the subject of present investigation.

Methods Employed: Enzymatic assays were carried out at various time intervals following the standard cold injury in cats, as used in this laboratory. CSF was obtained from the cisterna magna and from the lateral ventricles via cannulation of the aqueduct. The cats were sacrificed by exsanguination and brain tissues from edematous area and normal control hemisphere were removed for histochemical and biochemical enzymatic assays.

LDH activity was determined in the CSF by the method of Carbaud and Wroblewski, 1958. The LDH activity in brain tissue homogenates was assayed

by the method of Lowry. The isozymes of LDH in sera, CSF, and brain tissue were separated electrophoretically in starch gel. Tissue sections were stained histochemically by the method of Novikoff and Masek, 1958.

Major Findings: 1. The CSF removed from the ventricles via the aqueduct showed in the experimental cat at five days after the production of cold injury a 6-8 fold increase in the level of LDH activity. At 7 days this rise still persisted. The CSF from control cats in which the dura only was cut did not show this rise. Serum LDH did not increase in experimental animals.

2. In brain slices stained histochemically for LDH the astrocytes in white matter on the experimental side were enlarged and stained very intensely from 5-7 days and the changes appeared to persist at 14 days.

3. In homogenates of the white matter the activity of LDH at 5-7 days was significantly less in the experimental hemisphere than in the control one.

4. The pattern of LDH isozymes separated electrophoretically was similar in brain and CSF, but the sera pattern was distinct from the former two.

Significance to Bio-medical Research and the Program of the Institute: The evaluation of the enzymatic changes occurring during the development of brain edema contributes further information concerning this serious and frequent complication of many neurological and neurosurgical conditions.

Proposed Course of the Project: In order to determine if the pattern of LDH activity is an unique phenomenon or if other enzymes also exhibit similar changes, glutamic dehydrogenase (important in linking the metabolism of glutamic acid and other amino acids with the Krebs cycle) and glucose-6-phosphate dehydrogenase (an enzyme indicative of the activity of the pentose phosphate shunt) will be assayed in the brain tissue and CSF.

Honors and Awards: None

Publications: None

Serial No. NDB (I) - 66 SN/NP 1287 (c)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Comparative Protein and Enzyme Profiles of the Cerebrospinal Fluid, Extradural Fluid, Nervous Tissue, and Sera of Elasmobranchs.

Previous Serial Number: None

Principal Investigator: Lois E. Rasmussen, Ph.D.

Other Investigators: Reinhold A. Rasmussen, Ph.D.

Cooperating Units: Division of Biochemistry, Walter Reed Army Institute of Research, Washington, D. C.
Lerner Marine Laboratory, Bimini, Bahamas
Virginia Institute of Marine Science, Wachapreague, Virginia

Man Years

Total:	1.4
Professional:	1.0
Other:	0.4

Project Description:

Objectives: In view of the meager information available on the biochemistry of the body fluids and nervous tissue of the Elasmobranchs and the recent general interest in biology of these species the proteins and several enzymes of Elasmobranch sera, cerebrospinal fluid (CSF), extradural fluid (EDF), and brain were investigated. The proteins of these tissues were compared, by several electrophoretic techniques, to mammalian proteins. Several Elasmobranch enzymes were also compared to mammalian enzymes, both in total activity and in isozyme patterns.

Methods Employed: Various species of Elasmobranchs were obtained at the Lerner Marine Laboratory, Bimini, Bahamas and the Virginia Institute of Marine Science, Wachapreague, Virginia. Samples of CSF, EDF, sera, and brain tissue were removed from animals anesthetized with MS-222 and assayed for total protein, alkaline phosphatase (AP), serum glutamic oxaloacetic transaminase (SGOT), acetylcholine esterase (AChE), and lactic dehydrogenase (LDH) activity. In addition, the proteins were separated

by four different electrophoretic techniques and the LDH isozymes were separated in these four tissues by agar gel electrophoresis.

Major Findings: This study revealed both species characteristic distinctions among Elasmobranchs and characteristic distinctions between mammals and Elasmobranchs in the brain, CSF, EDF, and sera. In sera the gamma globulins of Elasmobranchs differed from mammalian gamma globulins in their more rapid mobility; the albumin fraction could be detected in only trace amounts in all species of Elasmobranchs; and the levels of LDH and SGOT activity were similar to mammalian levels, but the AP and AChE values were lower than mammalian sera.

The CSF and EDF protein electrophoretic patterns were both species and tissue specific. The CSF, LDH, and SGOT levels were elevated in comparison to these levels in mammalian CSF. The LDH isozyme pattern in Elasmobranchs was different than mammals in sera, EDF, CSF, and brain tissue. Most interestingly in several species, the CSF isozyme pattern closely resembled the brain pattern but not the sera pattern.

Our data showing an increased percentage of gamma globulin in the EDF in comparison to sera, qualitative differences between gamma globulin of EDF and sera, and low levels of LDH and SGOT in the EDF indicate that the EDF is a fluid of a different composition than plasma. Our studies indicate that in shark CSF both the protein content and level of LDH activity are significantly higher than in mammalian CSF. The similarity of the LDH isozyme pattern in brain and CSF as contrasted to sera leads to the speculation that perhaps the brain tissue may be a source of the LDH found in CSF.

Significance to Bio-medical Research and the Program of the Institute: Comparative studies on physiology of primitive species such as Elasmobranchs may elucidate basic mechanisms operative in mammals including man. The investigations carried out on protein and enzyme profiles in various body fluids and nervous tissue in sharks and rays provide a base-line for studies concerning the circulation and dynamics of the cerebrospinal fluid as well as the transport phenomena in the nervous system. The last subjects are within the framework of major research projects of our branch.

Proposed Course of the Project: This project is concluded. The findings were reported at the Symposium on Current Investigations on Elasmobranchs Biology, held in February 1966, in Bimini, Bahamas.

Honors and Awards: None

Publications: None

1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Psychological Evaluation of Temporal Lobe Operations.

Previous Serial Number: SAME

Principal Investigator: Herbert Lansdell, Ph.D.

Other Investigators: M. Baldwin, M.D., A. Ommaya, F.R.C.S.,
C-l. Li, M.D., Ph.D., N. Urbach, M.A.,
P. Fedio, Ph.D., J. Baker, B.A.,
J. Van Buren, M.D., Ph.D., B. Vest, M.S.,
A. Polcari, M.S.W., and E. Walker, M.S.W.

Cooperating Units: C.C. NINDB Social Work Section

Man Years

Total: 2.3

Professional: 1.3

Other: 1.0

Project Description:

Objective: To study neurosurgical patients with temporal lobe disorders with respect to intellectual abilities, visual and auditory perception, linguistic functions and other "personality" features.

Methods Employed: Intelligence and personality tests; aphasia, audiometric, and other specialized verbal tests; tests of visual perception.

The object-naming task of Project 1033(c) is being employed to test patients during stimulation through electrodes implanted in the temporal area, and during the cortical stimulation which occurs in conscious patients undergoing temporal lobe surgery.

Major Findings: A recent finding that certain sets of scores from the Wechsler-Bellevue Intelligence Scale (WB) inversely correlated with the extent of the neurosurgery in some of our "followup" cases encouraged us to develop computer programs to investigate the efficiency of factor weighted scores in this type of research. Three factors were derived from the standardization

data by Maxwell's method. The scores from Atwell & Wells's Vocabulary test and the first WB factor (Verbal Comprehension) correlated well with the amount of left temporal removals. The scores from Mooney's Closure Faces test and the two minor factors of the WB ("Perceptual Organization" and "Freedom from Distractibility") were affected by right temporal removals; and these impairments differed for the two sexes. Although the factor scores seem to be no more "useful" than scores from other tests in this type of research, they are important in providing some reference points for neuropsychological research with cognitive tasks. At least, these results discourage the view that "intelligence" or some "highest integrative process" is a function only of the brain as a whole.

Recently we demonstrated that left vs. right differences in the effects of unilateral temporal-lobe removals on certain personality measures were not the same for male patients as for female patients. One of these measures came from the well-known MMPI, and its conventional psychiatric diagnostic terminology seemed to be potentially misleading with neurosurgical patients. In an attempt to find an appropriate descriptive terminology for this MMPI measure an investigation was undertaken with the help of a social worker (AP) who has interviewed patients on the neurosurgical ward for over three years. Thirty-nine cases, with various disorders, were found who had been interviewed by AP and who had yielded "valid" MMPI records. AP was given the MMPI score for one third of the group and asked to estimate the values for the remaining two thirds. His values correlated better than chance with the obtained values. The experiment is being repeated with another social worker (EW) on a larger group of patients, few of which are in the first sample. The description of the MMPI measure by AP seems appropriate; possibly the second experiment will provide a similar description.

Significance to Bio-medical Research and the Program of the Institute. With careful selection of patients, relations between psychological test scores and extent of cerebral lesion can be established selectively for different loci and for each sex; these relationships have implications for physiological concepts of "higher mental" processes.

Proposed Course of Project: Continued testing of patients undergoing surgery; further development of computer programs to analyze other psychometric test results. Systematic analysis of the effects of cortical stimulation during surgery with the standardized naming and memory task.

Honors and Awards: None

Publications: Lansdell, H., and Urbach, N. Sex differences in personality measures related to size and side of temporal lobe ablations. Proc. Amer. Psych. Assoc. 1: 113-114, Sept. 1965.

- Serial No. NDB(I)-63 SN/CP 1032(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Psychological Effects of Subcortical Lesions Used
for Relief from Abnormal Movements.

Previous Serial Number: SAME

Principal Investigator: Herbert Lansdell, Ph.D.

Other Investigators: J. Van Buren, M.D., Ph.D., A. Ommaya,
F.R.C.S., C-1. Li, M.D., Ph.D., P. Fedio,
Ph.D., G. Ojemann, M.D., J. Baker, B.A.,
and B. Vest, M.S.

Cooperating Units: None

Man Years

Total: 1.4
Professional: .5
Other: .9

Project Description:

Objective: In comparison with the effects of other forms of neurosurgery, to investigate the impairments resulting from subcortical lesions and electrical stimulation with the aim of delineating subcortical from cortical factors in behavior.

Methods Employed: A battery of tests (the same as those used in Project Serial No. 67c) is given to patients before, two weeks after, and again a year or more after operation. Results are to be analyzed for differences according to loci of the lesions, side of operation, and sex of the patients.

The object-naming task of Project 1033(c) has been employed to test patients during stimulation through the intracranial electrodes in the thalamic and other subcortical areas. In addition to the standard naming procedure, the test incorporates a motoric and delayed-recall feature. In this form, the task permits an analysis of arrest errors and those related to dysnomia, memory impairment and loss of consciousness.

Major Findings: Preliminary data indicates that stimulation in the left superior thalamus is associated with a significant

degree of anomia. In contrast, stimulation in a homologous region in the right thalamus has failed to produce a similar disruption.

Significance to Bio-medical Research and the Program of the Institute: The research on patients with implanted electrodes demonstrates that clear lateralization of speech mechanisms exists at the subcortical level; these results also imply that cortical vs. subcortical distinctions in the hemisphere for speech are not as clear as has been commonly assumed.

Proposed Course of Project: Analysis of the psychometric data is in progress; research on the effects of intracerebral stimulation will continue.

Honors and Awards: None

Publications: None

- Serial No. NDB(I)-63 SN/CP 1033(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Phonemic Aspects of Dysnomia

Previous Serial Number: SAME

Principal Investigators: Herbert Lansdell, Ph.D. and
Paul Fedio, Ph.D.

Other Investigators: J. Baker, B.A. and G. Ojemann, M.D.

Cooperating Units: None

Man Years

Total: .1

Professional: .1

Other: .0

Project Description:

Objective: To investigate the phonemic aspects of induced dysphasia and normal naming errors.

Methods Employed: Filmstrips and a projector are used. The filmstrips contain three orders of pictures; the names of the pictured objects have a wide range of phonemes in the initial and final positions. The nouns have a high frequency in the English language. The projector has an attached miniature screen; there is automatic control of exposure time from 30 to less than 0.5 sec/frame.

Major Findings: Much of the data being accumulated with this apparatus are considered under project nos. 1032(c) and 401(c). Data solely relevant in this project are being accumulated with its use during the occasional carotid Amytal tests.

Significance to Bio-medical Research and the Program of the Institute: Future different types of data may continue to extend our knowledge of the phonemic aspect of speech disruption and to delineate this important clue about the physiological mechanisms of speech.

Proposed Course of Project: The apparatus will be used in these projects and the phonemic aspect of the results will be analyzed when sufficient data are obtained.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-66 SN/CP 1245(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland
New Project

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: EEG Learning Correlates using Intracranial Depth
Electrodes.

Previous Serial Number: NONE

Principal Investigators: Paul Fedio, Ph.D., George A. Ojemann,
M.D., and William H. Sheriff, M.A.

Other Investigators: H. Lansdell, Ph.D., J. Van Buren, M.D.,
Ph.D., A. Ommaya, F.R.C.S., J. Baker, B.A.,
J. Bryan, M.A., and M. Haddox, R.N.

Cooperating Units: None

Man Years

Total: .2

Professional: .1

Other: .1

Project Description:

Objective: To study the neural correlates of information
storage and retrieval during learning.

Methods Employed: An auditory list of paired-associates is
used as the learning task. During the testing procedure, simul-
taneous EEG is recorded from an electrode situated in the medial
temporal or thalamic region in patients with epilepsy or motion
disorders. Control periods contain resting levels and the
stimulus-response characteristics of the learning session. Auto-
correlograms and spectral analysis of the EEG data for the learning
stages and control periods are computed.

Major Findings: Not available; further compilation of data
necessary.

Significance to Bio-medical Research and the Program of
the Institute: Data available from patients following temporal
lobectomy show a significant learning deficit. The technique
employed in this project affords a more precise method for examin-
ing the role of these neural structures during the course of
learning.

Proposed Course of Project: To employ various learning tasks and develop programs for EEG data analysis.

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1965 through June 30, 1966
Branch of Ophthalmology, Intramural Research
National Institute of Neurological Diseases and Blindness

Ludwig von Sallmann, M.D., Chief

Changes in the staff during the past year necessitated reorientation of programs in several units of the Branch. Dr. P. O'Brien was appointed to the Section on Cell Biology to succeed Dr. S. Bonting who resigned in July of 1965. Dr. O'Brien is a glycoprotein chemist who is applying his experience in this field to studies of eye tissues. Dr. A. Lasansky became Head of the Laboratory for Electronmicroscopy filling a position which had been vacant since the death of Dr. Wanko in 1964. Dr. Lasansky has to his credit important contributions to retinal research which combine morphological and physiological approaches and which place him in a unique position for participating in the program of the Branch. Other new investigators of the Staff include Dr. R. Helmsen, a Staff Fellow in the Section on Chemistry; Dr. G. Wasserman, a Guest Worker in the Section on Physiology; Dr. S. Salceda, a Guest Worker from Manila in the Section on Cytology and Histopathology; and Dr. R. Mazlen, a Research Associate in the Section on Cell Biology. Dr. Kern terminated his assignment as Associate in the Visiting Scientist Program in September 1965 after a stay of one and a half years. Rearrangement of investigative efforts in the clinical program was also required due to the two-year turnover of clinical associates engaged in this work. The departure of Drs. Carr, Spaeth, and Green was keenly felt.

Eleven new projects are listed among the laboratory and clinical investigations and 13 are being continued. Fifteen were either terminated or temporarily inactivated.

In the period from July 1, 1965 to April 20, 1966, 146 patients were admitted to the Nursing Unit accounting for 6,685 in-patient days. The out-patient census lists 495 patients and 1,663 visits to the OPD. Consultation requests from other Institutes were answered and amounted to 1,279 exceeding by about 100 the figure of the last report period. The number of major operations rose from 26 to 63. Minor surgical interventions appeared to decrease because many of these are now performed in the treatment room.

Laboratory Investigations:

RETINA: The overall approach to basic studies of vision has been strengthened by the appointments of Dr. Lasansky and Dr. O'Brien. Even more than before, the research activities of the Branch center around the anatomy and function of the retina.

The most fundamental work is conducted in the Section on Physiology. It was shown for the first time that retinula cells of the *Limulus* eye produce a propagated potential and that the retinula cells and the eccentric cell of one ommatidium respond synchronously to a light stimulus. The train of impulses

travels rapidly in the large fibers of the optic nerve, which originate in the eccentric cells, and more slowly in the small fibers deriving from the retinula cells. Therefore, the impulses arrive at a common point with different delays. The regular sequence of repeating events may be utilized in the analysis of the sensory message.

It has been concluded from previous work on time constants of responses to light that two processes of response generation exist; one controlling the rising phase of the response and the other its decay phase. Both functions are temperature dependent to the same degree pointing to the involvement of chemical reactions. This assumption was recently confirmed with the use of metabolic inhibitors. Dinitrophenol abolished the visual response before it affected membrane potential, membrane conductance, and the nerve impulse.

In another study a comparison was made between the Hodgkin-Fuortes cascade model for visual responses in *Limulus* and the probabilistic model of Levinson by analyzing the responses evoked by flashes delivering only a few photons. The results obtained so far indicate that responses to absorption of a single photon fit better the characteristics of the Hodgkin-Fuortes model.

Responses of single receptor cells have been measured as a function of wavelength and energy, and action spectra have been constructed which include data from the near ultraviolet region of the spectrum. Different ommatidia seem to consist of two types of cells designated as alpha and beta cells. The alpha cells are most sensitive to light of a wavelength in the region of 525 nm. Their action spectrum resembles that of rhodopsin. The beta cells, in contrast, are equally sensitive to all wavelengths from 350 to 550 nm. Beyond this region the sensitivity falls off steeply. These cells possess a higher ultraviolet sensitivity than the alpha cells, and their action spectra do not correspond to the density spectrum of a single photo pigment.

A technique perfected for the mammalian eye permitted introduction of glass microelectrodes into the surface layer of the retina in the intact eyes of anesthetized monkeys. Positioning of the electrodes and the light stimulus is carried out under direct biomicroscopic control. The responses of single ganglion cells in the fovea and perifoveal area to very small monochromatic light spots indicated the presence of several types of ganglion cells. They differ from each other in the amount of rod and cone signals and in the degree of antagonistic color responses they receive. The analysis of a particular class of perifoveal ganglion cells, the "on-center" type, provided evidence for the first time that rod signals reach the cell more slowly than the cone signals. Differences in the light sensitivity of the receptors are associated with rod responses to dim light and cone responses to bright light stimuli. The time delays of rod and cone signals are probably determined in the receptors themselves, whereas rod and cone interaction may occur afterwards, presumably at the level of the amacrine cells.

In continuation of previous work on ultrafine structure and function of retinal glial cells, the barrier qualities of the retinal pigment epithelium were examined in the toad. The electrical potential and current were measured and the ionic fluxes determined by the use of radioactive tracers. It was

shown that the pigment epithelium is the site of origin of the resting potential of the retina. The most important finding of the study was the demonstration of a net flux of chloride ions in the direction from pigment epithelium to choroid which accounts for a great part of the short circuit current. An active transfer of bicarbonate ion could not be proven but the experiment suggested that bicarbonate is taken up preferentially by the inner surface of the pigment epithelial cells. The distribution of bicarbonate may serve in regulation of the pH in the extracellular fluid of the retina. Work was initiated in this laboratory on the fine structure on the blood retinal barrier, but results are not available as yet.

New and promising investigations on retinal metabolism, especially on glycoprotein synthesis, are underway in the Section on Cell Biology. Early results have shown that one enzyme operating at the first step of the synthesis of an amino sugar nucleotide is present in the bovine retina. This is L-glutamine-D-fructose-6-phosphate transaminase. Most important evidence has been obtained that the retina is indeed actively engaged in glycoprotein synthesis. An enzyme associated with the particulate fraction of beef retina is able to transfer N-acetylneuraminic acid (NAN), the terminal sugar of many glycoproteins, to a non-lipid high molecular weight acceptor. Galactose, the penultimate sugar of many glycoproteins, is incorporated by the same system and the addition of UDP-galactose to the incubation mixture increases the incorporation of NAN. The stimulating effect of galactose suggests that the addition of this sugar to the endogenous acceptor provides additional sites for the attachment of NAN, and that glycoproteins may have their sugar components added one at a time rather than as a preformed polysaccharide chain.

Whether an autoimmune mechanism can be operative in acute hemorrhagic retinopathies was further studied in the Section on Cytology and Histopathology. Monkeys which had received a single dose of guinea pig cord antigen together with the complete Freund adjuvant developed eye pathology in conjunction with experimental allergic encephalomyelitis. The eyes of monkeys of four age groups were ophthalmoscopically examined by Dr. Ronald Myers of the Perinatal Research Branch in Puerto Rico before the animals died or were sacrificed. The eyes of 22 experimental monkeys and 6 controls, which had been injected only with Freund adjuvants, were examined histopathologically. Of particular interest were the eyes of those animals which exhibited the first fundus pathology one or two days before the termination of the experiment. In addition to hemorrhages in different layers of the retina and signs of breaks of the walls of fine vessels, the presence of fibrinoid occlusion of capillaries and small venules was noted which may be the first step in the sequence of pathological events. A fibrin meshwork was seen around large vessels and aggregates of fibrin or fibrinoid occasionally occupied their walls. Later stages were characterized by destruction of the retina by the hemorrhages; some of the extravasates broke through into the vitreous or into the subretinal space. The incidence of severe vascular occlusive lesions was greatest in young monkeys but occurred also in animals one or several years old. Optic nerve pathology consisting mostly of perivascular granulomatous lesions were seen in all age groups regardless of the presence or absence of retinal involvement.

CORNEA and VITREOUS: The Section on Ophthalmic Chemistry reported progress in the physical chemical definition of beef corneal collagen. New examinations indicate that the molecular weight of the collagen polymer is much higher, and that of the collagen subunit much lower, than previously thought. The polymers form aggregates of high axial ratio. Effective separation of subunits will be necessary before comparative studies on different collagens of the eye during development and disease can be undertaken.

A new project in this laboratory dealt with physical and chemical characteristics of proteins in the vitreous hydrogel. Immunochemical examination of the rabbit's vitreous, supports the concept that many vitreous glycoproteins derive from related serum proteins. A drastic decrease of globulin levels in the rabbit's vitreous occurs with age.

As a side issue of the research in this Section examinations were performed by the use of physical chemical methods to estimate the molecular weight of DNA extracts from purified adenovirus Type II. A value of 34,000,000 was obtained instead of 22,000,000 previously reported.

Two new experimental studies in the Section of Cytology and Histo-pathology dealt with corneal transplantation. The viability of the endothelium of corneal homografts after various periods of storage of the donor material was examined using the rabbit as the experimental animal. This investigation has obvious practical implications. Corneal grafts remained clear when the donor material was stored as long as fourteen days. Twenty-one days of storage resulted in transiently or permanently opaque grafts. Cytological examinations showed a progressive loss of endothelial cells corresponding to the time of storage. The surviving endothelial cells proliferated as shown by mitosis and by H^3 -thymidine incorporation. Cell proliferation was still demonstrated two months after transplantation but was less marked than in the first post-operative week. Morphological changes occurred in the surviving endothelium which contained cells with giant nuclei particularly in later stages.

In another study attempts were made to use the cat for producing a corneal homograft transplant model in which rejection of the graft regularly developed without additional non-ocular desensitization necessary to produce the rejection of homografts in the rabbit. The experimental plan is to attempt suppression of the rejection process by inhibitory agents. The work is in an early stage as technical difficulties in this experimental animal are great but progress has been made to overcome complications by modifying the surgical procedure.

CHOROID: Occlusion of choroidal vessels, particularly those of the choriocapillaris is expected to cause damage to the structures of the retina, the nutrition of which depends on choroidal blood. By the use of a technique described last year it was shown in a sufficient number of cats that obstruction of choroidal vessels by latex spheres resulted in retinal damage of various degrees in relation to the completeness or extent of the occlusive lesion. The pigment epithelium of the retina responded in most instances to slight or moderate ischemia of the choroid by either hypertrophy or destruction.

In severe choroidal ischemia the outer four layers of the retina underwent degeneration. Slight disturbance of the choroidal circulation did not seem to affect the photoreceptors.

LENS: Work on cell population dynamics in the lens epithelium of the rat was extended to studies of age effects on the growth of the lens and its epithelial population, on mitotic activity and H^3 -thymidine incorporation, and on diurnal fluctuation of these two aspects of cell proliferation. A rapid increase of the epithelial area contrasted with a very small and transient increase of the number of epithelial cells. Mitoses and DNA synthesis decreased markedly with age. The extent of diurnal fluctuations of mitosis were similar at all ages, but there was a six hour shift in the timing of the periods of minimum and maximum activity in older animals. Information on these various factors is required for estimation of phases in the cell life cycle and this in turn allows meaningful studies of experimentally induced cataract.

Last year the characteristics of a new type of cataract produced in fish by prolonged feeding with thioacetamide, a rodent carcinogen, was described. This year attempts to produce the tumor-like lens damage in rats failed even when the thioacetamide feeding was continued for one year.

INTRAOCULAR PRESSURE: The possible existence of a blood-aqueous humor potential functioning in the process of aqueous humor formation was reexamined in the Section on Pharmacology. Experiments did not provide data indicating the presence of a potential gradient in the ciliary body. The secretory function of the ciliary epithelium is not negated by these findings, but a blood-aqueous potential is not connected with such a function if it exists at all in the anesthetized cat.

The elaborate technique developed in this Section for measurement of the intravascular pressure and flow rate in the eye together with the intraocular pressure was employed in studies of the effects of corticosteroid preparations on various ocular structures and functions, and on the action of different pharmacological agents. When certain steroid compounds were directly infused into eye arteries the intraocular pressure rose up to 80 mm Hg. Celestamyd^R was more potent than Decadron^R. Interestingly the vehicle of Celestamyd^R also caused an elevation of the intraocular pressure. Six other steroids, including hydrocortisone and betamethasone, did not produce high intraocular pressure. None of the tested steroids affected the caliber of iris vessels. The effective steroids inhibited the responses of iris-ciliary body preparations to epinephrine, pilocarpine, eserine and acetazolamide to various degrees and resulted in dilation of the pupils. The work is of interest at this time in view of the lively controversy concerning steroid effects on the intraocular pressure.

Clinical Investigations:

RETINA: Newly developed electrophysiological and psychophysical techniques were employed successfully to distinguish between tapeto-retinal degenerations and other retinopathies. The use of the Ganzfeld stimulus instead of the

Maxwellian view facilitated and improved standard procedures of electroretinography. Modifications of static perimetry and the use of colored stimuli permitted recognition of incipient retinal receptor pathology at a time when other methods failed to disclose abnormalities. For patients treated with chloroquine such early diagnosis of retina dysfunction is important since in this stage the damage seems to be reversible. The elaborate testing system developed over several years in the Branch led to an increased number of patient referrals from other Institutes and Eye Centers.

UVEA: Systemic therapy with the antifolic methotrexate was continued in patients with steroid resistant anterior uveitis and was extended to the treatment of sympathetic ophthalmia. The number of patients studied is as yet too small to evaluate the effectiveness of the antimetabolite therapy. Some patients showed slow but definite regression of the peripheral fundus lesion and absorption of vitreous opacities, but the results were equivocal in other instances. One patient with sympathetic ophthalmia improved dramatically in response to the first injection of the antifolic drug, although two patients in the later stage of the same disease did not benefit from the medication.

The usefulness of chemotherapy with Daraprim and a sulfa drug was confirmed in many patients admitted to the Branch with the presumptive diagnosis of acquired toxoplasmosis. Ophthalmologists, previously critical of this therapy, have reversed their viewpoint. The failure of other types of treatment in controlling this infection was documented in a patient whose blind painful eye with secondary glaucoma had to be removed. An overwhelming number of toxoplasma cysts in various stages of viability were scattered throughout the retina. Behcet's disease, a relapsing chorioretinitis leading usually to complete loss of visual function, was studied again in several patients by adding anticoagulant therapy to the usual administration of steroids. In some patients relapses appeared to be delayed or prevented, but more cases and longer observation periods are necessary before a definite statement can be made.

GLAUCOMA: Most of the well organized studies on glaucoma patients or normal volunteers were carried out last year. Since final results were not available at the time of last year's report they are briefly mentioned now.

It was shown in a study of the water drinking test that, in about 20 percent of patients, the rise of intraocular pressure precedes the fall of serum osmolality and, more important, that measurements carried out at 15 minute intervals were necessary to avoid overlooking abnormal pressure rises.

Close analysis of data dealing with effects of Dexamethasone instillation on the intraocular pressure of normal volunteers and glaucoma patients indicated that, when the drug was used for three to four weeks, the pressure increased significantly in all instances. The coefficient of aqueous outflow decreased in 56 percent of normals and 67 percent of glaucoma patients. The water drinking test became positive in about 50 percent of the normal volunteers.

Although, as a rule, systemic use of steroids does not cause elevation of the intraocular pressure, one observation showed the opposite can be true. A patient referred to NIH because of unexplained elevation of intraocular pressure was found to have applied a steroid ointment to his legs over a long period of time for treatment of a skin lesion. When the skin treatment was discontinued the intraocular pressure and outflow facility returned quickly to normal.

Hydromethylprogesterone is an anti-inflammatory steroid supposedly without effect on the intraocular pressure. In studies on 15 glaucoma and 7 normal volunteers no increase of intraocular pressure followed one month's treatment with the compound whereas the same subjects had reacted with pressure rises to local Dexamethasone medication previously.

OCULAR CHANGES IN SYSTEMIC DISORDERS: Crystals in the cornea are well known manifestation of congenital cystinosis. The presence of a peripheral retinopathy in these children was described for the first time. It will be of particularly great diagnostic value in cases where this lesion precedes the corneal complications. The retinopathy is characterized by a zone of depigmentation with clumps of pigment of various forms. The retinal lesion was observed in all eleven patients of the series, but was not found in adults with this disease. The histopathology in two patients confirmed the involvement of the pigment epithelium of the retina.

1. Ophthalmology Branch
2. Section on Ophthalmology
Physiology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Design and Construction of Ophthalmic Instruments;
Research in Psychophysical Methods of Evaluating Vision

Previous Serial Number: SAME

Principal Investigator: Ralph D. Gunkel, O.D.

Other Investigators: Peter Gouras, M.D.

Cooperating Units: None

Man Years

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: To make improvements or modifications of instruments used in clinical and experimental ophthalmological work. Specifically, to design and/or construct such devices as are suggested or required by current projects and to follow through in their use.

Methods Employed: A number of further additions and modifications have been made to the apparatus used by Dr. Gouras in his studies of electroretinography, d.c. potentials and single unit measurements.

It was found desirable to improve the brightness and quality of the second beam which had been added to the photic stimulator a year or more ago. This involved dismantling the large Xenon lamp housing, milling a 2½ inch hole in one side and constructing a condenser system to fit it. This second beam was brought into the fundus camera which is the core of the apparatus by means of a partially reflecting mirror critically placed inside an existing aperture in the optical system. This permits a background light of any color or brightness to be projected into the living eye while a light stimulus of any size, color, brightness or duration is superimposed upon it. An electrically controlled shutter was also incorporated in the second beam. The success of this system suggested a critical experiment in which small spots of the same

or different color are projected on the retina from each beam in a known location with respect to the electrode. The interesting results and implications are discussed in a paper by Dr. Peter Gouras.

In an effort to attain a logical and convenient standard apparatus for clinical electroretinography and electrooculography, a 24-inch integrating sphere constructed some two years ago has been utilized. Since uniform stimulation of the entire retina, standard brightness, and elimination of peripheral distractions are desired, the integrating sphere (Ganzfeld) appeared to offer the best solution. A Grass photic stimulator was removed from its parabolic reflector housing and equipped with diffusing filter and cells for neutral density or colored filters. This assembly was inserted in an aperture in the top of the sphere, outside the patient's field of view when his head is positioned by the chinrest. The arrangement appears to be entirely satisfactory for electroretinography and is now used routinely.

In order to use the same apparatus for electrooculography, it was necessary to devise some simple means of controlling fixation as the subject shifted his gaze from straight ahead to 30° to one side and back again. It seemed possible that better control might be maintained if the subject were simply instructed to follow a projected spot of light as it moved to one side, paused the desired period, moved to the other side, paused and returned, continuing for as long as the test continued. A device meeting these requirements was designed and constructed, but the resulting oculogram appeared quite unconventional, being a sinusoidal wave of considerable amplitude. Since the sine wave response could not be completely interpreted at this time, the device was set aside in favor of a pair of miniature red lights which are manually turned off and on in proper sequence by an external switch. The subject is instructed to look at whichever light is on, and with the more or less sudden eye movements, the conventional electrooculogram is obtained.

Measurements of dark adaptation and retinal (rod and cone) thresholds continue to be clinically useful. Some further interest was stimulated by a recent publication in which the threshold test was described as being helpful in detecting early retinal damage resulting from ingestion of chloroquine. About 150 of these tests have been done in the past year, many of them to obtain baseline sensitivity levels before starting chloroquine therapy or to evaluate subsequent fluctuations.

A series of more typical or interesting retinal profile studies has been selected to show the findings in different types of retinal abnormalities. This paper will be presented for possible publication within the month.

Numerous occasions have arisen in which it would have been useful to have a human eye with dilated pupil readily available for testing, demonstrating, or practicing some ophthalmic procedure. Sometimes a suitable patient is available, but more often there is some reluctance to justify dilatation without an obvious benefit to the patient. Schematic eyes are available which can be used for practice retinoscopy and fundus photography, but in

most ways they do not approach similarity to the human eye and they are completely unsuited for the use with the slit lamp or indirect ophthalmoscope. After considerable study and some consultation with technicians who make prosthetic eyes and others who are familiar with plastic materials and techniques (art department) a model is nearing completion which will appear remarkably lifelike. The physical dimensions, refractive properties, and general appearance will contribute to the usefulness of this artificial eye. Mr. Howard Bartner, Chief of the Medical Illustration Section also had need for such a model and has devised techniques for reproducing vascular, retinal and iris details with remarkable fidelity.

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

It would appear that the ready ability to translate ideas into instrumentation has been helpful to several projects in the Ophthalmology Branch and may have suggested others.

Proposed Course of Project: It is proposed that this project be continued in its present flexible form.

Honors and Awards: None

Publications:

Carr, R.E., Gouras, P., and Gunkel, R.D.: Chloroquine retinopathy: early detection by retinal threshold test. Arch. Ophthal. 75: 171-178, Feb., 1966.

1. Ophthalmology Branch
2. Section on Ophthalmology
Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Activity of Visual Receptor Organs

Previous Serial Number: SAME

Principal Investigator: M.G.F. Fuortes, M.D.

Other Investigators: None

Cooperating Units: Marine Biological Laboratory
Woods Hole, Massachusetts

Man Years

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: The aim of the present research is to identify the processes responsible for activation of visual receptor cells.

Methods Employed: Research has been performed using the same electro-physiological methods as in previous years. For some experiments, these methods have been modified in order to permit the study of two units simultaneously.

Major Findings: The experiments performed last year on the electrical interaction between ommatidial cells have been continued. By recording simultaneously from two cells in the same ommatidium, it has been observed that the small spikes which can be led off from retinula cells are always synchronous with the large spikes recorded from the eccentric cell of the same ommatidium. By contrast, no interaction is found between cells belonging to different ommatidia. Contrary to reports by other authors, it has been found that both the large and the small nerve fibers in the optic nerve (originating in eccentric and retinula cells, respectively) can conduct nerve impulses. Since impulses are synchronous in all cells of the same ommatidium, it seems probable that following illumination, identical trains of impulses travel rapidly down the axon of one eccentric cell fiber and more slowly along a dozen retinula cell fibers. This repetition of identical patterns reaching

a common point with different delays may be a useful arrangement for the analysis of the features of the sensory message.

Two years ago it was noted that responses to light can be reproduced approximately by a formula containing two time constants. This suggests that two processes are important for generation of responses: one controlling their rising phase and the other controlling their decay. Last year it was observed that the effects of changes of temperature on visual responses can be reproduced by the theoretical formulation assuming simply that temperature changes both the time constant of rise and the time constant of decay in the same proportion. The relation between time constants and temperature was found to be compatible with the assumption that both processes are chemical reactions. This suggestion was confirmed this year by experiments making use of enzymatic poisons; it was shown that drugs such as Dinitrophenol (DNP), which interfere with oxidative phosphorylation, abolish (reversibly) the visual response before they affect appreciably membrane potential, membrane conductance or the nerve impulses of the visual cell. These results strengthen the view that visual responses are brought about by chemical reactions and that the organization of these reactions is in series, as represented by the model proposed by Dr. Alan Hodgkin in 1964.

In a recent article discussing Hodgkin's cascade model, Dr. J. Levinson of the Bell Telephone Laboratories suggested that an alternative arrangement could lead to very similar results. In brief he proposed that generator potentials are composed of "quantal responses" and that one quantal response occurs when n particles converge upon a site which has absorbed one photon. The shape of the generator potential would then be controlled by the scatter of the latency of the individual quantal responses. The two models can be distinguished in principle by the results obtained with very dim illuminations. A study of responses evoked by flashes delivering only few photons to the preparation was performed during the summer. The results of this study are not unequivocal, but they suggest that the response to absorption of one single photon has the characteristics predicted by Hodgkin's cascade model and not those anticipated by Levinson's probabilistic model. The sharp discrete waves often seen in dark adapted preparations seem to be a consequence of, rather than the basic components of, generator potentials.

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

The model proposed by Dr. Hodgkin was an important step forward because it suggested how the processes leading to vision are organized, but it said little or nothing on the physical nature of these processes. The findings described in the present report may prove to be of some important because for the first time they reveal the probable nature of visual processes. The effects of temperature on visual responses are consistent with the view that responses are brought about by chemical reactions, but might be equally consistent with a number of other interpretations because temperature influences in a similar manner a large variety of processes. The later observations showing that poisons such as DNP abolish the response to light gives more direct support to the view that chemical reactions are required for excitation of

visual cells. The results obtained so far are compatible with the interpretation that light produces responses in visual cells by triggering a sequence of enzymatic reactions whose end product is a substance capable of changing the properties of the cell's membrane.

Proposed Course of Project: In the presence of evidence suggesting a chemical origin of visual responses, it may be useful to study what chemical agents in addition to DNP may affect production of visual responses. It may be interesting to study substances affecting ATP metabolism or respiration or glycolysis. One may hope in this manner to confirm the need for chemical reactions and to identify some of the reactions involved.

Honors and Awards: None

Publications:

Borsellino, A., Fuortes, M.G.F., and Smith, T.G.: Visual responses in Limulus. Cold Spring Harbor Symp. Quant. Biol. XXX: 429-443, 1965.

Fuortes, M.G.F.: Effect of temperature on visual responses in Limulus. In Nye, P. (Ed.): Proc. Symp. Information Processing in Sight Sensory Systems. Pasadena, Cal., California Institute of Technology Press, 1965 (in press).



Serial No. NDB(I)-63 O/OPS 1012(c)

1. Ophthalmology Branch
2. Section on Ophthalmology
Physiology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Electrophysiologic and Psychophysical Studies of Tapeto-Retinal Degenerations - Clinical and Experimental

Previous Serial Number: SAME

Principal Investigator: Peter Gouras, M.D.

Other Investigators: J. Brooks Crawford, M.D.
Ralph D. Gunkel, O.D.

Cooperating Units: None

Man Years

Total:	0.7
Professional:	0.3
Other:	0.4

Project Description:

Objectives: To study by the use of electrophysiologic and psychophysical techniques specific characteristics of the abnormal visual systems in man and animals.

Methods Employed: Electrophysiological testing procedures, such as the electroretinogram and the electrooculogram, are used to study patients and experimental animals with various types of retinal degenerations. In patients this is coupled with psychophysical examinations, such as perimetry, dark adaptation and retinal profiles.

Patient Material: Patients have been obtained from both the outpatient and ward services of the Ophthalmology Branch and also referred from other institutes of NIH. Normal subjects were obtained from the Normal Control Program of NIH.

Major Findings: A sensitive psychophysical test for chloroquine retinopathy has been introduced which demonstrates that damage from this drug can elude standard testing procedures.

A new method of eliciting clinical ERGs, the ganzfeld technique, has been developed and promises to be an important step in relating the amount of retinal damage with the electrical response of the eye.

Significance to Bio-medical Research and the Program of the Institute: The mechanism underlying these ocular disorders is being investigated and may contribute to a better understanding of their pathogenesis.

Proposed Course of Project: Patients with various forms of retinal degenerations will be continued to be studied as they appear in the clinical service using the new "ganzfeld" technique. Studies are also being carried out to detect the site of damage of chloroquine and heavy metals on the retina in experimental animals.

Honors and Awards: None

Publications:

Carr, R.E., Gouras, P., and Gunkel, R.D.: Chloroquine retinopathy: early detection by retinal threshold test. Arch. Ophthal. 75: 171-178, Feb., 1966.

Carr, R.E., and Gouras, P.: Clinical electroretinography. J.A.M.A. (in press), 1966.

Serial No. NDB(I)-63 O/OPS 1016(c)

1. Ophthalmology Branch
2. Section on Ophthalmology
Physiology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Electrophysiological Studies of Mammalian Retina

Previous Serial Number: SAME

Principal Investigator: Peter Gouras, M.D.

Other Investigators: None

Cooperating Units:

Man Years

Total:	1.5
Professional:	0.9
Other:	0.6

Project Description:

Objectives: To determine the cells responsible for generating the electrical activity of the retina following light stimulation and to understand their coding of visual information.

Methods Employed: Micro- and macro-electrodes are introduced into the eyes of animals in order to determine the sources of the light induced potentials of the retina. The responses of different retinal elements are separated either by suitable control of the wavelength, energy, spatial distribution and time function of the light stimulus or by the production of retinal degeneration. Retinal degeneration is later studied by light microscopy. Recently it has become possible to introduce fine glass micro-electrodes to the retinal surface of the intact eye of the anesthetized Rhesus monkey. The responses of single cells are recorded to spots and patterns of monochromatic light. Both the microelectrode and the light stimuli can be clearly observed on the retinal surface. Cells in the foveal and perifoveal areas of the retina, in particular, are studied. The eyes can be examined repeatedly over many months.

Major Findings: Three distinct functional types of ganglion cells have been found so far in the central primate retina: 1) Cells whose high frequency spontaneous activity is initially inhibited by light, 2) Cells with little spontaneous activity which are initially excited by light. Both of

these cells receive information from both rod and cone receptors. 3) Cells which are excited at one end and inhibited at the other end of the visible spectrum. The latter cells appear to mediate an opponent mechanism of color vision in the primate retina.

The study of rod and cone receptor interaction on the Type 2 perifoveal ganglion cell has revealed an interesting new aspect of retinal function. Although both rods and cones deliver their signals to the same ganglion cell, the times at which these signals take to reach the ganglion cell are quite different. The rod signals are sensitive and slow; the cone signals are insensitive but quick. Therefore, response to dim stimuli are slow and determined entirely by the rods. Responses to brighter stimuli are quicker and determined by the cones. The earliest signals to excite the ganglion cell leave a transitory refractoriness in their wake. Therefore when rods and cones are stimulated simultaneously, the earlier cone signal arriving at the ganglion cell has a greater chance of producing excitation than the later rod signals. Activity recorded from before the ganglion cells and representing bipolar and receptor activity show the same delay between rod and cone signals but no evidence of interaction. The conclusion is that the time delays of the rod and cone signal are determined before the bipolar cells, presumably in the receptors, themselves, whereas the rod-cone interaction occurs afterwards, presumably at the level of the amacrine cells.

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

Such animal experimentation provides a means of correlating function with anatomy at the cellular level in a way that is impossible with the human retina.

Proposed Course of Project: To answer the following questions about retinal function:

- 1) Do the retinal connections of a perifoveal ganglion cell change during visual adaptation or centrifugal fiber stimulation?
- 2) Are the bipolars connected to both rods and cones?
- 3) What is the role of the horizontal cells? In the latter experiments I shall have the assistance of Dr. Arnaldo Lasansky.

Honors and Awards: None

Publications:

Gouras, P., and Link, K.: Rod and cone interaction in dark-adapted monkey ganglion cells. J. Physiol. 184: 777-789, 1966.

1. Ophthalmology Branch
2. Section on Ophthalmology
Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Spectral Sensitivities of Receptor Cells in the Lateral Eye of Limulus

Previous Serial Number: NONE

Principal Investigator: Gerald S. Wasserman, Ph.D.

Other Investigators: M.G.F. Fuortes, M.D.

Cooperating Units: None

Man Years

Total:	1.1
Professional:	0.8
Others:	0.3

Project Description:

Objectives: To confirm an early report of the existence of two classes of ommatidial cells in the lateral eye of Limulus with different action spectra and to extend the range of observations into the near ultraviolet region of the spectrum.

Methods Employed: The eyes are sectioned so as to expose the ommatidia; using micropipettes, intracellular responses from single receptor cells are measured as a function of wavelength and energy. Equal-response action spectra are constructed from these data.

Major Findings: Two types of cells are found:

Alpha cells - These cells have action spectra which agree well with the density spectrum of rhodopsin, which is the only photopigment thus far extracted from the rhabdome of this eye. The most sensitive wavelength is in the neighborhood of 525 nm.

Beta cells - The action spectra of these cells do not resemble any photopigment spectra; beta cells are almost (± 1 dB) equally sensitive to all wavelengths from 350 to 550 nm, whereupon sensitivity drops rapidly. The absolute sensitivities of individual cells penetrated with a micropipette

vary over a range of 10 dB and not enough data have been collected to definitively establish the relative sensitivities of alpha cells and beta cells. However, the data on hand suggest that beta cells are less sensitive than alpha cells in the visible spectrum (which agrees with the earlier report) and more sensitive in the near ultraviolet.

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

The ability to extract a complete, replicable equal-response action spectrum from a single receptor cell is unique to the eyes of invertebrates. Such information is of value both in understanding the molecular basis of vision in general and in answering questions about color vision in particular.

Proposed Course of Project:

The current plan of research is to take spectra from a number of cells sufficient to determine the relative sensitivities of alpha and beta cells and then to try to fractionate the beta cell action spectrum by chromatic bleaching.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-56 O/CH 302(c)

1. Ophthalmology Branch
2. Section on Cytology and
Histopathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Electron Microscopic Studies on Tissues of the
Eye, Such as Epithelium, Fibers and Capsule of
the Lens, and the Conjunctiva

Previous Serial Number: SAME

Principal Investigator: Theodor Wanko, M.D.

Other Investigators: Ludwig von Sallmann, M.D.
Bolivar J. Lloyd, Jr., B.S.

This project is terminated until an additional investigator
joins the Section and can reactivate the project.

1. Ophthalmology Branch
2. Section on Cytology and
Histopathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Autoradiographic Studies of Cellular
Proliferation in Ocular Tissues

Previous Serial Number: SAME

Principal Investigators: Ludwig von Sallmann, M.D.
Patricia Grimes, B.A.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	2.1
Professional:	1.2
Others:	0.9

Project Description:

Objectives: To study the life cycle of cells in the lens epithelium, specifically, to investigate the influences of age and diurnal variations in cell division in order to establish the relation between epithelial proliferation and formation of lens fibers and to provide a basis for the analysis of the effects of drugs on this population.

Methods Employed: The procedures used in the study of mitosis and DNA synthesis in the lens epithelium have been described in detail in earlier reports. To determine the size of the epithelial population in normal rats of different ages flat mounts of the epithelium were projected on a sheet of paper at a linear enlargement of 30 times. The equatorial, preequatorial, and central areas were traced and measured with a planimeter. In each preparation, the number of nuclei in

small microscopic fields of known area were counted at a magnification of 1500 times in the densely populated equatorial zone and 960 times in the preequatorial and central areas. The average nuclear count from 10 fields of each zone was employed to calculate the population density for each zone according to the following formula: Zone population = number of cell/field area x zone area. The sum of the number of cells in the three zones was taken as the total population.

Major Findings: Direct measurements of the area of flat preparations of the epithelium demonstrated an increase of size with age which closely paralleled the enlargement of total surface area with age calculated by other workers. Increase of the area covered by the epithelial layer was, therefore, a reliable index of lens growth. Between 2 weeks and 8 weeks of age epithelial area expanded by 65 per cent but the population grew by only 14 per cent. At 6 months of age the epithelial area was 80 per cent larger with no further change in population size. The area measured at 1 year was slightly greater than that at 6 months but the number of cells had fallen during this interval so that the population equaled that of 2 week old rats. During this growth period cell nuclei became more widely separated and their size appeared larger demonstrating that the epithelial layer adapted to growth of the lens by spreading and flattening of the cells. The stability of population size attained several weeks after birth indicates that for a portion of adult life, at least, the lens epithelium functions as a steady state renewal system with cell birth balanced by loss of cells through differentiation.

Study of the effects of age on cell proliferation and on diurnal variations of both mitotic activity and ^3H -thymidine incorporation demonstrated the need for caution in interpreting data relating to the life cycle of cells in vivo. We observed a decrease in mitotic activity in the lens epithelium of rats killed in the forenoon between 1 day and 1 year of age. Determination of the number of ^3H -labeled cells in the same preparations showed, however, that ^3H -thymidine incorporation fell much more drastically. This phenomenon was explained to some extent by the existence of a pattern of diurnal variations in mitotic activity which was altered by age. The

forenoon is a period of relatively low mitotic activity in young animals but is a time of peak activity in older animals. No consistent pattern of diurnal variations in ^3H -thymidine incorporation was seen in 6 week, 6 months, or 1 year old animals. Sampling over the day at intervals shorter than 6 hours may reveal a periodicity in DNA synthesis which was undetected in these experiments.

Significance to Bio-medical Research and the Program of the Institute: The lens epithelium is a relatively simple cell system particularly well suited to study of the life-cycle of the cell. Knowledge of the population dynamics of this tissue is necessary to promote a better understanding of the normal development of the lens and of cataractogenesis. Results of investigations of cell proliferation in the lens epithelium may be applicable to studies of more complex cell systems.

Proposed Course of the Project: The investigation will be extended to the study of effects of other cataractogenic agents in an attempt to define the role of epithelial damage in lens opacification.

Honors and Awards: None

Publications:

von Sallmann, L., and Grimes, P.: The effect of triethylene melamine on DNA synthesis and mitosis in the lens epithelium. Docum. Ophthal. 1966, In press.

Grimes, P., and von Sallmann, L.: Interference with cell proliferation and induction of polyploidy in rat lens epithelium during prolonged myleran treatment. Exper. Cell Res. 1966, In press.

Serial No. NDB(I)-65 O/CH 1212(c)

1. Ophthalmology Branch
2. Section on Cytology and Histopathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Retina and Optic Nerve Pathology in Autoimmune Encephalomyelitis of Rhesus Monkeys of Different Ages

Previous Serial Number: SAME

Principal Investigator: Ludwig von Sallmann, M.D.

Other Investigators: Ronald Myers, M.D.

Cooperating Units: Laboratory of Perinatal Physiology
NINDB

Man Years

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives: To study the sequence and nature of the pathologic events in eye tissues of Rhesus monkeys which had been injected at various ages with a single dose of guinea pig cord antigen.

Methods Employed: The clinical neurological observations by Dr. Ronald Myers of the Laboratory of Perinatal Physiology included frequent ophthalmoscopy. Three animals were housed and examined at NIH where photographic documentation of the retinal lesions in one monkey was possible. Both eyes of 22 Rhesus monkeys were received for histological studies accompanied by description of the eye ground findings by Dr. Myers. After gross examination of the opened globes, the

eyes were embedded in celloidin and sectioned. In addition to routine techniques, fibrin, glia, axon, myelin, PAS and other special stains were used.

Major Findings: The most striking changes involved the retina and optic nerve of the majority of animals. The uvea showed minor inflammatory lesions considered secondary to the vascular retinopathy. In five monkeys the first ophthalmoscopically visible changes preceded death by one or two days. Examination of these eyes permitted identification of the nature of the earliest lesions. Widespread hemorrhages in various layers of the retina and disruption of the walls of small and medium-sized veins were associated with occlusion of the lumen of venules and capillaries by fibrinoid or fibrin clots. Large vessels showed accumulation of fibrin material in their walls and in parts of the lumen and a fibrin meshwork around the vessels. Inflammatory cells were absent. In later stages destruction of the retina by massive hemorrhages with occlusion of large vessels occurred. The hemorrhages extended into the vitreous and the subretinal space. This was noted particularly often in the youngest age groups consisting of new born to three months old monkeys, while in monkeys one year old or older the incidence of severe vascular occlusive lesions was lower. The perivascular granulomatous inflammatory changes in the optic nerve developed in most animals independent of age. As a rule, the histopathology paralleled the clinical funduscopic findings. In some of the optic nerves focal demyelination coexisted with preserved axons.

Significance to Bio-medical Research and the Program of the Institute: This is the first observation of a severe vasculo-occlusive retinopathy in animals with experimental allergic encephalomyelitis. In the present study, the clinical course was followed from the onset and histologically fibrinoid obstruction of small vessels and per rhexin hemorrhages in the retina were documented. Retina and optic nerve pathology was correlated with the age of the animals. Although an autoimmune mechanism has not been proven to underlie vascular retinopathies of the human eye, the observations in the EAE monkeys point to the possibility of such occurrence.

Proposed Course of the Project: When the histopathologic studies of brains are available (Dr. Lerner) it is planned to correlate the eye pathology with that in the central nervous system.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 O/OC 1216(c)

1. Ophthalmology Branch
2. Section on Cytology and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Thioacetamide-Induced Cataract in Trout

Previous Serial Number: SAME

Principal Investigator: Ludwig von Sallmann, M.D.

Other Investigators: Patricia Grimes, B.A.
Eleanor M. Collins

Cooperating Units: None

Man Years

Total: 0.3
Professional: 0.15
Others: 0.15

Project Description:

Objectives: To produce a thioacetamide cataract in rats similar to that induced in trout by prolonged feeding of the carcinogenic agent, in order to investigate further the pathogenesis of this unusual cataract.

Methods Employed: Thirty-five male rats of the Osborne-Mendel strain weighing approximately 100 gms were placed on a diet which contained 0.04% thioacetamide. Twelve additional animals served as controls. Body weight and food consumption were recorded and the lenses of the animals were examined with the biomicroscope at regular intervals. Rats were killed in groups of two after one, two, four, eight, and twelve weeks. The livers and eyes of these animals were examined histologically. The remaining rats were kept on the thioacetamide diet for one year.

Major Findings: The livers of the experimental rats demonstrated typical signs of thioacetamide toxicity. Enlargement of the nuclei and nucleoli of the liver cells was followed by severe cirrhosis and the development of cholangiocarcinomas in many cases. No abnormalities of the lens were observed during the one year observation period. Probably the cataractogenic effect of thioacetamide in trout and its carcinogenic effect in the rat liver represent different mechanisms of drug action in the two species.

Significance to Bio-medical Research and the Program of the Institute: The invasive proliferation of the lens epithelium observed in the thioacetamide-induced cataracts of trout is of particular interest in view of the fact that cancer of the lens has never been observed in vivo. It was hoped that if the cataract could be produced in rats a more complete study could be made of the relation between the carcinogenic and cataractogenic properties of thioacetamide.

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

Honors and Awards: None

Publications:

von Sallmann, L., Halver, J. E., Collins, E., and Grimes, P.: Thioacetamide-induced cataract with invasive proliferation of the lens epithelium in rainbow trout. Cancer Res. 1966, In press.

Serial No. NDB(I)-65 O/CH 1223 (c)

1. Ophthalmology Branch
2. Section on Cytology and
Histopathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 31, 1965 through June 30, 1966

Project Title: Studies on the Enhancement of Contrast in
Isolated Particles and Thin Sections

Previous Serial Number: SAME

Principal Investigator: Bolivar J. Lloyd, Jr.

Other Investigators: None

This project is terminated because of the change of research
interest of the new head investigator of this laboratory.

1. Ophthalmology Branch
2. Section on Cytology and
Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Fine Structure of Toad Retinal Capillaries

Previous Serial Number: NONE

Principal Investigator: Arnaldo Lasansky, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives: To investigate the morphological basis of the blood-retinal barrier.

In the toad the retinal capillaries lie within the vitreous gel on the inner surface of the neural retina. This situation led to hypothesize that these capillaries might not have glial cell covering. If a blood-retinal or blood-vitreous barrier were found, it would indicate that the capillary wall is the structure responsible for the restriction to diffusion of solutes.

Methods Employed: Conventional electron microscope techniques are used. The permeability of retinal capillaries is tested by studying the penetration within the vitreous humour and retina of some of the dyes or tracer substances classically employed in experiments on the blood-brain barrier.

Major Findings: Only preliminary observations have been made. It is already clear however that a glial cell covering is absent. The wall of retinal capillaries in the toad is formed by endothelial cells, pericytes and a basement membrane as is usually found in other capillaries. Externally to these elements only vitreous components are observed. The inner limiting membrane of the retina is immediately adjacent but the footplates of Müller cells do not surround the capillaries.

In addition, experiments dealing with the permeability of toad retinal capillaries to trypan blue have been performed. This dye has been selected as a starting tool because it is the most commonly used test substance for investigations on the blood-brain barrier. There was a lack of penetration of trypan blue into either the vitreous humour or the retina, indicating that toad retinal capillaries behave similarly as capillaries in the retina of other species and in the brain.

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

The purpose of this study is to determine what role, if any, do Müller cells (the main glial elements in the neural retina) have in mediating exchanges of ions and metabolites at the level of retinal capillaries.

Proposed Course of Project: The preliminary observations described will be extended and a detailed analysis of the fine structure of the endothelial cells will be performed. Since it seems that the capillary wall is the site of the barrier phenomenon, studies will also be conducted in toad skeletal muscle capillaries for comparison purposes.

The observations on permeability to trypan blue will be complemented by employing other tracer substances, since the limitations of using dyes that are adsorbed to plasma proteins are very well known.

Honors and Awards: None

Publications: None

1. Ophthalmology Branch
2. Section on Cytology and
Histopathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Studies on the Function of the Retinal Pigment Epithelium

Previous Serial Number: M-NB-3

Principal Investigator: Arnaldo Lasansky, M.D.

Other Investigators: None

Cooperating Units: **Laboratory of Neurobiology, NIMH**

Man Years

Total:	0.75
Professional:	0.75
Other:	0.00

Project Description:

Objectives: The pigment epithelium appears to be the only major diffusion barrier interposed between choroid and neural retina. The main objective of this work is to provide information on the possible role of the pigment epithelium in controlling the flow of ions and metabolites into and out of the retina. Pigment epithelium cells are non-neuronal neuroectodermic elements which can be regarded on the basis of structural and ontogenetical considerations as analogous to the ependymal cells lining the choroid plexuses. It is therefore hoped that the results obtained may prove relevant to such problems as the functional properties of glial cells and the mechanisms of regulation of the inner milieu in the central nervous system.

Methods Employed: A technique has been devised by which the pigment epithelium and choroid of the toad eye are dissected free of the sclera and neural retina without damaging the pigment epithelium cells. The membrane constituted by the pigment layers of the eye is then mounted in a specially designed flux chamber and the electrical potential and current are measured and related to the ionic fluxes as determined by means of the use of radioisotopic tracers. This methodology is essentially identical to that used in studies on other biological membranes, as frog skin and toad bladder. The eye of a South-American toad (Bufo arenarum, Hensel) was initially used, but then the main results were confirmed in Bufo marinus which is the only species utilized presently.

Major Findings: (1) The pigment layers of the eye generate "in vitro" and during many hours a d.c. potential of 20-30 mV, pigment epithelium surface positive, and a short-circuit current of 20-40 μ amp cm^{-2} . This observation supports the hypothesis (W. K. Noell, 1953) that the pigment epithelium is the site of origin of the resting potential of the retina.

(2) In the absence of an electrochemical potential difference, a net flux of Cl^- ($0.90 \mu\text{Eq. cm}^{-2} \text{hr}^{-1}$) in the direction pigment epithelium to choroid is found. This net flux accounts for a major fraction of the short-circuit current, but not for all of it. The non-chloride fraction of the short-circuit current remains unaccounted for after measuring the fluxes of Na^+ , K^+ and Ca^{++} .

(3) Considering that bicarbonate is the only other ion in the saline solution utilized attempts were made to investigate the possibility that a net transfer of bicarbonate might exist which would explain the non-chloride fraction of the current. Manometric determinations of bicarbonate content in the bathing solution do not lend support to this view. The results however suggest an uptake of bicarbonate at the pigment epithelium surface.

Significance to Bio-medical Research and the Program of the Institute: The existence of a net flux of Cl^- in the absence of an electrochemical potential difference is indicative of an active transport of this ion from pigment epithelium to choroid. This finding gives support to the idea that the pigment epithelium has a major role in influencing the composition of the ionic environment in the neural retina. However, the implications of chloride transport in relation to the function of the neural retina remain unknown.

More work is needed in order to have a better understanding of the fate of the bicarbonate ion at the epithelial surface of the membrane. The indications that pigment epithelium cells take up bicarbonate from the bathing solution suggest the existence of a mechanism for regulation of the pH of the extracellular fluid in the neural retina.

Proposed Course of Project: Previous technical difficulties in avoiding exchanges of C^{14}O_2 with the atmosphere have been eliminated and some preliminary experiments have been done measuring $\text{C}^{14}\text{O}_3\text{H}^-$ fluxes. These initial results suggest a net flux of the pair $\text{C}^{14}\text{O}_2 - \text{C}^{14}\text{O}_3\text{H}^-$ from pigment epithelium to choroid but further experimentation is required. The chemical determinations of bicarbonate will be repeated but this time without the use of a flux chamber so that several membranes can be maintained in the same incubation medium, thereby increasing the magnitude of the changes in bicarbonate concentration and the sensitivity of the method.

Honors and Awards:

Dr. Lasansky was invited to participate in the IJ Symposium on the "Structure of the Eye" at the 3th International Congress of Anatomy in Wiesbaden, Germany.

Publications:

Lasansky, A., and Fisch, F.W.: Potential, current and ionic fluxes across the isolated retinal pigment epithelium and choroid. J. Gen. Physiol. (in press), 1966.

Lasansky, A., and Fisch, F.W.: Studies on the function of the pigment epithelium in relation to ionic movement between retina and choroid. In Rohen, J. (Ed.): Structure of the Eye. Schattauer-Verlag, Stuttgart (in press), 1966.

1. Ophthalmology Branch
2. Section on Cytology and
Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on the Endothelium of the Corneal Homograft

Previous Serial Number: NONE

Principal Investigator: Salvador R. Salceda, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To investigate the fate of the endothelium of stored corneas in homotransplantation and to compare the findings with those reported on fresh corneal homografts.

Methods Employed: Whole-thickness corneal homotransplantation was carried out on young-adult New Zealand albino rabbits, using females as donors and males as recipients. Donor corneas were stored as eyeballs in + 4°C moist chambers for as long as three to four weeks, before transplantation. Feulgen or thionin-stained whole flat mounts of the corneal endothelium were prepared. Occasionally, silver nitrate and alizarin red were used to delineate cell boundaries. The donor endothelium was identified by the sex-chromatin present in female cells and by isotopic labeling of the cells with tritiated-thymidine at some prior time.

Population estimates of the endothelial cells were made from the average number of nuclei in a field of known size counted at a magnification of 800 times. By comparing the cell population of the 5.5 mm. corneal graft at the time of transplantation as estimated either from the opposite cornea of the same donor animal or from the remaining rim of the donor cornea, with that following transplantation at a period before host endothelium migration into the donor graft, it was possible to estimate the amount of cell loss.

Major Findings:

Clinical: Permanently transparent corneal grafts were invariably obtained from donor materials stored for as long as 14 days in + 4°C moist chambers. Of six corneal grafts transplanted after 21 days of storage, two were opaque when the animals were sacrificed one week after grafting, two became clear three weeks after surgery and two were permanently opaque with only partial clearing in the periphery two to three months after surgery.

Microscopic: The endothelium of the stored donor corneas in + 4°C moist chambers underwent progressive morphological changes, including loss of the sex-chromatin details. As early as the 2nd storage day, nuclear clumping especially at the perinuclear area, was noted and was most pronounced by the end of the first storage week. Swelling and vacuolation of the nuclei which could already be demonstrated within the first storage week, were more generalized at the end of the 14th storage day. In general the nuclei were poorly stained with thionin and Feulgen. The sex-chromatin could no longer be demonstrated with Feulgen stain and in only about 25 percent of the cells with thionin. The endothelium of corneas stored for 21 to 22 days showed changes ranging from generalized loss of nuclear details to actual nuclear disintegration. In some specimens, only islands of pyknotic and disintegrating nuclei were found.

Post-operative estimates of the endothelial cell population in the grafts indicated a progressive loss from both the fresh and stored corneas. A week following transplantation, fresh grafts showed a loss of approximately 14 percent of the cells and grafts stored seven days, a loss of 34 percent. The slow, progressive cell loss continued and by the end of the first post-operative month, only 45 percent of the endothelial cells in the seven days stored corneas had survived, while in the fresh, 80 percent still remained. The intriguing observation however was that in all four grafts stored for 14 days before transplantation, the amount of cell loss was significantly less than those of the seven days stored corneas.

In the 21 days stored corneas, the findings were not consistent. In opaque grafts examined one week after transplantation, there was 100 percent loss of the endothelium, as evidenced by the absence of cells containing sex-chromatin and/or isotope labels. Similar absence of the donor endothelium was noted in initially opaque grafts that subsequently cleared up three weeks after transplantation. However, a couple of grafts that were clear when the animals were sacrificed one month after transplantation still showed some surviving endothelium containing sex-chromatin.

Actual invasion by host endothelial cells occurred only in corneas transplanted after 21 days of storage that exhibited massive loss of cells. One month after transplantation no migrating host cells were present in the periphery of the graft when donor corneas stored for two weeks had been used.

The surviving endothelium of the donor graft actively participated in the "repair" of the homograft. Cells undergoing DNA synthesis evidenced by

incorporation of tritiated-thymidine and undergoing mitosis were demonstrable both in the peripheral and central zones of the graft. Although, cellular proliferation was most marked within the first post-operative week, it could be seen sometimes in grafts examined one to two months after transplantation.

Surviving endothelial cells showed 9 to 29 percent increase in nuclear size. Increasing numbers of cells with giant nuclei were found especially in specimens examined one month after transplantation.

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

(1) The project is undertaken in connection with the research training of the principal investigator as a U.S. Public Health Service International Fellow.

(2) In keratoplasty, the ultimate objective is a clear corneal graft. The role of the endothelium in the maintenance of corneal transparency is well recognized, therefore, efforts at evaluating the suitability of the graft material has been shifted in recent years to the assessment of the viability of the endothelium. As many methods of graft preservation are investigated, more and more of the "stored" corneas are expected to be used in clinical practice. Comparative studies on the behavior of the cell components of the fresh and "stored" corneal grafts may show how they differ from the point of view of survival. Finally, it is hoped that by this method of study, it may be possible to quantitatively correlate cell survival to the state of corneal graft transparency and thereby determine how much of the donor cells must be viable and functioning at the time of transplantation to assure graft success.

Proposed Course of Project: More data are necessary from corneal donor materials grafted after 14 to 21 days + 4°C moist chamber storage, especially in those cases that remain opaque. This is necessary in confirming what seems to be indicated by present data that survival of 55 to 65 percent of the donor endothelium within the first post-operative week is required to assure graft success.

Furthermore, it is planned to study the function of the endothelial cells with giant nuclei that are increasingly demonstrable in the homografts. How they are related to the over-all process of "repair" is at present a matter of conjecture.

Honors and Awards: None

Publications: None

1. Ophthalmology Branch
2. Section on Ophthalmology
Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Physical Chemistry of Corneal Collagen

Previous Serial Number: SAME

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.3
Professional:	0.7
Other:	0.6

Project Description:

Objectives: To determine the physical and chemical parameters related to the molecular structure of corneal collagen with the view of examining these for significance in the morphology of the cornea.

Methods Employed: Collagen has been extracted from the bovine cornea, purified, and fractionated on the basis of salt solubility. The preparations have been studied by amino acid analysis, analytical ultracentrifugation, viscometry, and optical rotation. The properties of subunits resulting from denaturation in 5M guanidine or 8M urea have been similarly studied.

Major Findings: New studies indicate that the weight average molecular weight of the collagen polymer is much higher than was previously thought and that a marked degree of polydispersity exists in these preparations. Data from sedimentation velocity and intrinsic viscosity studies, when combined with the molecular weight data, indicates that the polymer forms aggregates of very high axial ratio. This is consistent with the configuration of corneal collagen fibres as indicated in electron micrographs. Studies on the molecular weight of corneal collagen subunits suggest that these are much lighter in molecular weight than anticipated. Effective separation of the subunits will be necessary before their relationship to the native molecule can be ascertained.

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

This project represents a continued study of the relationship of the molecular structure of certain proteins of ophthalmic origin to the structure and function of the tissues of their origin. It provides a base for comparative studies of different collagens in the eye, and for studies of the role of collagen in corneal development and in corneal pathologies.

Proposed Course of Project: Continued studies on the subunits of corneal collagen are planned, with particular emphasis on the nature of the cross-link and the relationship of the subunits to the native molecule. Studies on the development of collagen in the chick embryo cornea and on the collagen of the normal and pathological human cornea will be continued if the present development of data analysis systems will permit meaningful interpretation of results. Studies are also planned on vitreous body and scleral collagen.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 O/OCH 1210(c)

1. Ophthalmology Branch
2. Section on Ophthalmology
Chemistry
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Chemistry of Rhodopsin

Previous Serial Number: SAME

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: None

No new work was done on this project this year because of lack of suitable facilities for continuous work on light sensitive material and absence of personnel experienced in lipo-protein research. This project will be resumed in the future.

Serial No. NDB(I)-65 O/OCH 1217(c)

1. Ophthalmology Branch
2. Section on Ophthalmology
Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Physical Biochemistry of Model Gel System

Previous Serial Number: SAME

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: Jules A. Gladner, Ph.D.
Laboratory of Biophysical Chemistry,
NIAMD

Insufficient work was done on this project this year to warrant reporting. The project should be completed in the following year.

Serial No. NDB(I)-66 O/OCH1292(c)

1. Ophthalmology Branch
2. Section on Ophthalmology
Chemistry
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Physical Chemistry of Nucleic Acids

Previous Serial Number: NONE

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: James A. Rose, M.D.

Cooperating Units: Laboratory of Biology of Viruses, NIAID

Man Years

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: Methods used in the past for estimation of the molecular weights of large nucleic acids have all involved assumptions which are not necessarily valid. The purpose of this study is to obtain meaningful values using ultracentrifugal techniques without recourse to limiting assumptions.

Methods: DNA extracted from purified adenoviruses is being used for the present studies. The DNA is characterized with respect to sedimentation velocity in the ultracentrifuge. The molecular weight is determined by sedimentation equilibrium studies in the ultracentrifuge using the meniscus depletion technique of Yphantis.

Major Findings: DNA from adenovirus type 2 was found to have a sedimentation coefficient of 31 S, indicating a molecular weight of 22,000,000 if the molecular weight is estimated with previously used formulas. Sedimentation equilibrium yields a value of 34,500,000. This value was obtained for the number, weight, and Z-average molecular weights of some preparations, indicating probable homogeneity, and increasing confidence in the value.

Significance to Bio-medical Research and the Program of the Institute: Adenoviruses produce respiratory and ocular infections in man and cause tumors in experimental animals. Further definition of the molecular character of adenovirus DNA's is relevant to an understanding of specific cellular alterations induced by infection with these viruses.

Proposed Course of Project: Further studies will be carried out on the DNA's of several adenoviruses. The methods employed will be used to examine DNA from other organisms in order to compare the molecular weights obtained by a rigorous technique with those previously estimated.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-66 O/OCH 1293(c)

1. Ophthalmology Branch
2. Section on Ophthalmology
Chemistry
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Chemistry of Vitreous Proteins

Previous Serial Number: NONE

Principal Investigator: Ralph J. Helmsen, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	1.0
Others:	None

Project Description:

Objectives: To determine physical and chemical parameters related to the molecular structure of vitreous proteins with the view of examining these for significance in the morphology of the vitreous body.

Methods Employed: Two lines of investigation have been undertaken: immunochemical examination of the globulin in developing rabbit vitreous and isolation of the protein from the vitreous of cattle. The former preparation has been studied by immunodiffusion as well as by gel diffusion technique of Oudin. Ammonium sulfate precipitation and column chromatography are used to fractionate and isolate the bovine vitreous proteins. Analytical ultracentrifugation, intrinsic viscosity, optical rotatory dispersion, amino acid analysis and carbohydrate analysis are used for characterization.

Major Findings: A protein has been detected in rabbit

vitreous which gives a precipitin test with a specific sheep antisera against piece III of 7 S gamma globulin derived from rabbit serum. The presence of this gamma globulin like substance in the vitreous of the seven day old animal lends credence to the concept that many vitreous glycoproteins may be derived from their serum counterparts. The concentration of the protein in the eye of the week old rabbit is 0.8 - 1.5 mg/ml which is approximately one-fourth the value in the serum at that stage of development. On the basis of the hexosamine content of 7 S gamma globulin in rabbit serum the constituent in the vitreous represents nearly 25 per cent of the total amino sugar in the connective tissue. At the age of four months the level of the globulin has diminished seven-fold in the vitreous while the concentration of the protein in serum has doubled.

Significance to Bio-medical Research and the Program of the Institute: This project represents the initial study by the investigator into the relationship of the molecular structure of the soluble proteins of the vitreous body to the structure of this avascular connective tissue. It provides the base for comparison of the level of these glycoproteins and possible alterations in their tertiary structure in various disease states affecting the vitreous.

Proposed Course of Project: Further purification of a bovine vitreous gamma globulin-like protein is being pursued and the results obtained from the physicochemical examination of that material will be correlated with the information obtained by immunochemical studies with the rabbit. Experiments will be undertaken with allotypic rabbits to determine whether gamma globulin in the young vitreous was acquired by passive transfer of antibodies from mother to the foetus or whether synthesis of the protein occurred in an ophthalmic tissue.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 O/CB 1209(c)

1. Ophthalmology Branch
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Mode of Action of Rhodopsin

Previous Serial Number: SAME

Principal Investigator: Sjoerd L. Bonting, Ph.D.

Other Investigators: Alex Bangham, M.D., Department of
Physiology
A.R.C. Institute of Animal Physiology,
Cambridge, England

This project is complete.

Serial No. NDB(I)-65 O/CB 1211(c)

1. Ophthalmology Branch
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Studies on Glycoprotein Synthesis

Previous Serial Number: SAME

Principal Investigator: Paul J. O'Brien, Ph.D.

Other Investigators: Elizabeth F. Neufeld, Ph.D.

Cooperating Units: Laboratory of Biochemistry and
Metabolism, NIAMD

Man Years

Total: 0.4

Professional: 0.2

Others: 0.2

Project Description:

Objectives: To determine how the sugar residues of glycoproteins are added to the polypeptide core. To study the enzymatic reactions involved and the control mechanisms that regulate these reactions. To determine how altered, incomplete or defective glycoproteins might result from a disturbance of these reactions.

Methods Employed: The microsomal fraction of rat liver is isolated. The transfer of radioactive sugars from activated sugar nucleotides to endogenous acceptors is measured. The labeled products are isolated from the microsomes and identified by immunoelectrophoresis. Transfer to heterologous acceptors is also measured. The effect of protein synthesis inhibitors is studied.

Major Findings: This work completed a project begun in 1964. The results are essentially the same as in the last

report, with the exception of item two which gives positive identification of the products.

1) Liver microsomes are capable of transferring N-acetyl neuraminic acid (NAN) from CMP-NAN to an unfinished glycoprotein bound to the microsomes. The transfer reaction probably represents the final step in the synthesis of the carbohydrate side chains of these glycoproteins since NAN is the terminal sugar of many glycoproteins. 2) The products of this reaction are solubilized by sonication and identified as plasma glycoproteins on the basis of their immunoelectrophoretic properties. Antiserum to rat plasma proteins produces labeled precipitin bands in the alpha and beta globulin regions. 3) The K_m for CMP-NAN is 2×10^6 M. 4) Puromycin, injected into rats 45 minutes before the removal of the liver, reduces the level of endogenous acceptor by 85% without affecting NAN transfer to exogenous acceptors. 5) The transfer to exogenous heterologous acceptors implies that the transferase is not specific for proteins made by its own cell type. Rather, the specificity lies in the protein synthesizing system of the cell.

Significance to Bio-medical Research and the Program of the Institute: These findings indicate that the terminal sugar of glycoproteins is added in a single enzyme step rather than as part of a preformed polysaccharide chain. In addition, the transfer to exogenous heterologous acceptors implies that the transferase is not specific for proteins made by its own cell type. Rather, the specificity lies in the protein synthesizing system of the cell.

This project was undertaken as a model system for subsequent studies of glycoprotein synthesis in the eye.

Proposed Course of Project: Completed.

Honors and Awards: None

Publications:

O'Brien, P. J., Canady, M. R., Hall, C. W., and Neufeld, E. F.: Transfer of N-acetylneuraminic acid to incomplete glycoproteins associated with microsomes. Biochim. Biophys. Acta. 117: 331-341, 1966.

Serial No. NDB(I)-66 O/CB 1294(c)

1. Ophthalmology Branch
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Synthesis of Sugar-Containing Polymers
in Retina

Previous Serial Number: NONE

Principal Investigator: Paul J. O'Brien, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total: 1.3

Professional: 0.8

Others: 0.5

Project Description:

Objectives: To determine whether beef retina is capable of synthesizing polysaccharides and glycoproteins. To determine if transfer enzymes are present that will catalyze the incorporation of simple sugars into high molecular weight acceptors, to determine if some of the products of these reactions are identical to glycoproteins of the vitreous humor.

Methods Employed: Subcellular particulate fractions of retina are isolated and incubated with various radioactive sugar nucleotides. Trichloroacetic acid-insoluble radioactivity is measured in a scintillation counter. Labeled products are also extracted with detergents or sonication and compared with the glycoprotein components of the vitreous humor by immunoelectrophoresis.

Major Findings: 1) N-acetylneuraminic acid (NAN) is transferred from CMP-NAN to a non-lipid high molecular weight

acceptor associated with the particulate fraction. The enzyme is also particulate. This reaction probably represents the terminal step in glycoprotein synthesis. 2) Addition of UDP galactose to the incubation mixture stimulates the incorporation of NAN. 3) Galactose (^{14}C) is incorporated from (^{14}C) UDP galactose by the same system. 4) The data in 2) and 3) suggest that the addition of galactose (the penultimate sugar of many glycoproteins) to the endogenous acceptor provides additional sites for the attachment of NAN (the terminal sugar of many glycoproteins). 5) N-acetylglucosamine (NAG) is transferred from UDP-NAG to the particulate acceptors. As yet UDP-NAG has not been shown to stimulate the incorporation of galactose or NAN.

Significance to Bio-medical Research and the Program of the Institute: These findings indicate that glycoproteins in general may have their sugar components added to the protein core one at a time rather than as a pre-formed polysaccharide chain. Furthermore they strongly suggest that the retina is actively engaged in glycoprotein synthesis.

Proposed Course of Project: To determine the characteristics (co-factors, K_m values, etc.) of the enzymes detected thus far. To determine the nature of the acceptor(s). To demonstrate the identity or non-identity of the acceptor(s) for NAN and galactose. To determine whether the products resemble vitreous humor glycoproteins. To determine if the enzymes are capable of altering bovine plasma proteins to produce vitreous glycoproteins. To examine the affects of pharmacologic agents on these enzymes. To detect the presence, if any, of enzymes transferring other sugars to macromolecules.

Honors and Awards: None

Publications: None

1. Ophthalmology Branch
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Aminosugar Metabolism in the Retina

Previous Serial Number: NONE

Principal Investigator: Roger G. Mazlen, M.D.
Paul J. O'Brien, Ph.D.

Other Investigators: None

Cooperating Unit: None

Man Years

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: To study overall aminosugar metabolism in the retina. To isolate and characterize the retinal enzymes involved in aminosugar nucleotide biosynthesis. To study the enzymatic reactions involved, and the control mechanisms that regulate these reactions. To determine any significant differences in the retinal enzymatic pathway of aminosugar nucleotide synthesis as compared to that of other tissues. To determine the role of aminosugar metabolism in retinal glycoprotein synthesis.

Methods Employed: Bovine retinal homogenates are used to obtain a crude supernatant source of soluble enzymes. Ammonium sulfate fractionation, sephadex column chromatography, and dialysis are used to purify the soluble enzymes. The enzyme is then incubated with its specific substrates and its characteristics studied as to: activity, kinetics, pH dependency, inhibitory feedback (and/or inhibitory compounds), and stability.

The hydrolysis of aminosugars and aminosugar nucleotides is followed using labeled substrates, paper chromatography, strip scanning and scintillation counting. Enzyme activity is assayed by specific colorimetric and radioactive methods.

Major Findings: (1) The enzyme L-glutamine-D-fructose-6-phosphate transaminase has been demonstrated to be present in the bovine retina. It exhibits inhibition by UDP-N-acetylglucosamine similar to that described in rat liver. The partially purified enzyme preparation appears to catalyze a slight breakdown of UDP-N-acetylglucosamine in contrast to no breakdown noted in rat liver preparations. (2) Its activity versus pH, and activity versus protein concentration curves are similar to those reported for rat liver enzyme and E. coli enzyme. The activity versus time curve of the enzyme is significantly different than that of the N. crassa enzyme and E. coli enzyme. (3) The crude high speed supernatant enzyme, and the partially purified enzyme exhibit significantly greater stability than that previously reported for similar bacterial and rat liver preparations. (4) The bovine retinal enzyme activity, for the crude high speed supernatant fluid, is comparable to activities reported for beef and calf liver. (5) Preliminary studies of the retinal enzyme's reaction kinetics show some similarities to, and some differences from the rat liver enzyme kinetics.

Significance to Bio-medical Research and the Program of the Institute: Results of the outlined project will add to the existing information on retinal metabolism, and in particular, the metabolism of aminosugars. It is of interest to determine whether or not the retina, or other eye tissues, can synthesize glycoproteins, and this study will attempt to provide information for that purpose. This project will, in its completion, yield information as to how all inhibited, altered, denatured, or absent enzymes of the pathways previously discussed, in retina, might disturb retinal aminosugar, and glycoprotein synthesis (if it is endogenous). From such information, it is conceivable, some hypotheses might be formed as to the types of pathological lesions which would derive from such deranged retinal metabolism.

Proposed Course of the Project: To study, in retina, each enzymatic step of the pathways of biosynthesis of UDP-N-

acetylglucosamine and CMP-N-acetylneuraminic acid. To determine if the bovine retina possesses the capability of synthesizing the previously mentioned sugar nucleotides, which are known to be frequently incorporated into glycoproteins and other macromolecules.

The presence of these enzymes in the pigmented epithelium will be studied.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-56 O/OPH 301(c)

1. Ophthalmology Branch
2. Section on Ophthalmology
Pharmacology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Study on the Pharmacodynamics of Various
Agents Affecting the Intraocular Pressure

Previous Serial Number: SAME

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total: 1.4

Professional: 0.5

Others: 0.9

Project Description:

Objectives: To determine the pharmacodynamics of agents able to alter the intraocular pressure with a view toward finding more effective compounds and to possibly further the understanding of mechanisms which maintain the intraocular pressure.

Methods Employed: All agents investigated were examined on the cat eye. The preparations used were: (1) The intact living eye. (2) The arterially perfused enucleated eye. (3) The arterially perfused, isolated iris-ciliary body and iris preparations. The various functions measured were the intraocular pressure, iris artery pressure, and perfusate flow rate.

Major Findings:

Steroid Effects on Intra-ocular Pressure: Some glucocorticoids

are known to produce an elevation of IOP in humans when applied locally. In studying the problem of glaucoma or the mechanisms which regulate the IOP, this observation may offer clues to the mechanisms by which the IOP is regulated. Topical administration of these steroids to animals has met with little success in causing the IOP to become elevated; this could be due to the animal removing the drug or perhaps to the fact that the drug concentration does not build up to sufficient levels to produce the effect. In the experiments reported here high concentration levels were achieved by perfusing the steroid directly into the arterial supply to the eye. The ophthalmologic preparations Celestamyd^R and Decadron^R were found to produce a rise in eye pressure from beginning levels of 15 mm Hg to as high as 80 mm Hg. The preparation Celestamyd^R was more potent in this regard than Decadron^R. The vehicle of Celestamyd^R alone also produced a rise in IOP, but the magnitude of the rise was not as great as with the complete preparation. The diluent of Decadron^R was not available for study. The following steroids were also tested and found to have no ocular hypotensive effect: hydroxymesterone, methylprednisolone, hydrocortisone, fluorometholone, and betamethasone disodium phosphate.

Steroid Effects on Intra-ocular Blood Vessels: None of the steroids tested had a direct effect on the blood vessels of the iris, i.e. they did not produce vasoconstriction or vasodilatation. All the steroids were tested for their ability to antagonize the activity of other pharmacologic agents such as epinephrine, pilocarpine, eserine, and acetazolamide. They arterially perfused through the iris-ciliary body preparation for ten minutes, prior to the administration of both steroid and test drug. The effective steroids were capable of inhibiting the responses of all of these test agents and therefore are classified as negative myotropic agents. Celestamyd^R was the most potent steroid in this group, followed by Decadron^R, the vehicle of Celestamyd^R, methylprednisolone, and hydroxymesterone. Betamethasone disodium phosphate, hydrocortisone and fluorometholone were without effect.

Steroid Effect on Pupil Size: Steroids which have been reported to produce a rise in IOP, in humans, also dilate the pupil. These observations were also made in the experiments above. Celestamyd^R, its diluent, and Decadron^R all caused the pupil to become larger: the remainder of the

drugs were without effect.

Significance to Bio-medical Research and the Program of the Institute: The glucocorticoids are the first group of agents which can produce a rise in IOP of various magnitudes, in many instances producing pathologic changes which mimic those of glaucoma. On this basis the activity of this series of compounds are of great interest. The steroids which elicited the highest IOP pressures were also the most potent in producing elevations of IOP in the enucleated, arterially perfused cat eye. On the assumption that the mechanism for the IOP rise in the cat eye is the same as that of the human, we now may have a useful experimental model upon which to conduct further research. This model offers two distinct approaches toward the study of IOP: 1. Mechanisms which are involved in producing pathologic elevations of IOP and 2. Search for compounds which may more efficiently produce a lowering of eye pressure.

The fact that the glucocorticoids have no direct effect on the artery of the iris-ciliary body indicates that this mechanism can not be involved in the pathogenesis of the elevated IOP. The negative myotropic effect of the steroids does not appear to be involved either since no exogenous vasoactive substances were administered which could have been inhibited by the steroids.

Proposed Course of Project: Examination of the eye after the administration of the glaucomogenic steroids by arterial perfusion demonstrates marked bowing of the iris, and shallowing of the anterior chamber. Cessation of arterial perfusate flow does not cause an immediate fall in IOP, indicating therefore that the rate of aqueous humor outflow is markedly decreased. From the appearance of the eye, the decrease in outflow may be ascribed to the decrease in anterior chamber depth which is brought about by an increase in vitreous volume. This possibility will be investigated. In addition we intend to study the activity of some of the known antiglaucoma agents with this preparation to see whether the procedure can be employed for the screening of potentially useful agents.

Honors and Awards: None

Publications:

Macri, F. J., Dixon, R. L., and Rall, D. P.: Aqueous humor turnover rates in the cat. I. Effect of acetazolamide. Invest. Ophthalm. 4: 927-934, Oct. 1965.

Macri, F. J., Dixon, R. L., and Rall, D. P.: Aqueous humor turnover rates in the cat. II. Effect of ouabain and chlorothiazide on aqueous humor turnover rate. Invest. Ophthalm. In press.

Serial No. NDB(I)-59 O/OPH 600(c)

1. Ophthalmology Branch
2. Section on Ophthalmology
Pharmacology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Role of Vasculature in the Maintenance
of Intraocular Pressure

Previous Serial Number: SAME

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigators: Walter E. Morgan, III, M.D.

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.7
Others:	0.2

Project Description:

Objectives: To determine if and by what mechanisms the dynamics of the intraocular vasculature affect the eye pressure.

Methods Employed: Ciliary body potentials were measured in intact living cat eyes as well as in the enucleated arterially perfused preparation. The method used by Miller was employed. This consisted of placing an agar-KCl filled glass electrode in the anterior chamber of the eye and a similarly filled polyethylene tube into a vein. The potentials were measured by a Keithley Electrometer Model 600A and recorded on an Esterline-Angus milliammeter.

Major Findings: Approximately 75 determinations for blood-aqueous potential difference (BAPD) were made in the anesthetized cat and 5 determinations in the isolated perfused eye. In the intact eye, a mean value of 4.26 mV was obtained

whereas in the enucleated eye the value was essentially zero. The in vivo measured potentials were not affected by the administration of ouabain (0.06 mg/kg) or of acetazolamide (100 mg/kg), even though these doses have been demonstrated in this laboratory to effectively decrease aqueous humor formation. The second indication that the measured potentials are unrelated to the process of aqueous humor formation is the fact that a cell poison (osmium tetroxide) when injected directly into the ciliary body had no immediate deleterious effect on the potential measured. Moreover, the injection of a toxic, electrically non-conducting substance (Tygon paint) into the blood vessels of the ciliary processes also caused no change in the measured potential. The implication of these findings is that the potential measured in vivo does not originate from the ciliary processes; confirmation stems from the fact that in the in vitro eyes, ciliary body potentials of any appreciable magnitude are not found.

Significance to Bio-medical Research and the Program of the Institute: It has been reported by a number of investigators that the ciliary body potential measured by the technique reported here is strong evidence for the concept that aqueous humor is a secretion and that the short circuit current measured using this potential gives reliable information about the secretory functions of some ions, such as sodium, and the involvement of Na-K dependent ATPase. The result of our study does not negate the possibility that aqueous humor is a secretion but does indicate strongly that a potential gradient assumed by many investigators to be responsible for the activity is not generated in the ciliary body, and therefore, cannot be involved in this activity. Unless drugs such as acetazolamide and ouabain are inhibitory of secretory processes which are not electrogenic in nature it would appear unlikely that these agents could lower intraocular pressure by inhibition of aqueous humor secretion.

Proposed Course of Project: No further work in the measurement of blood-aqueous humor potential is anticipated, since this does not appear to be related with the formation of aqueous humor. Our previous findings indicate that antiglaucoma agents of different classes act by constricting the iris artery. It is hoped that we will be able to study this problem further to determine the reason for the common response.

Honors and Awards: None

Publications:

van Alphen, G., and Macri, F. J.: The entrance of fluorocein into the aqueous humor of the cat eye. Arch. Ophthal. 75: 247-253, Feb. 1966.

Macri, F. J., Politoff, A., Rubin, R., Dixon, R. L., and Rall, D. P.: The preferential vasoconstrictor properties of acetazolamide on the arteries of the choroid plexus. Int. J. Neuropharmacol. 5: 109-115, March, 1966.

Politoff, A., and Macri, F. J.: Pharmacological differences between isolated perfused arteries of the choroid plexus and of the brain parenchyma. Int. J. Neuropharmacol. 5: 153-162, April, 1966.

Serial No. NDB(I)-63 O/OPH 1018(c)

1. Ophthalmology Branch
2. Section on Ophthalmology
Pharmacology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: A Study on Enucleated, Arterially Perfused
Human and Cat Eyes

Previous Serial Number: SAME

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigators: None

No work was done on this project this year due to the
unavailability of human eyes.

Serial No. NDB(I)-65 O/OPH 1214(c)

1. Ophthalmology Branch
2. Section on Ophthalmology
Pharmacology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The In Vitro Effect of Corticosteroids on the
Intraocular Muscles of the Cat and Monkey

Previous Serial Number: SAME

Principal Investigator: Rudolf Kern, M.D.

Other Investigator: Frank J. Macri, Ph.D.

This project is terminated. The principal investigator is no longer with the NIH and is continuing the work in Switzerland at the University Eye Hospital in Zurich.

Serial No. NDB(I)-65 O/OPH 1215(c)

1. Ophthalmology Branch
2. Section on Ophthalmology
Pharmacology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Studies on Extraocular Muscles. (a) A Comparative Pharmacologic-Histologic Study of the Rabbit. (b) The In Vitro Effect of Sympathomimetic Agents on the Cat, Monkey and Rabbit.

Previous Serial Number: SAME

Principal Investigator: Rudolf Kern, M.D.

Other Investigators: None

Publications: Kern, R.: A comparative pharmacologic-histologic study of slow and twitch fibers in the superior rectus muscles of the rabbit. Invest. Ophthal. 4: 901-910, Oct. 1965.

This project is complete.

Serial No. NDB(I)-58 O/OC 500(c)

1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Investigation of the Effects of Topically Administered Steroids on the Intraocular Pressure. Examination of Aqueous Humor Dynamics

Previous Serial Number: SAME

Principal Investigator: George L. Spaeth, M.D.

Other Investigators: Ludwig von Sallmann, M.D.

This project has been temporarily discontinued because of a change in personnel. It will be resumed in the future.

1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Methotrexate Therapy of Selected Patients with Uveitis

Previous Serial Number: SAME

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.5
Professional:	0.5
Others:	0.0

Project Description:

Objectives: This project was continued since its inception in 1963. The therapeutic effect of methotrexate has been extended to other types of refractory uveitis in addition to chronic cyclitis.

Methods Employed: (1) Methotrexate, 25 mgm per M^2 of body surface area was given intravenously on a schedule of every four days. The clinical course of the patient was followed closely and the systemic effects of methotrexate were documented by blood counts and blood chemistries.

(2) The effect of methotrexate on an independently induced local inflammation in these patients was continued with the use of the skin-window technic. The volar surface of the forearm was abraded to the papillary layer of the corium with a Bard-Parker blade to sample the cellular exudates in this area, serial changes of cover slip were done at various intervals up to 72 hours. Wright's stain was used on the preparations and a 400 cell differential counts were performed on each preparation.

Major Findings: Sixteen patients have received methotrexate since the project was initiated.

Of the 10 patients treated in the cyclitis group, 9 showed definite evidence of improvement during the course of therapy. The remaining patient's

response to the antimetabolite was questionable but flare-up of his uveitis did not occur. Although some degree of relapse was noted on cessation of therapy, one patient did show complete remission after a single 6 week course of intermittent treatment. She has now been followed for 27 months without any trace of residual inflammation. Her visual acuity in the affected eye has remained 20/15 compared to her pretreatment acuity of 20/200. Another patient was maintained on daily oral methotrexate (2 mgm) for 1½ years with good control of his uveitis and lack of any ill effects on his general health. Treatment was suspended when he expressed desires to enlarge his family. Chromosome studies obtained at this time were normal. His spouse recently gave birth to a healthy 9 lb. baby boy.

Of the three cases of sympathetic ophthalmia treated so far, one showed dramatic improvement soon after instituting methotrexate. During maintenance therapy successful cataract operations have restored his vision to normal and his glaucoma has since become controlled without medication. Whereas upwards of 50 mgm of prednisone could not control the inflammation prior to methotrexate, his uveitis has been in complete remission for the past 16 months on an equivalent 12 mgm dose of the same steroid. Two other cases with sympathetic ophthalmia did not respond to this therapy.

Studies and treatment are currently being instituted in three more patients. One of these being treated is a case of uveal effusion syndrome.

It is concluded from these studies that antimetabolites may have a definite place in the armamentarium for the treatment of uveal inflammatory diseases. More additional studies are indicated particularly in view of the mode(s) of action of these drugs and their proper utilization in selected patients with refractory uveal diseases.

Significance to Bio-medical Research and the Program of the Institute: This project is within the scope of our uveitis program to pursue and uncover more effective means of treating uveal inflammatory diseases. It is hoped that such efforts would help us to a better understanding of the pathogenesis of uveitis.

Proposed Course of Project: The present project will continue. Studies have been initiated in the laboratory to study the effect of Methotrexate on endogenous production of immune globulins as well as the behavior of immune cells in the presence of this antimetabolite.

Honors and Awards: None

Publications:

Wong, V. G., Hersh, E. M., and McMaster, P. R. B.: Treatment of a Presumed Case of Sympathetic Ophthalmia with Methotrexate. Arch. Ophthal. (in press), 1966.

1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Effect of Experimental Choroidal Ischemia
on the Retina

Previous Serial Number: SAME

Principal Investigator: Robert H. Collier, M.D. \

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.3
Professional:	0.3
Others:	0.0

Project Description:

Objectives: The purpose of this experimentive study is to determine the effects resulting from extensive embolic ischemia of the choroid without altering the retinal circulation.

Methods Employed: The technique consists of an intra-vascular injection of latex emboli via a polyethylene canula. A total of 63 cat eyes have been operated upon to date. Of this number 23 demonstrated the embolic occlusion of the choroid. In an additional 10 eyes, the suspending media alone (propylene glycol was used to hold the emboli of latex in suspension to prevent plugging of the canula) was injected in a manner identical to the procedure using the latex microspheres. In another four eyes, the same operation was performed with saline (0.85%) substituted for the latex suspension as a control on the operative trauma. In three eyes the latex embolic media was injected onto the surface of the retina to preclude any possible inherent toxicity of the embolic

suspension. The eyes were used to develop and perfect the technique of choroidal embolic occlusion. This included also the selection of the proper material to produce the occlusion of the choroidal vessels.

Major Findings: The selective nature of this technique in producing a sector of choroidal ischemia without altering the retinal vasculature, in contrast to previous studies, permits one to investigate and interpret the role the choroid plays in the nutrition of the retina and other tissues of the eye. The results indicate: (1) With severe, sectorial ischemia of the choroid, there is a sharp demarcated loss of the external layers of the retina consisting of the outer four layers of the neurosensory retina. (2) With minimal ischemia, only the photoreceptors are affected. (3) The pigment epithelium undergoes hypertrophy and loss of the normal monolayer pattern in 20 out of the 23 eyes. (4) A serous detachment of the retina ensued in 14 of the 23 eyes. (5) Microcystoid changes occurred in the pars plana and ciliary processes in the chronic animals.

Significance to Bio-medical Research and the Program of the Institute: A variety of clinical disorders of the eye have been attributed to a vascular deficiency or ischemia of the choroid, but specific evidence documenting such clinical impressions as well as knowledge of the normal function of the choroid is lacking. As part of the Institute's investigation of vascular disorders of the eye, this study attempts to understand the role that the choroidal circulation plays in the nutrition of the eye.

Proposed Course of Project: The project is being completed and about to be reported.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 O/OC 1218(c)

1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Ocular Manifestations in Carcinoid Syndrome

Previous Serial Number: SAME

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: Kenneth Melmon, M.D.
Experimental Therapeutics Branch, NHI

This project is complete.

Serial No. NDB(I)-65 O/OC 1219(c)

1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Ocular Complications of Chloramphenicol
Therapy

Previous Serial Number: SAME

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: Paul Lietman, M.D.
Pediatric Metabolism Branch, NIAMD

This project is complete.

Serial No. NDB(I)-65 O/OC 1220(c)

1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Ocular Manifestation of Ehlers-Danlos Syndrome

Previous Serial Number: SAME

Principal Investigator: William R. Green, M.D.

Other Investigators: Alvin Friedman-Kein, M.D.
Dermatology Branch, NCI
William Banfield, M.D.
Laboratory of Pathology, NCI

Publications: Green, W.R., Friedman-Kein, A., and Banfield,
W.: Angioid streaks in Ehlers-Danlos syndrome.
Arch. Ophthal., 1966. In press.

This project is complete.

Serial No. NDB(I)-65 O/OC 1221(c)

1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Clinical Aspects of Fabry's Disease
(Glycolipid Lipidosis)

Previous Serial Number: SAME

Principal Investigator: George Link Spaeth, M.D.

Other Investigator: Phillip Frost, M.D.
Dermatology Branch, NCI

Publications: Spaeth, G.L., and Frost, P.: Fabry's disease.
Its ocular manifestations. Arch. Ophthal.
74: 760-769, Dec. 1965.

This project is complete.

Serial No. NDB(I)-65 O/OC 1222(c)

1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Ocular Manifestations of Chlorpromazine and
Other Phenothiazines

Previous Serial Number: SAME

Principal Investigator: J. Brooks Crawford, M.D.

Other Investigators: None

This project has been terminated because none of the experimental animals developed toxic cataracts and no patients with ophthalmologic abnormalities secondary to phenothiazines were available for study.

1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Maintenance of Anterior Chamber Depth During Anterior Segment Surgery

Previous Serial Number: None

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: Robert H. Collier, M.D.

Cooperating Units: None

Man Years

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To devise a technic whereby the anterior chamber depth can be maintained during surgical aspiration of congenital cataracts.

Methods Employed: A 27-gauge hypodermic needle connected to a 10-ml saline-filled syringe is engaged to one end of a PE #10 polyethylene tubing (approximately 12" long). Another needle, broken from its attachment to the hub, is threaded with its blunt end into the opposite end of the synthetic tubing. Following a knife-needle incision the needle is entered into the anterior chamber. The infusion is graded by the assistant to maintain desired chamber depth during the surgical procedure.

Major Findings: Two suspected cases of congenital rubella with bilateral cataracts were operated with the aspiration technic. The latter method was chosen in attempts to isolate rubella virus from the aqueous humor and separately from the lenses. The surgeon aspirated the cataracts in-toto with a 19 gauge needle attached to a syringe. By the controlled infusion termination of surgery due to anterior chamber loss was prevented. Recovery of rubella virus from the aqueous humor and lens matter was achieved.

In animal studies, aspiration of normal rabbit lenses was performed. Although not tried clinically as yet, the technic could be utilized for aspiration of congenital "hard" cataracts as a one-stage procedure.

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

This introduces a simplified, practical, and inexpensive method for control of chamber depth during surgery of the anterior segment of the eye. It enhances the efficacy of the aspiration technic for congenital cataracts.

Proposed Course of Project: The project will continue in an attempt to evaluate the potential of the method as an adjunct in congenital glaucoma surgery.

Honors and Awards: None

Publications: None

1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Peripheral Retinopathy of Cystinosis

Previous Serial Number: NONE

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: Paul Lietman, M.D.
Jarvis Seegmiller, M.D.

Cooperating Units: Arthritis and Rheumatism Branch, NIAMD

Man Years

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: To evaluate, correlate, and characterize the ocular manifestations in patients with congenital cystinosis or Lignac-Fanconi Syndrome.

Methods Employed: All patients were seen in consultations. The various ocular changes as occurred in cystinosis were documented either by photography or by medical illustrations. Ophthalmic psychophysical tests were performed on approximately half of the children examined. Autopsy material was made available for histologic studies from two such patients. For a comparative study two patients with the adult form of this disorder and two other cases of adult Fanconi Syndrome were also evaluated.

Major Findings: Nine patients with congenital cystinosis were examined ophthalmologically and all were found to have a similar retinopathy. The lesions in the eye generally extended throughout the entire circumference of the retinal periphery. The fundus changes were characterized by a generalized depigmentation which often assumed a patchy distribution. Superimposed on this light background of the retina were pigment clumps which were homogeneously distributed. The pigment clumps varied in size from about 1/10 disc diameter to a very fine peppery-like stippling. In some areas the pigment was grouped in a circular fashion to form small ringlets of pigment. The intensity of the pigmentation faded posteriorly so that the region posterior to the equator was generally devoid of abnormal pigmentation.

Deposition of crystals in the cornea in cystinosis is well known. This change was present in all but one case in the present series. The existence of associated fundus abnormalities or retinal pathology in this disorder is a new observation. Pathologic examination in two additional patients with this condition demonstrated extensive degeneration and loss of retinal pigment epithelium corresponding to the area of retinopathy. Psychophysical tests (electroretinography, electro-oculography and adaptometry) were performed in several of these patients. The responses were normal.

The existence of this retinopathy appears to be characteristic to congenital cystinosis; it was absent in the adult form of the disease. The retinal pigment disturbance may represent the earliest ocular manifestation of this disorder. One of our youngest patients was found to have rare iris crystals in the absence of corneal deposits and the above mentioned peripheral retinopathy; on this basis a presumptive diagnosis of Lignac-Fanconi Syndrome was made. Subsequent buffy coat and bone marrow aspirate examinations were positive for cystine crystals.

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

This is the first description of a retinopathy associated with congenital cystinosis. It was found to be present in all eleven cases that were examined ophthalmologically. Moreover, there is suggestive evidence the peripheral retinopathy is the earliest ocular manifestation of this disease. Early recognition of the syndrome is important, for future effective therapy of this disorder may depend on such treatment before the onset of irreparable systemic damage.

Proposed Course of Project: To identify the crystalline material found deposited in the ocular structures of these affected patients.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-66 O/OC 1298(c)

1. Ophthalmology Branch
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Suppression of Corneal Graft Rejection with Protein Synthesis Inhibitors

Previous Serial Number: NONE

Principal Investigator: William S. Gilbert, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total: 0.25

Professional: 0.25

Other: 0.00

Project Description:

Objectives: To create a full thickness corneal homograft transplantation model in which graft rejection consistently develops after technically successful operations without additional non-ocular sensitization of the host animal, and to study the suppression of the rejection process by various inhibitory agents.

Methods Employed: Full thickness corneal grafts are performed on cats utilizing varying suture techniques. An experimental group of animals is treated for varying intervals prior to, concomitant with, and following, transplantation surgery with chloramphenicol. The ultimate fate of the graft is studied by gross and biomicroscopic observations during the clinical course of the transplant and by histologic studies of the cellular responses in the corneal, perilimbal, and uveal tissue.

Major Findings: To date, operations on animals and a number of enucleated globes have been performed to perfect the technical aspects of grafting surgery in the cat cornea. While the immediate postoperative appearance has been satisfactory, extensive corneal edema and dense neovascularization have followed in the wake of sloughing sutures and wound edge over-riding. More recent experience indicates that incorporation of overlying suture technique will obviate these earlier complications.

Significance of Bio-medical Research to the Program of the Institute: Animal experiments in the literature to date have utilized models which assure cornea rejection by additional sensitization of animals with skin transplants, and control of rejection phenomena has been attempted with systemic administration of potentially toxic agents with which clinical experience has been gained in human renal transplant situations. It would be useful to have available less toxic but effective pharmacological agents to suppress graft rejection in human cornea surgery. On the basis of successful animal experimentation with skin homografts and some suppression of immune Wessely corneal precipitin rings in experimental animals there is reason to suspect that chloramphenicol may hold some promise for suppression of corneal graft rejection, via systemic or local routes of administration. Additional experimental evidence indicative of local antibody production and cell transformation in the eye dictates the trial of local administration of immunosuppressive agents. For such purposes, the experimental animals should not be sensitized or challenged with donor antigens by non-ocular routes.

Proposed Course of the Project: Following the accumulation of an adequate series of control animals, chloramphenicol will be administered according to different schedules by intramuscular, topical, or subconjunctival routes. Histopathological and immunochemical studies will be performed to observe associated changes at the serologic and cellular levels. Additional inhibitors of protein synthesis may be evaluated.

Honors and Awards: None

Publications: None

ANNUAL REPORT

July 1, 1965 through June 30, 1966

Branch of Electroencephalography and Clinical Neurophysiology
National Institute of Neurological Diseases and Blindness

Cosimó Ajmone Marsan, M.D., Chief

Summary of Program Activity

1. Clinical Diagnostic Service

This Branch has continued to provide, as in the past, a clinical-diagnostic service for all the patients in the Clinical Center requiring an EEG examination, either as part of their routine work-up or as part of specific investigative projects in which the Branch (or Institute) has no direct interest. This form of activity represents a considerable portion of the over-all activity of the Branch monopolizing more than half of the time of the technical and secretarial and about one-third of that of the professional staff.

From the time the last report was prepared (March 31, 1965) to that of the present report (March 31, 1966) a total of 1605 examinations have been carried out in the EEG Laboratory (on both in- and out-patients), following referrals from the various Institutes, and specifically:

<u>Institute</u>	<u>No.</u>	<u>%</u>
NINDB	1025	54.0
NCI	231	14.4
NIMH	118	7.3
NIAMD	76	4.7
NIAID	87	5.4
NHI	63	3.9
NIDR	5	0.3
Total	<u>1605</u>	<u>100</u>

The large majority of referrals has been, as always, from NINDB and, especially, from the Branch of Neurological Surgery. A number of these examinations have included repeated studies in patients with chronically implanted electrodes. To the above total one should add 20 electrocorticograms which were obtained from the exposed cerebral cortex in the course of surgical procedures for the relief of seizures.

This form of service activity provided by the Branch in the last year has not been as satisfactory as in the past. Not only the monthly average of EEG examinations (about 134) has decreased by about 12 in comparison with the monthly average of the previous year, but the over-all technical quality of the records has not always been up to the standards set in the past and/or the examination procedure, occasionally, has not been carried beyond the routine stage.

This situation has resulted from a series of circumstances beyond our control; i.e., an unexpected - and unavoidable - acute shortage in the technical staff. This started with the much regretted, premature death of the supervisor technician, Mrs. Maureen Berkeley who had very efficiently occupied this position in the Branch since 1955.

The situation, still critical at the time this report is prepared, is expected to improve in the near future.

2. Research activity. Two of the research projects which had been started during the previous fiscal year have been completed. Some of the results have already appeared in published form and the remaining ones are currently in press. These projects deal with an investigation of various electrical properties of cortical neurons in resting conditions, in the course of inhibitory post-synaptic potentials and under the effect of topically applied strychnine. Using single and double intracellular micro-pipettes it has been possible to determine the resistance and time constant of the neuronal membrane as well as to estimate its capacitance and the relative contribution of, respectively, the somatic and dendritic portions of the membrane. In the same neurons, during the peak of antidromically elicited inhibitory post-synaptic potentials, the membrane conductance was found to be increased over two-fold that observed in resting conditions. These characteristic changes in conductance appear to persist - at least in some cells - even after the voltage changes which accompany the IPSP have been depressed or abolished by strychnine. This finding is in support of previous results from this Laboratory, suggesting a more direct action of the drug upon the neuronal membrane rather than (or in addition to) the commonly accepted pre- or subsynaptic action. The same findings bear indirectly on the essence of the epileptogenic effects of strychnine and on the problem of experimental epilepsy in general. In the same series of experiments, observations were also made on the post-effects of polarizing currents applied through the neuronal membrane.

It would appear that anodal break responses are quite different from the phenomena that might occur following the gradually subsiding IPSPs; these observations would not seem to support the hypothesis of a crucial "phasing" role of the IPSP in the genesis of rhythmical activity in the CNS.

After the completion of the two preceding projects an analogous one has been recently started in collaboration with the spinal cord section of the Laboratory of Neurophysiology, NINDB. In this project the same technique and analysis are applied toward the investigation of the basic physical properties of the membrane of the spinal cord motoneurons. No major findings are as yet available.

Another project deals with the study of the electrical activity that can be recorded by means of chronically implanted electrodes from various cortical areas and sub-cortical structures in man. On the basis of 162 tracings obtained in 40 epileptic patients it has been possible to provide additional evidence on the great limitations of routine scalp EEG and on the distortions resulting from the latter technique. Depth or direct cortical electrography permits the identification of very discretely localized epileptiform patterns but also demonstrates the high complexity and extensive distribution that generally characterize the epileptogenic process in temporal-parahinal structures. These and other findings have been presented - upon official invitation - at a recent International Symposium on Stereoecephalotomy and are currently in press, although the main project is not considered completed.

The other projects are not of a research - experimental nature but rather consist of critical analyses or synthetic reviews of neurological topics. The first deals primarily with a survey of the papers and monographs on epilepsy which have been published in the past year. This critical review of the world literature on this neurological disorder (in which our Institute is particularly interested) has been carried out as thoroughly as possible. The highly condensed form and rather superficial treatment of the material are the consequence of the number of publications (about 1100). This work, to be published as a chapter in the 1966 volume of "Progress in Neurology and Psychiatry" represents a follow-up of similar reviews that have been prepared in the three previous years. This is, however, the last year and there are no plans to undertake analogous projects in the future since the review of the continuously growing

literature would require too much time. It should be pointed out, on the other hand, that this type of work (as unrewarding and as unimaginative as it can be) is nevertheless becoming more and more indispensable in this as well as in other fields of investigation; somebody has to do it and, to be useful, it should be complete, accurate and should be carried out by a competent investigator with good library facilities.

The second project consists of essays of a more critical-synthetic nature, always in the general field of epilepsy. The work was undertaken following formal invitations to participate, with a specific contribution, in two International Symposia. In one it was the matter of providing the physiological bases for the new classification of epileptic seizures, recently proposed by a Committee under the sponsorship of the International League against Epilepsy. This contribution has recently appeared in published form. The other deals with a survey and discussion of the basic, cellular mechanisms in the epileptic process and of the mode of action of agents and drugs commonly employed to produce experimentally epileptogenic foci in the cerebral cortex of animals. This report, based in great part on material collected from our Laboratory, was presented at the Symposium on "Comparative and cellular pathophysiology of epilepsy" held in Czechoslovakia, September 1965, and is currently in press in the proceedings of said Symposium.

The last review-type project deals with the systematic-functional organization of the thalamus and with a discussion and speculation on some of the phenomena or functions which are likely to involve (or be the expression of) thalamo-cortical integration. The thalamus represents an interesting cerebral structure which is known to subserve and/or participate in a large number of different functions. Its complexity is unfortunately enhanced by the existence of a varied and confusing nomenclature of its numerous nuclear components and it was therefore considered useful to present (and provide some rational basis for) their different names and classifications, with emphasis on their functional role. This study has recently appeared in published form.

3. Other activities and organizational aspects. Training in clinical electroencephalography has been provided for one doctor. Both from a quantitative and a qualitative standpoint, this form of activity of the Branch leaves much to be desired. Some of the reasons responsible for this situation are "chronic" in character and have been elaborated upon in the past Annual Reports. In the last year the situation has worsened due to the above-mentioned crisis in the technical staff resulting in an acute shortage of fully trained personnel. Bureaucratically slow steps are currently in progress to correct this situation. The Chief of the Branch only wishes to stress once again the importance of this professional training program in clinical EEG, especially in view of the shortage of competent electroencephalographers still affecting this country. This is a too well known fact and one of which the Branch Chief has been particularly aware as Chairman of the Board of Qualification in clinical electroencephalography of the American EEG Society; a position he has occupied for the last three years.

Other official ("extramural") positions of the Branch Chief include that of Delegate for the American EEG Society to the International Federation of Societies for Electroencephalography and Clinical Neurophysiology (position from which he has recently resigned) and that of Chief Editor for the Americas and the Far East of the international monthly publication "Electroencephalography and Clinical Neurophysiology". Besides the above-mentioned Symposia, he has been invited to participate in that organized by the Fulton Society on "Frontal lobe" (Vienna, Sept. 1965) and in the Conference on Sleep organized by the Association for Research in Nervous and Mental Diseases (New York, Dec. 1965). From the very beginning of the program and up to March 1966, he has served as a consultant in the OIR International Fellowship Program.

4. Program considered for the near future. A certain amount of modernization and expansion of equipment is under consideration, while the chronic problems of space will probably persist, unchanged, at least in the next two years. The main field of investigation of the Branch is not expected to differ significantly, at least in its broad lines, from that of the recent past.

Serial No. NDB(I)-61 EEG/OC 800 (c)

1. Electroencephalography
and Clin. Neurophysiology
- 2.
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Depth recording in man

Previous Serial Number: Same

Principal Investigators: C. Ajmone Marsan and K. Abraham

Other Investigators: J. Van Buren

Cooperating Units: Branch of Neurological Surgery, NINDB

Man Years: (NINDB-EEG only)

Total: 0.85

Professional: 0.50

Other: 0.35

Project Description:

Objectives: To investigate the pattern characteristics of the electrical activity of various subcortical structures in relation to the patterns observed at the cortical level in "normal" and pathological conditions. Also to establish criteria of practical (diagnostic-localization) value for the classification and treatment of epileptic disorders.

Methods employed: The study is carried out routinely in all those patients (from the Branch of Surgical Neurology) in which there has been "chronic" implantation of multiple-contacts electrodes as a preliminary step to therapeutic surgery. The patients belong to two groups of neurological disorders: a) involuntary movements and dyskinesias in general and b) various forms of epilepsy. Records are obtained (from three to six in each patient) under various conditions and are subsequently analyzed.

Major findings: This is a long range project which depends, among other factors, on a large amount of material which, for obvious reasons, is not readily available (the number of patients which are suitable for this study being necessarily limited).

Some of the data relating to seizure cases have been processed. These data were derived from 162 records obtained in 40 patients including 28 presenting partial seizures with complex symptomatology (or temporal lobe epilepsy), 6 affected by partial seizures with elementary symptomatology (focal cortical epilepsy) and 6 with generalized or other types of seizures. Due to the relatively small number of cases and to the inadequate number of samples of activity from any given subcortical structure but, chiefly, due to the very nature of this study in which each patient represents an individual problem case with almost unique features, it has not been possible to formulate generalizations of diagnostic-nosologic value. The study has, however, permitted to determine a number of interesting facts: a) pointing out the great limitations of routine scalp EEG; b) emphasizing the high complexity of the epileptogenic process in practically every case investigated; c) demonstrating the excellent discriminating properties of the method which permits the detection of even a very discretely localized epileptiform activity; d) demonstrating the reciprocal effects between local and distant background activity and epileptiform discharges etc.

Significance to Bio-medical Research and the Program of the Institute: The project is directly related to one of the main programs on the diagnosis, classification and treatment of epileptic disorders and to the general problem of functional characteristics and interrelationships of various brain structures.

Proposed Course of Project: Part of the findings have been presented at the International Congress in Vienna at the Symposium on Stereoecephalotomy to which one of the principal investigators (C. Ajmone Marsan) has been officially invited. The published report is currently in press. The project continues, however, along the same general guidelines and it is expected that the analysis of at least part of the records from additional cases should be completed in the coming fiscal period.

Honors and Awards: None

Publications:

Ajmone Marsan, C. and Abraham, K.: Considerations on the use of chronically implanted electrodes in seizure disorders. Confin. Neurol., 1966, (in press)

Serial No. NDB(I)-65 EEG/OC 1225(c)

1. Electroencephalography
and Clin. Neurophysiology
- 2.
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Nocturnal sleep. Implanted electrode study

Previous Serial Number: Same

Principal Investigators: K. Abraham and F. Snyder

Other Investigators: C. Ajmone Marsan

Cooperating Units: Adult Psychiatry Br., NIMH

Man Years: (NINDB-EEG only)

Total:	0.5
Professional:	0.3
Other:	0.2

Project Description:

Objectives: This study is concerned with the topographical distribution of sleep patterns in various cerebral structures in man during different stages of nocturnal sleep, with particular emphasis on the REM (rapid eye movement) phase.

Patient material and methods: Subjects included in this study are patients with extrapyramidal disorders or epilepsy, with multiple, chronically implanted electrodes in various cortical and deep structures. The electrical activity from these electrodes is recorded continuously throughout the night (7-8 hours), with simultaneous recording of muscle-tone and eye movements.

Major findings: Data are collected at a slow rate in view of the nature of this study and those available are still in the process of being analyzed. Some results should be expected in the course of the coming fiscal period during which the project will be continued.

Significance to Bio-medical Research and the Program of the Institute: This study is a continuation of the work previously

done, and partly still in progress in this Institute. Sleep patterns have not yet been clearly defined in various sub-cortical structures in man. It is hoped, that this study, although performed on a patient material with cerebral disease, will help to determine "normal" patterns of sleep in those structures examined and possibly provide some clue to the mechanism of nocturnal seizures. Emphasis is particularly on the REM (paradoxical, low voltage fast or rhombencephalic) sleep which is suggestively related to dreaming and is currently a subject of intensive studies in several centers.

Proposed course of project: All available and suitable patients will be studied with similar techniques, and the available data analyzed.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 EEG/CN 1226(c)

1. Electroencephalography
and Clin. Neurophysiology
- 2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Electrical constants of neurons in the motor
cortex of cat

Previous Serial Number: Same

Principal Investigators: H. D. Lux and D. A. Pollen

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.85
Professional:	0.40
Other:	0.45

Project Description:

Objectives: Within the last decade much work has focused on intracellular recording of synaptic drives upon neurons in the mammalian cortex. Whereas the complex cortical anatomy makes the task of untangling synaptic organization formidable enough, the problem is even further complicated when there is lack of knowledge of those physical properties (such as membrane resistance, capacitance, time constant, rectification properties and soma-dendritic conductance ratios) which set limits on the "output" function of a given cell in response to synaptic input.

These basic data must be provided not only for understanding the physiology of the "normal" cell but for considering the possible types of changes that may occur in the membrane properties of a cell after it has become "epileptic". Hence this study also attempts to see whether any of the above properties are altered in cortical neurons exhibiting excessive or "epileptic-like" discharges after strychninization.

Methods: Experiments were done in barbiturate anesthetized cats using glass micropipettes for intracellular penetration.

When single electrodes were used for both stimulating and recording, a Wheatstone bridge was employed. "Double micro-electrodes" have also been successfully inserted into some cortical neurons, and in these experiments current was passed through one electrode while using the other for voltage recording.

Major findings: The voltage changing curves for cortical neurons appear to deviate significantly from the form expected for simple exponential curves. It is postulated that such deviations are caused by variable degrees of dendritic dominance. The precise soma-dendritic conductance ratios according to the theory of Rall are being determined.

Regarding rectification properties, the current-voltage relationship does not depart much from a linear relationship in the normal physiologic range of membrane potential levels, and such small departures as have sometimes been found are still being evaluated for possible significance. All the data have been analyzed and the project is completed. The results have very recently appeared in the published form.

Here is a brief summary of the findings:

Single and double intracellular microelectrode techniques were used to determine the electrical membrane properties of neurons in the motor cortex of barbiturate anesthetized cats. The total soma-dendritic membrane resistance for large Betz cells was $6.7 \pm 1.4 \text{ M}\Omega$; no rectification was found within the physiological range. The membrane time constant of Betz cells, determined on the basis of a "cable" model was $8.4 \pm 1.4 \text{ msec}$.

Capacitance estimations were based on the assumption of a homogenous membrane current density in a cell soma ("sphere" model). The data ($0.74 \pm 0.28 \text{ }\mu\text{F}$) compared with histological soma surface estimations yield unusually high estimations for specific capacitance ($20\text{-}40 \text{ }\mu\text{F}/\text{cm}^2$). It seems therefore likely that a much greater portion of the current is spreading in dendritic regions than crossing the somatic membrane area and in turn, that application of a "cable" model in the electrotonus of cortical neurons is justified. There is evidence for relatively high specific membrane resistances (in the order of $4000\text{-}8000 \Omega \text{ cm}^2$), which enhances the role of distant dendrites.

Significance to Bio-medical Research and the Program of the Institute: These data will allow a better understanding of

the physical properties of "normal" cortical neurons. This in turn may allow us to further understand the functions of such cells, the similarities or differences with other central neurons previously studied, and will provide a base for seeing whether alterations in physical properties occur and are responsible for the excessive neuronal discharges of experimental epilepsy. This in turn may help in our understanding of the basic disorder in human epileptic states.

Proposed course of project: Project completed.

Honors and Awards: None

Publications:

Lux, H. D. and Pollen, D. A.: Electrical constants of neurons in the motor cortex of cat. J. Neurophysiol. 29 (2): 207-220, 1966.

Serial No. NDB(I)-65 EEG/CN 1227(c)

1. Electroencephalography
and Clin. Neurophysiology
- 2.
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Cortical neurons; conductance changes during
IPSPs and evaluation of anodal break and post-
inhibitory rebound

Previous Serial Number: Same

Principal Investigators: D. A. Pollen and H. D. Lux

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.85
Professional:	0.40
Other:	0.45

Project Description:

Objectives: These experiments have been undertaken to determine the extent and duration of the membrane conductance changes during induced post-synaptic potentials (IPSPs) in neurons of the motor cortex of the cat; and to determine the respective contribution of both membrane conductance change and increase in membrane level to the inhibitory effect itself.

Secondly, it is at present an open question as to what extent post inhibitory membrane changes may contribute to the recycling mechanisms which seem to be involved in the genesis of the main cortical rhythms. Anodal break phenomena after passage of rectangular currents have been evaluated in different cells at different membrane levels. In order to ascertain the significance of rebound in a physiological situation these data are re-evaluated in view of the dampening effect of the prolonged inhibitory transmitter action.

Thirdly, the possibility is investigated that changes in the membrane properties of cortical neurons may be involved in

the transition of a cell from normal to "epileptic" during the production of experimental "epileptiform" states as induced by topical application of strychnine. A related study (Serial No. NDB(I)-65 EEG/CN 1226(c)) deals with the possible changes in the physical properties of the resting membrane. This project deals with possible changes in the subsynaptic membrane subserving the IPSP and with their possible alterations under the effect of strychnine.

Methods: Experiments were done in barbiturate anesthetized cats using glass micropipettes for intracellular penetration. When single electrodes were used for both stimulating and recording, a Wheatstone bridge was employed. "Double micro-electrodes" have also been successfully inserted into some cortical neurons, and in these experiments current was passed through one electrode while using the other for voltage recording.

Major findings: Increases in ion conductance of over two fold that of the neuron at the resting membrane level occurred during the peak of antidromically induced inhibitory postsynaptic potentials (IPSP) in neurons of the motor cortex of barbiturate anesthetized cats. The conductance increases gradually declined during the subsiding phase of the IPSP indicating a prolonged but gradually declining transmitter release or action.

Conductance increases in some cells remained high, after strychnine had depressed the voltage change associated with the IPSP; it therefore seems possible that strychnine, in addition to pre- and subsynaptic actions affects postsynaptic sites by altering an IPSP equilibrium level.

Single spikes may occur as part of an anodal break response at the end of sufficiently strong rectangular polarizing currents. No evidence was obtained that the same phenomenon occurred to any significant degree following the gradual subsidence of the IPSP.

An elaboration of these results and of other data which might be pertinent to the problem of experimental epilepsy has also been carried out following the invitation of the Chairman of the Program Committee of the American Epilepsy Society to participate in a Symposium on "Triggering mechanisms in epilepsy". The same report has been prepared for publication and is currently in press.

Significance to Bio-medical Research and the Program of the Institute: This and the associated work (see preceding

project, Serial No. NDB(I)-65 EEG/CN 1226(c)) provide data about the physical properties of the membrane both in the resting state and during inhibitory synaptic action. It is also hoped that the "epileptogenic" action of strychnine can be elucidated.

Proposed course of project: The project is now completed. The results have been written up in an article which is currently in press.

Honors and Awards: None

Publications:

Pollen, D. A. and Lux, H. D.: Conductance changes during inhibitory postsynaptic potentials in normal and strychninized cortical neurons. J. Neurophysiol. 29(3): 1966 (in press)

Pollen, D. A. and Lux, H. D.: Intrinsic triggering mechanisms in focal paroxysmal discharges. Epilepsia (Amst.), 7: 1966 (in press)

Serial No. NDB(I)-66 EEG/CN 1299(c)

1. Electroencephalography
and Clin. Neurophysiology
- 2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Epilepsy - A critical review of the literature
for 1964-1965

Previous Serial Number: None

Principal Investigator: C. Ajmone Marsan

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.2
Other:	0.3

Project Description:

Objectives: As implied in the title of the project this is a review work. The project has been undertaken upon specific invitation by the editor of "Progress in Neurology and Psychiatry" (1966).

Methods: -

Major findings: The project is based on a survey of about 1100 papers on the various aspects of seizure disorders (experimental epilepsy, etiology, pathology, pathogenesis, clinical and EEG features, experimental pharmacology, medical and surgical treatment, epidemiology, social and medico-legal aspects etc.) published throughout the world in 1965 and in the second half of 1964.

Significance to Bio-medical Research and the Program of the Institute: Besides the direct interest of both Branch and Institute in this field of neurological disorders, this relatively small project should prove of great practical usefulness in an era of over-publication which greatly hampers any individual attempt at bibliographic retrieval and documentation.

Proposed course of Project: This work which is a follow-up of similar projects carried out in the last three years, has been completed and will form the Chapter on "Epilepsy" in the above mentioned volume.

Honors and Awards: None

Publications:

Ajmone Marsan, C.: Epilepsy. In Spiegel, E. A. (Ed.): Progress in Neurology and Psychiatry. New York, Grune & Stratton, 1966, Chapt. VIII (in press)

Serial No. NDB(I)-66 EEG/CN 1300(c)

1. Electroencephalography
and Clin. Neurophysiology
- 2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Basic mechanisms in epilepsy

Previous Serial Number: None

Principal Investigator: C. Ajmone Marsan

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.35
Professional:	0.30
Other:	0.05

Project Description:

Objectives: This project is of an analytical-critical nature. It was undertaken, in part, upon the specific request by the Secretary General of the International League against Epilepsy. The League has recently sponsored the formulation of an international classification of epileptic seizures (Gastaut, H. et al, Epilepsia (Amst.) 1964, 5: 297-306) and the principal investigator was assigned the task of providing a neurophysiological basis to this newly proposed classification which is essentially based on clinical-semeiological and electrographic criteria.

The results of this critical essay have been presented at the 10th Meeting of the International League against epilepsy in Vienna, Sept. 1965 and the report has recently appeared in a published form.

At about the same time the principal investigator was invited to participate in the International Symposium on "Comparative and cellular pathophysiology of epilepsy" held in Liblice (Prague), Sept. 20-24, 1965, with the request he contribute an analysis of the basic mechanisms of seizure susceptibility. A report was therefore prepared based on (published and

unpublished) personal findings and on those of various collaborators on experimental epilepsy. In this report some of the agents and procedures commonly used to induce seizure activity have been analyzed and discussed particularly in relation to the different type of their epileptogenic effects; the problem of the "epileptic" neuron was again discussed in an attempt to establish whether there is such an entity and whether its abnormal form of activity can be considered truly pathognomonic, as well as to evaluate the participation of excitatory and inhibitory phenomena in its epileptiform manifestations. Also analyzed - among other phenomena - were the development of the epileptic focus and the transition from inter-ictal to organized, self-sustained ictal activity. This report is currently in press.

Methods: -

Major findings: -

Significance to Bio-medical Research and the Program of the Institute: This critical analysis deals with a subject in which both this Branch and the Institute have been directly interested for many years.

Proposed course of Project: This project has been completed.

Honors and Awards: None

Publications:

Ajmone Marsan, C.: A newly proposed classification of epileptic seizures. Neurophysiological basis. Epilepsia (Amst.) 6: 275-296, 1965.

Ajmone Marsan, C.: Microstructural mechanisms of seizure susceptibility. In Servit, Z. (Ed.): Comparative and Cellular Pathophysiology of Epilepsy, a Symposium. Excerpta Medica, Amsterdam, 1966, (in press)

Serial No. NDB(I)-66 EEG/CN 1301(c)

1. Electroencephalography
and Clin. Neurophysiology
- 2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The thalamus. Data on its functional anatomy
and on some aspects of thalamo-cortical
integration.

Previous Serial Number: None

Principal Investigator: C. Ajmone Marsan

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.15
Professional:	0.1
Other:	0.05

Project Description:

Objectives: This project consists of 1) an analytical review and synopsis of the anatomical features of the thalamus and 2) of the survey and discussion of a number of neurophysiological phenomena which can be considered as a manifestation of thalamo-cortical integration or which might justify the existence of the latter as a basic concept in CNS physiology.

Methods: -

Major findings: In view of the different nomenclature and great complexity of the thalamic structures an attempt was made to present them under various classifications each based on somewhat different criteria and each providing information of a different nature (anatomo-topographical, phylogenetic, functional etc.), using as much as possible a uniform nomenclature or providing cross-references to simplify the identification of any given nucleus.

The spontaneous electrical activity of the cortex, various aspects of sensory mechanisms and certain types of epileptic

disorders are the entities which have been analyzed and discussed from the point of view of thalamo-cortical integration.

Significance to Bio-medical Research and the Program of the Institute: Thalamo-cortical integration plays a primary role in various fields of neurophysiology. Different alterations of thalamo-cortical mechanisms are probably at the basis of a number of neurological disorders.

Proposed course of project: This project has been completed and the published article has recently appeared.

Honors and Awards: None

Publications:

Ajmone Marsan, C.: The thalamus. Data on its functional anatomy and on some aspects of thalamo-cortical integration. Arch. ital. Biol. 103: 847-882, 1965.

Serial No. NDB(I)-66 EEG/CN 1302(c)

1. Electroencephalography
and Clin. Neurophysiology
- 2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Impedance measurements of motoneurons by
means of separated double microelectrodes

Previous Serial Number: None

Principal Investigator: D. H. Lux

Other Investigators: P. G. Nelson

Cooperating Units: Spinal Cord Section, Lab. of Neurophysiol.,
NINDB

Man Years: (NINDB-EEG only)

Total:	1.1
Professional:	0.6
Other:	0.5

Project Description:

Objectives: This project is concerned with clarifying the physical properties of spinal cord motoneurons, such as input resistance, soma-dendritic conductance ratios and membrane capacitance and rectification.

Methods employed: Experiments are carried out in the spinal cord of cats. The technique includes AC-impedance measurements with independent electrodes and measurements of membrane potential slopes by application of constant current steps (to check the results obtained in cortical cell - see other project by Lux and Pollen, this Report).

Major findings: Project has been started recently. No findings are available as yet.

Significance to Bio-medical Research and the Program of the Institute: As in the case of the other, related, projects (see this Report) it is felt that a knowledge of the basic physical properties of the neuronal membrane is essential for

the interpretation and understanding of the various forms of neuronal activity in either normal or abnormal situations.

Proposed course of project: Experiments to be continued.

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1965 through June 30, 1966
Laboratory of Neuroanatomical Sciences
National Institute of Neurological Diseases and Blindness

Alfred J. Coulombre, Chief

Introduction

The scope of the program of the Laboratory of Neuroanatomical Sciences during FY 1966 is suggested by the number of structures which were under investigation (muscle spindle, lens, vestibular afferent and efferent systems, cochlear nuclei, superior olivary complex, nuclei of the lateral lemniscus, inferior colliculus, medial geniculate body, cerebral cortex, locus coeruleus, nucleus dorsalis raphe, area ventralis tegmenti, substantia nigra, olfactory bulb, smooth muscle of the vas deferens and iris, ependyma), by the number of animal types studied (rat, chinchilla, cat, rabbit, monkey, mouse, chick), by the variety of techniques which were employed (electron microscopy, scintillation counting, autoradiography for both light and electron microscopy, macro and micro surgery, spectrophotometry, electrophoresis, etc.), and by the fact that over 30 research questions were answered. Despite the scope of the program the resources of the Laboratory were this year committed in a more focused way to specific problem areas (subcellular structure, cytoarchitecture, trophic interactions, morphogenesis and regeneration). Some problems in each of these areas were simultaneously under attack in different Sections of the Laboratory, against different conceptual backgrounds and with different techniques. It is not common that single individuals, or even different individuals in the same working unit, possess simultaneously, on the one hand, the background and skills necessary for the type of cytoarchitectural information revealed by such techniques as the Golgi, the Marchi, the Nauta, and on the other hand, the battery of techniques and concepts associated with electron microscopy. This Laboratory is providing increasingly strong leadership in the field in relating the findings of both types of approach to give an integrated picture of the structure of the nervous system at all levels of resolution. This year, for example, the auditory system received attention in three Sections (from the cytologic, cytoarchitectural and morphogenetic points of view). This sharing of an object of study, without significant overlap in the mode of analysis, has provided scope for individual initiative, has led to collaboration across Section lines and will, it is hoped, prove an added stimulus to those who share common interests and attack common problems. This increase in the relatedness among groups of projects is reflected in a decrease in the number, and an increase in the complexity, of the projects reported by the Laboratory as a result of this year's activity.

Another example of the manner in which the program of the Laboratory is changing in structure is the increase of interest in, and work on, problems of morphogenesis, trophic interaction and regeneration. The Laboratory is concerned not only with the structure of the adult vertebrate system, but is also increasingly involved with the changes in structure and organization

which occur during development and regeneration, and with the mechanisms which guide and channel these changes. These emerging areas of concentration are in no way freezing the program of the Laboratory. Rather, they represent a commitment of resources to present opportunities. At the level of its individual projects the program remains broadly diversified and flexible.

Investigators in the Laboratory are making increasingly judicious use of collaboration with each other, with investigators in other units of the National Institutes of Health, and with colleagues in other institutions. Such cooperative undertakings have enabled us to tap first-rate talent for specific purposes and to use such talent for just the period of time required by our programs. Collaborative undertakings range from those involving investigators within the same Section to international collaboration. Some collaborative ventures have required less than a work-day, while others are of an indefinite duration. Such activities have proved increasingly beneficial to the programs of our Laboratory since they accelerate progress by supplementing our pool of talent when and where it is needed, and since they increase flexibility. New techniques and instruments continue to be developed as the need arises. An example of a technical advance is the application of a permanganate fixation procedure to the problem of demonstrating dense-cored synaptic vesicles in certain autonomic terminals. An example of new instrumentation is the development, in cooperation with the Technical Development Section, of the "tissue plotter" which harnesses an X-Y plotter to the stage of a compound microscope in such a fashion that objects seen at high resolution through the microscope can be plotted spatially relative one to another with great accuracy.

Among the honors accorded members of our staff was the conferring of the degree of Docteur Honoris Causa on Dr. Grant Rasmussen by the Free University of Brussels on 13 November 1965 in recognition of his many research contributions to the analysis of the auditory system. In addition, Dr. Kent Morest, now of the Department of Anatomy, Harvard Medical School, was honored by the American Association of Anatomists when he received the C. Judson Herrick Award for his study of the anatomy of the medial geniculate body which he undertook and completed as a Research Associate in this Laboratory.

The research activities of the Laboratory can be categorized in several different ways to bring out different aspects of the program. In the annual report for FY 1965 the program was presented in terms of the major sensory, central, and motor systems which were then under investigation. The present report distinguishes those studies which concern themselves principally with the structure of the nervous system from those studies which deal more with the dynamic interactions which occur among the components of the sensory, nervous, and motor systems. This separation is made largely for reasons of convenience since structural and functional considerations can rarely be considered separately.

I. Analyses of structure

A. Cytology

1. Auditory and vestibular systems: The auditory and vestibular systems are under intensive investigation at all levels of organization from the sensory input to ascending and descending pathways. Building upon a previous light microscopic study an electron microscopic analysis of the trapezoid body and anterior ventral cochlear nucleus has revealed two types of endings. Small, presumably efferent, endings containing small synaptic vesicles persist following destruction of the spiral ganglion. Large caliciform endings which make multiple synaptic contact with the dendrites and cell bodies of the neurons of these nuclei, contain relatively larger synaptic vesicles, disappear upon destruction of the spiral ganglion, and are endings of the cochlear nerve fibers. The identification on a structural basis of two types of nerve terminals raises the possibility that in this system facilitation and inhibition are mediated by different neurotransmitters.

2. Olfactory system: A hitherto undescribed synapse, the dendrodendritic synapse, has been discovered in the glomerulus and external plexiform layer of the olfactory bulb. In addition, it was discovered that some small periglomerular neurons were enveloped by the flattened soma of adjacent neurons. These observations have provided a morphological basis for such neurophysiological phenomena as lateral inhibition in the olfactory bulb. It is anticipated that similar arrangements may exist in other sensory systems.

3. Autonomic nerve endings: The study of the ultrastructure of autonomic nerve endings on smooth muscle continues in collaboration with related chemical and pharmacological investigations in other laboratories. Modification of a potassium permanganate fixation procedure has opened the way for the identification of individual nerve terminals bearing granular synaptic vesicles. It now appears possible to distinguish such terminals from other types. Hitherto, reliable identification of adrenergic nerves was available only at the level of resolution provided by the light microscope. The technical advance made in this Laboratory during the past year makes it possible to identify individual terminals at the high levels of resolution provided by the electron microscope. The new technique will permit resumption of an investigation of the structural effects of the monoamine oxidase inhibitor pargyline on the nerve endings of the rat vas deferens.

In general, work on the innervation of smooth muscle, which has been carried on in this Laboratory over the past several years, is providing detailed information concerning the types of nerve fibers which innervate smooth muscle in different regions of the body and is revealing a detailed picture of the relationship between the nerve terminal and the muscle cell.

In addition to studying the distribution of monoamine containing cell organelles in the peripheral nervous system a survey was made of the distribution of monoamines in the central nervous system. Combined electron microscopic and autoradiographic analysis of rat brains revealed that exogenous epinephrine becomes neuronally localized, but that it is not associated with any specific cell organelle. Serotonin, on the other hand, appears to localize in certain nerve endings. These findings point to

possible differences in the storage forms of monoamines in different parts of the nervous system.

4. Pathways of transport in the brain for large molecules; During fiscal year 1965 electron microscopic observation of the distribution of ferritin which had been experimentally introduced into the ventricles revealed the pathway by which this large molecule moved across the ependyma and became distributed among the several compartments of the brain tissue. Among other mechanisms responsible for the transport of this protein molecule pinocytosis was found to be of cardinal importance. During fiscal year 1966 the Laboratory undertook to further describe and define this important process which is responsible for the movement of many solutes across cell boundaries, not just in the brain but in nearly every tissue of the body. It was found that pinocytotic activity continues in the ependyma for a considerable period following the death of animal. Similarly, pinocytotic activity persists in living animals even in the presence of sufficiently high concentrations of inhibitors (iodoacetate and n-ethyl-maleimide) to severely damage the ependymal cells. Since pinocytotic activity continues even in severely damaged cells, it is planned to study the formation of the pinocytotic vesicles in membraneous isolates from living cells in order to determine the minimal morphological requirements for pinocytosis. Should it be possible to duplicate this phenomenon in a cell-free system the way would clearly be open for studying not just the morphological, but also the energetic requirements of this process.

B. Cytoarchitecture: Neuroanatomy continues to add to the knowledge of the major and minor neuronal pathways which interconnect nuclear stations in the central nervous system. Recently increasing importance has been given, in addition, to studies of synaptology. Information concerning the types, number, cells of origin and spatial distribution of synapses is increasing our understanding of the organization and functions of the nervous system.

1. Auditory system: The study of the auditory-vestibular afferent and efferent systems including the receptors is still in progress in the chinchilla and cat. It is sought to establish at the synaptic level the interconnections which exist among the cochlear nuclei, the superior olivary complex, the nuclei of the lateral lemniscus and the inferior colliculus, as well as the auditory reflex connections with certain motor nuclei and the reticular formation. This is an undertaking of some magnitude and the studies and the analysis of data are not yet far enough along to warrant a report at this time. It is important to realize that our knowledge of the functional organization of these systems, and of the interrelationships which exist between them must ultimately rest upon a detailed analysis of this structural organization.

In FY 1966, as a result of collaboration with Professor Jean E. Desmedt, Director, Laboratory of Pathophysiology of the Nervous System and Brain Research Unit of the Free University of Brussels, the Laboratory launched a detailed analysis of the ascending and descending auditory connections in primates. This work is building upon extensive past experience in

the analysis of the cytoarchitecture of the auditory system in lower mammals. Thus far, strategic lesions have been placed in 30 cynomolgus monkeys. The brains of these animals are currently being processed and analyzed. Collaboration with Professor Desmedt promises to be particularly fruitful since he is directing his considerable abilities and unique laboratory facilities to a physiological analysis of the primate auditory system based upon the anatomical findings. This work promises to yield the most detailed analysis of the auditory pathways yet available for a form this close to man. During the past year we have placed unusual emphasis upon studies of the auditory system. Nearly half of the professional staff of the laboratory became involved in some important way in studies of this system. Three of the four Sections of the Laboratory contributed significant resources to this undertaking. During the year two of the scientists studying the auditory and vestibular systems received notable honors because of their work in this Laboratory. No other unit of the National Institutes of Health currently has a commitment this large to the analysis of form and function of the auditory system.

2. The muscle spindle: The structure and composition of the muscle spindle began to receive attention during FY 66. The work begun by Dr. James Stephens in the Section on Neurocytology held considerable promise of revealing the fine structure of muscle spindle. Unfortunately, this work must now be suspended because Dr. Stephens, a Guest Worker, has had to return to his home institution owing to illness. The Section on Experimental Neurology recently began to apply a battery of histochemical techniques to the muscle spindle. This work will continue, and an effort will be made to determine the distribution of glycolytic and oxidative enzymes. In addition, the effects of denervation and tenotomy upon the histochemical profile will be studied. One goal of this study is to determine why intrafusal muscle in spindles is so remarkably resistant to a wide variety of myopathies.

II. Interactions in and among the sensory, central and motor systems:

A. Trophic interactions:

1. Nerve-muscle relationships: It has long been known that most skeletal muscles require an intact innervation to maintain them at full size and function. The Laboratory has now carried the analysis of this trophic interaction onto the chemical level. Two endpoints (cholinesterase activity and the electrophoretic pattern of muscle protein) have been used to assess the effects of denervation and of reinnervation of skeletal muscle by foreign nerves. Denervation causes a rapid drop in cholinesterase activity which is independent of the level at which the nerve is transected. Physiotherapy did not retard the rate of loss of cholinesterase activity in these cases. Spinal cord transection also resulted in a significant diminution of cholinesterase activity, but the effect was less severe than after a peripheral nerve lesion. Cholinesterase activity associated with sole plate regions remains constant during growth of the muscle, whereas the activity in regions of muscle lacking sole plates increases in proportion to the growth of the muscle.

The soluble proteins of red and white muscles differ qualitatively and quantitatively. Following denervation the protein pattern of red muscle is unaltered, whereas that of white muscle assumes a pattern that is qualitatively and quantitatively similar to that of red muscle. When denervated red muscle is reinnervated by nerve fibers that normally supply a white muscle, the protein pattern becomes transformed into that characteristic of the white muscle. Tenotomy produces no change in the protein pattern in either type of muscle. These studies and others in progress in the Section on Experimental Neurology indicate that nerve fibers exert a profound and highly specific chemical influence on the muscle fibers which they innervate. Because of their importance in understanding function and dysfunction in the neuromuscular system, the Laboratory will continue to place major emphasis upon chemical studies of trophic interaction.

2. Visual system: During the past year about a fourth of the resources of the Laboratory were committed to an analysis of those factors which control size, shape and orientation of the tissues of the developing vertebrate eye. For the most part the work has been conducted with the chick embryo. It was found that the volume of the lens of the chick embryo increases exponentially throughout the period of incubation. However, from at least the 5th day of incubation onward, there is a progressive decrease in the rate of growth. The lens fiber mass contributes most importantly to the increase in lens volume. This increase in the volume of the lens fiber mass during development is brought about by an increase in the number of its cells, and by an increase in the mean volume of the cells. The lens fiber mass contains between 20,000 and 30,000 lens fibers on the fifth day of incubation. New lens fibers are recruited into the lens fiber mass at the equator. They are added rapidly during early development, and progressively more slowly at older ages. By the 20th day of incubation there are nearly 300,000 fibers in the lens fiber mass. The enlargement of lens cells to become lens fibers is brought about, at least in part, by the synthesis within them of increasing amounts of lens protein. The total lens protein increases exponentially and at a rate higher than that of the increase in lens volume. As a consequence, the amount of protein per unit volume of lens increases as development proceeds. This increase in lens protein concentration is accompanied by a progressive relative loss of water and by an increase in specific gravity of the lens.

The increase in volume of the growing lens is under the control, at least in part, of the neural retina. Removal of the neural retina from the eye early in development results in a severe depression of lens growth. Replacement of a portion of neural retina into eyes from which it has been removed sustains rates of lens growth which are closer to normal. It remains to be determined to what extent this control has been exerted by regulating the number of lens cells which enter lens fiber population, and to what extent control of growth is mediated by regulating the volume of the individual cells (e.g. by controlling protein synthesis).

A previous project demonstrated that a reasonably well formed lens can develop from lens epithelium alone when this tissue is substituted for the lens early in development. During the past year it was demonstrated

that the ability of the lens epithelium to respond in this way to the eye environment decreases progressively with advancing age.

During FY 66 mathematical formulations were developed with which the shape of the lens can be quantitated. As a result it has been shown that the shape of the lens changes progressively with embryonic age in such a fashion that its posterior surface becomes more and more convex relative to the anterior curvature of the lens. This quantitative descriptive study of the development of the lens shape is preliminary to an analysis of the factors that regulate lens shape during embryonic development.

During the past year a good deal of attention was given to the lens considered as a target for influence by surrounding tissues. It is planned to continue studies of the interactions among all the tissues of the developing eye by considering each tissue in turn as both a target of influence by surrounding tissues, and a source of influence affecting neighboring tissues. As a result of this type of analysis, the Laboratory has been constructing a flow sheet which describes, in some detail, the complex chain of interactions among the tissues of the developing eye to shape it as an optical instrument. It is hoped that further studies will permit us to identify the specific mechanisms by which these influences are mediated.

B. Morphogenesis:

1. Auditory system: During the past two years the Laboratory has undertaken to determine the distribution in time and space of the terminal mitoses in 18 cell type populations in the developing inner ear of the mouse. The study involved injection of single pulses of tritiated thymidine into pregnant mice at known times during gestation, followed by autoradiographic analysis of the inner ear of the offspring after they had matured. Specially designed computer programs permitted analysis of cell population dynamics during embryological development of the inner ear. The developmental period during which the terminal mitoses occur is shorter in the cochlea than in the vestibular apparatus. Receptor cells, supporting cells and ganglion cells do not divide after the third postpartum day. In contrast, the connective tissue and Schwann cells continue to divide for at least 7 days after birth. Two spatial patterns of terminal mitosis were discovered in the cochlea. The hair cells and their supporting cells undergo terminal mitosis in a wave which begins at the apex of the cochlea on the 12th day of gestation and sweeps down the cochlea, reaching its base on the 16th day of gestation. The wave of terminal mitosis in the spiral ganglion cell population, in contrast, starts at the base on the 12th day of gestation and ceases at the apex on the 15th day of gestation. This study was conducted with genetically inbred mice. It establishes a normative base line for similar analyses of approximately 25 strains of mice, each bearing a different point mutation affecting the ear in a slightly different way. Such genetic dissections of the developing ear should tell us much concerning normal and abnormal morphogenesis of the auditory and vestibular systems. It is already possible to indicate that an insult to the auditory system during the relatively brief time during development when

the sensory cells are undergoing terminal mitosis could produce extensive permanent damage. In the vestibular system, on the other hand, terminal mitoses occur over a longer period of time, and acute insult would not be expected to produce as serious a defect.

2. Regeneration: The laboratory continues to study the factors influencing the rate of regeneration of peripheral nerves and degree of reinnervation of peripheral tissues. Different nerves in the same, or in different, species of animals have been found to have remarkably similar rates of regeneration. Similarly, the rate of maturation of the action potential amplitude is the same in the cat vagus nerve as in the rat sciatic nerve, and is unrelated to the action potential amplitude that the nerve is ultimately capable of achieving. In another study the micro method for the determination of choline acetylase activity is being used in an attempt to measure the density of innervation in normal, partially denervated and reinnervated muscle. Should it prove successful, this method could have both theoretical and clinical applications.

III. Future of the program: The statement of our future plans which was spelled out in the FY 65 Annual Report remains valid. The programs represented by the four existing Sections will continue in their present directions. In addition, further strength will be added in the area of morphogenesis of the nervous system over the next three years.

Serial No. NDB (I)-60LNS/FN 712

1. Neuroanatomical Sciences
2. Section on Functional
Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The ascending and descending auditory connections in the primates.

Previous Serial Number: SAME

Principal Investigator: Grant L. Rasmussen

Other Investigators: Prof. Jean Desmedt, Universite Libre de Bruxelles, Brussels, Belgium

Cooperating Units: Universite Libre de Bruxelles, Brussels, Belgium

Man Years

Total:	1.2
Professional:	0.5
Other:	0.7

Project Description:

Objectives: In general, to extend previous studies of the ascending and descending system of lower mammalian subjects to the primate which possesses anatomical features more similar to that of man. In 1965 the specific objective was a study of auditory and related cerebral cortical regions that project corticofugally to the medial geniculate body and inferior colliculus. This project was initiated in the laboratory of Prof. Jean Desmedt of the Universite Libre de Bruxelles in 1965 for the purpose of establishing an anatomical basis for follow-up physiological studies on the interaction of the higher set of ascending and descending conducting neurons in the process of hearing.

Methods Employed: Experimental neuroanatomical methods were employed following removal by suction of different parts of the superior temporal gyrus and surrounding cortical area. Corticofugal degenerated fibers are traced in serial sections

stained by an axonal degeneration method to lower auditory centers and the relative population of fibers originating from the variously located cortical lesions is evaluated. Thirty cynomolgus monkeys were used in this experiment.

Major Findings: Not all of the thirty monkey brains have been processed histologically and observations are too incomplete to give major findings at this time.

Significance: Anatomical knowledge of the cortical auditory connection in the monkey need be obtained in order to design appropriate physiological experiments for testing the functional role of the descending auditory system.

Proposed Course of Project: To extend anatomicophysiological collaborative studies with Prof. Desmedt on the higher auditory levels and to continue to study independently the auditory circuit of lower levels of the brain stem of the primate.

Honors and Awards: None

Publications: None

Serial No. NDB (I)-60 LNS/FN 713

1. Neuroanatomical Sciences
2. Section on Functional
Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A study of the auditory vestibular afferent and efferent systems including the receptors in the chinchilla and cat.

Previous Serial Number: SAME

Principal Investigator: Grant L. Rasmussen

Other Investigators: Dr. Catherine Smith, Washington University
Saint Louis, Missouri

Cooperating Units: Washington University, Saint Louis, Missouri

Man Years

Total: 2.3
Professional: 0.3
Other: 2.0

Project Description:

Objectives: To continue to explore and reveal unknown anatomical neuronal connections of the afferent and efferent divisions of the auditory system; to gain more information about the anatomical and functional interrelationships of these two systems. The immediate problem under study is to establish at the synaptic level the interconnections existing between the cochlear nuclei, superior olivary complex, the nuclei of the lateral lemniscus and the inferior colliculus as well as the auditory reflex connections with certain motor nuclei and the reticular formation.

Methods Employed: The newer and more effective techniques are chiefly relied upon for demonstration of axonal, preterminal and terminal degeneration. Also the Golgi and the histochemical method of Koelle, which we have modified to advantage, are heavily relied upon to furnish information not otherwise obtainable. Since the efferents of the cochlear nerves exhibit a much higher

concentration of acetylcholinesterase than the afferents it is possible to differentiate in histological preparations the two functionally different types of fibers.

Major Findings: No major findings revealed beyond those reported last year and which will appear this year as a chapter in a book entitled "Sensorineural Hearing Processes and Disorders."

The collaborative project with Dr. Catherine Smith, Department of Otolaryngology, St. Louis, Missouri has been active but no major findings can be reported at this time.

Significance: Knowledge of the neuronal relationships existing at the synaptic level between the afferent and efferent systems is basic to an understanding of the neuromechanism of hearing. Such information is essential for a foundation upon which to design physiological experiments for testing the functional significance particularly of the descending conduction system.

Proposed Course of Project:

1. The EM and light microscope collaborative study of the efferent innervation of the vestibular receptors will be continued with Dr. Smith.

2. The afferent and efferent auditory connections of the pons and midbrain of the chinchilla and cat will be investigated more thoroughly this year.

Honors and Awards: Degree of Docteur Honoris Causa conferred by Universite Libre de Bruxelles, November 13, 1965 in recognition of research contribution on the Auditory System.

Publications: None

Serial No. NDB (I)-65 LNS/FN 1229

1. Neuroanatomical Sciences
2. Section on Functional
Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Fine structure of afferent and efferent nerve endings in the cochlear nucleus of normal and experimental animals.

Previous Serial Number: NDB(I)-65 LNS/NC 1229

Principal Investigator: Thomas Reese, Harvard University,
Boston, Mass.

Other Investigators: Grant L. Rasmussen

Cooperating Units: Harvard University, Boston, Mass.
Dept. of Anatomy

Man Years

Total:	0.5
Professional:	0.2
Other:	0.3

Project Description:

Objectives:

1. To determine the ultrastructural difference between the cochlear nerve afferent endings in different subdivisions of the cochlear nucleus and those of efferent fibers originating from higher auditory centers previously established by light microscopic studies of G. L. Rasmussen.
2. To study ultrastructural alterations in the nerve cells deprived of synapses.

Methods Employed:

1. The afferent type endings are to be identified by ultrastructural alterations resulting from destruction of a spiral ganglion in chinchilla after different post-operative intervals and their time course degenerative process studied.
2. To study similarly the efferent endings of neurons

originating from higher auditory centers following destruction of the superior olive. Comparable areas of the cochlear nucleus of unoperated side is used as a control. The brain is to be perfused-fixed with osmium tetroxide to insure good material for electron microscopic study. The EM studies will be correlated with experimental results obtained from light microscopic studies.

Major Findings: Studies thus far on the normal anterior ventral nucleus reveal two distinct type of synapses based on size of vesicles. In experimental animals, a preliminary study reveals that the typical calciform endings of the cochlear nerve possessing the larger vesicles disappear 30 to 60 days postoperatively while the remaining unaltered synapses on the deafferented cell possess only the smaller vesicles. A preliminary report appears in the Anatomical Record, vol. 154, p. 408, 1966.

Significance: This study provides much needed information concerning ultrastructural alteration that accompany deafferentiation of nerve cells of the brain and particularly the identification of different functional types of nerve endings on the basis of ultrastructural dissimilarities.

Proposed Course of Project: This project is presently being carried on with Dr. Reese at the Harvard Medical School. Dr. Reese will return on duty with NIH September, 1966 and will extend this collaborative study in the Section on Functional Neuroanatomy with Dr. Rasmussen. Synaptology of the interstitial, posterior ventral and other subdivisions of the cochlear nucleus are to be studied.

Honors and Awards: None

Publications: None

1. Neuroanatomical Sciences
2. Section on Experimental
Neurology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Regeneration in the peripheral nervous system

Previous Serial Number: Same

Principal Investigator: Lloyd Guth

Other Investigators: R. Wayne Albers

Cooperating Units: Laboratory of Neurochemistry, NINDB

Man Years

Total:	.5
Professional:	.2
Others:	.3

Project Description:

Objectives: To study the factors influencing rate of regeneration and degree of reinnervation of peripheral tissues.

Methods Employed: (1) At various times after crushing the vagus nerve of the cat, the nerve was stimulated electrically. The farthest distance along the nerve at which an action potential could be recorded was taken as an estimate of the distance that the fibers had regenerated. The rate of nerve regeneration was computed by plotting the distance regenerated as a function of postoperative time. (2) Choline acetylase is an enzyme that is largely confined to the neural portion of the neuromuscular junction (in contrast to cholinesterase which appears largely on the muscular side of the neuromuscular junction). Studies are under way using a micromethod for enzyme determination to see whether the choline acetylase activity can be used to indicate the density of innervation in normal, partially denervated and in reinnervated muscle.

Major Findings: (1) The rate of regeneration of the vagus nerve of the cat is remarkably similar to that of the sciatic of the rat and the sural, tibial or peroneal of the rabbit. The rate of maturation of action potential amplitude is the same in the cat vagus as the rat sciatic and is unrelated to the amplitude that the nerve is ultimately capable of achieving.

(2) The micromethod of choline acetylase determination is a very sensitive one and readily applied to muscle homogenates. Within two weeks after denervation the choline acetylase activity falls to about 10% of normal in contrast to cholinesterase which remains at about 40% of normal at this stage.

Significance: (1) This is the most accurate method available for studying the rate of nerve regeneration, and investigators in other institutions are already using it to study the effects of lipid extracts and hormones on the rate of nerve outgrowth. (2) The study is in too early a stage to make predictions, but we are hoping to develop a technique that can be applied to the study and diagnosis of human diseases as well as one that will be useful to the more theoretical aspects of cell biology.

Proposed Course of Project: (1) This phase of the work has been completed and will be discontinued for the time being. (2) The choline acetylase study will be continued actively during the coming year.

Honors and Awards: None

Publications:

Guth, L. and Jacobson, S.: The rate of regeneration of the vagus nerve of the cat. Exper. Neurol. 14: 439-447, April 1966.

Serial No. NDB(I)-63 LNS/EN 1054

1. Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: "Trophic" functions of the peripheral nervous system

Previous Serial Number: Same

Principal Investigator: Lloyd Guth

Other Investigators: R. Wayne Albers

Cooperating Units: Laboratory of Neurochemistry

Man Years

Total:	2.8
Professional:	0.8
Others:	2.0

Project Description:

Objectives: To investigate the mechanism by which the peripheral nerve influences the metabolism of its end-organ.

Methods Employed: (1) The changes in cholinesterase activity of muscle were measured after cutting the nerve near or far from the muscle. The effects of physiotherapy on cholinesterase activity of denervated muscle were determined. Cholinesterase content of muscle after spinal transection was measured. (2) Protein patterns of normal, denervated, and cross-reinnervated red and white muscle were examined electrophoretically.

Major Findings: (1) Denervation produces a 60% loss in cholinesterase activity within one week of denervation. Little change ensues subsequently. The level at which the nerve was transected has no effect on the rate of loss of enzymatic activity. Physiotherapy did not retard the rate of loss of cholinesterase activity. Spinal cord transection also results in a significant diminution of cholinesterase activity but the effect is less severe than after a peripheral nerve lesion. During normal growth of muscles of adult rats the cholinesterase activity associated with the sole plates does not change,

whereas the activity of regions of muscle lacking sole plates increases in proportion to the growth of the muscle. (2) The soluble proteins of red and white muscle differ qualitatively and quantitatively. Following denervation the red muscle is unaltered whereas the white muscle assumes a pattern that is qualitatively and quantitatively similar to that of red muscle. When the red muscle is reinnervated by nerve fibers that normally supply a white muscle, the protein pattern becomes transformed to that characteristic of the white muscle. Tenotomy produces no change in protein pattern of either muscle.

Significance: (1) The changes in cholinesterase activity after these various operations are quite distinct from the changes in miniature endplate potentials or acetylcholine sensitivity after the same operations. Since acetylcholine appears to be the trophic agent responsible for regulating the MEPP's and the ACh sensitivity, it appears therefore that acetylcholine is not the neurohumor regulating the cholinesterase activity of muscle. (2) Eccles has shown that the nerve regulates the speed of contraction of muscles. The present experiments extend Eccles' hypothesis by showing that the nerve regulates the protein metabolism as well as the physiological activity of muscle. These results are consistent with Eccles' belief that the action of the nerve is a trophic one and is unrelated to the electrical impulse "traffic" of the nerve.

Proposed Course of Project: (1) These studies will be continued and extended during the coming year. (2) The electrophoretic analysis of cross-innervated muscle will be subjected to a more thorough analysis this year. Protein synthesis will also be studied directly using in vitro biochemical procedures to determine the biochemical lesion in denervated muscle.

Honors and Awards: None

Publications:

Guth, L. and Brown, W. C.: The sequence of changes in cholinesterase activity during reinnervation of muscle. Exper. Neurol. 12: 329-336, Aug. 1965.

Guth, L. and Brown, W. C.: Changes in cholinesterase activity following partial denervation, collateral reinnervation and hyperneurotization of muscle. Exper. Neurol. 13: 198-205, Oct. 1965.

Serial No. NDB(I)-66 LNS/EN 1303

1. Neuroanatomical Sciences
2. Section on Experimental
Neurology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Histochemical characteristics of muscle sensory systems

Previous Serial Number: None

Principal Investigator: Herbert Yellin

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives: To study morphological and histochemical characteristics of receptors in normal and atypical vertebrate muscle.

Methods Employed: Normal, denervated and tenotomized muscle are being studied by standard histological and histochemical techniques, particularly those demonstrating the disposition and relative levels of glycolytic and oxidative enzymes.

Major Findings: An enzymatic profile similar to that of the surrounding extrafusal muscle has been confirmed for muscle spindle intrafusal fibers of rat. Studies of the effects of denervation and tenotomy upon this histochemical profile are underway.

Significance: Muscle sensory organs in general, and intrafusal muscle of spindles in particular, are resistant to a wide variety of myopathies. The nature of the difference between extrafusal and intrafusal muscle is as yet undisclosed.

Proposed Course of Project: This project will continue a major portion of the coming year's work.

Honors and Awards: None

Publications: None

1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1964 through June 30, 1966

Project Title: The innervation of smooth muscle

Previous Serial Number: Same

Principal Investigator: Keith C. Richardson

Other Investigators: P. Taylor

Cooperating Units: Laboratory of Chemical Pharmacology, National Heart Institute

Man Years

Total:	1.0
Professional:	1.0
Others:	.0

Project Description:

Objectives: To study the ultrastructure of autonomic nerve endings on smooth muscle in collaboration with chemical and pharmacological investigations.

Methods Employed: Standard electronmicroscopical techniques of fixation and epoxy resin imbedding. Chemical estimation of radioactivity in living and fixed tissues following injection of H³-norepinephrine.

Major Findings: The ultra-structural identification of adrenergic autonomic axons by their content of granular (dense-cored) vesicles, as proposed in previous work on the rabbit iris, could not be confirmed by other workers for the rodent iris or the guinea-pig vas deferens in which, after osmium tetroxide fixation, only the agranular vesicles lacking a dense core were found. This discrepancy has now been cleared up by the use of potassium permanganate instead of osmium. The granular vesicles are found within axons adjacent to the dilator muscle of the rodent iris and within all regions of the guinea-pig vas deferens smooth muscle. Moreover the proportion of granular to agranular vesicles is very high in all tissues examined with this fixative, suggesting that artefactual extraction of the material within granular vesicles may occur

in very thin tissues like the rodent iris even with osmium tetroxide which is instantaneously reduced by catecholamines. Experiments are in progress on the rat heart to determine the degree of retention of H^3 -norepinephrine, administered intravenously 3-4 hrs. previously, by various fixatives. The results indicate so far that osmium tetroxide fixation retains about 63% of the exogenous NE, glutaraldehyde about 50% and permanganate 30-40%. The dense core of the granular vesicle must be preserved by permanganate by virtue of its protein and lipid content (as in cellular membranes) and not solely by its catechol amine content. Experiments are in progress to measure quantitatively the proportion of granular to agranular vesicles in vas deferens nerve endings following permanganate fixation as compared with osmium or glutaraldehyde fixation. The work outlined in Project NDB(I)-64 LNS/NC 1232 will have to be repeated with permanganate fixation.

Significance: While the fluorescence light microscopical technique of Falck and Hillarp provides a reliable histochemical identification of adrenergic nerves, there are many aspects of autonomic innervation which require study at the ultra-structural level, such as the intermingling of adrenergic and cholinergic axons within the individual strands of the autonomic ground plexus and the precise relationships of adrenergic varicosities and endings to the effector cells. The establishment of what appears to be a more reliable electronmicroscopical technique will further progress in this field.

Proposed Course of Project: To complete a series of studies on the fine structural effects of stimulation and of monoamine oxidase and COMT inhibition.

Honors and Awards: None

Publications: None

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The uptake of ferritin by the ependyma of the rat brain

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: None

This project has been completed and findings have been published.

Publications:

Brightman, M. W.: The distribution within the brain of ferritin injected into cerebrospinal fluid compartments. I. Ependymal Distribution. J. Cell Biol. 26: 99-123, July 1965.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The distribution within the brain of ferritin
injected intraventricularly

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: None

This project has been completed and findings have been
published.

Publications:

Brightman, M. W.: The distribution within the brain of
ferritin injected into cerebrospinal fluid compartments.
II. Parenchymal distribution. Am. J. Anat. 117: 193-220,
Sept. 1965.

Serial No. NDB(I)-65 LNS/NC 1230

1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Localization of monoamines in brain

Previous Serial Number: Same

Principal Investigator: Nicholas J. Lenn

Other Investigators: E. W. Maynert, University of Illinois

Cooperating Units: University of Illinois

Man Years

Total:	.75
Professional:	.75
Other:	0.0

Project Description:

Objectives: Nerve cells of various parts of the brain were studied in order to discover the morphological aspects of the localization of monoamine within these cells. There were no special ultra-structural characteristics in normal and drug-treated animals. Therefore, autoradiographic studies have been started to localize the sites of incorporation of exogenous norepinephrine and serotonin.

Methods Employed: Rat brain tissue has been fixed with solutions of osmium tetroxide or glutaraldehyde, and embedded in epoxy resin. Thicker sections are studied with the light microscope after alkaline toluidine blue staining. Thin sections from selected regions were examined with the electron microscope after staining with heavy metal salts. For autoradiography, thicker sections are mounted on glass slides, and thin sections on copper grids, coated with Ilford L-4 emulsion, exposed for varying periods, and developed. The specimens for autoradiography include brain from animals receiving intraventricular norepinephrine, brain slices incubated in vitro in norepinephrine, and nerve ending particle preparations (with Dr. Maynert) incubated with serotonin.

Major Findings: The normal fine structure of the locus coeruleus, nucleus dorsalis raphes, area ventralis tegmenti, and

substantia nigra, with particular attention to the neuronal perikarya of these regions, has been elucidated. Statistical estimates of the number of granular vesicles in the nucleus dorsalis raphe before and after pargyline administration have been made on the basis of random samples. Preliminary results of the autoradiographic studies indicate neuronal localization of norepinephrine in brain slices, and localization of serotonin to a limited proportion of nerve ending particles.

Significance: The association of monoamine content with electron microscopically demonstrable structures is well established in the peripheral nervous system and the adrenal medulla. The usefulness of autoradiography in confirming this association was established in previous studies from this laboratory. The possibility that monoamines in neuronal perikarya of the central nervous system are associated with other cytoplasmic structures is important in questioning any generalization about the storage forms of monoamines. The autoradiographic studies help to elucidate the initial movements of the compounds in question, and thus provide understanding of their later biochemical and pharmacological fate, as revealed by other methods.

Proposed Course of Project: The studies described above will be discontinued as Dr. Lenn will be leaving the NIH in June 1966.

Honors and Awards: None

Publications:

Lenn, N. J.: Electron microscopic observations on monoamine-containing brain stem neurons in normal and drug-treated rats. Anat. Rec. 153: 399-406, Dec. 1965.

Serial No. NDB(I)-65 LNS/NC 1231

1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The fine structure of the olfactory bulb.
I. Glomerulus

Previous Serial Number: Same

Principal Investigator: Thomas Reese

Other Investigators: Milton W. Brightman

Cooperating Units: None

Man Years

Total:	.45
Professional:	.20
Others:	.25

Project Description:

Objectives: To study the fine structure of the olfactory bulb in the rat.

Methods Employed: Olfactory bulbs were fixed with buffered osmium tetroxide and glutaraldehyde by vascular perfusion. Thin sections were examined by light and electron microscopy.

Major Findings: A hitherto undescribed synapse, viz., dendro-dendritic, has been found in the glomerulus and external plexiform layer. The envelopment of one small periglomerular neuron by the flattened soma of an adjacent neuron is a second novel feature.

Significance: The observations have already provided a morphological basis for such phenomena as lateral inhibition in the olfactory bulb and, it is anticipated, may be repeated in other sensory systems.

Proposed Course of Project: Completion of the manuscript for publication and systematic examination of the other strata in the olfactory bulb.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 LNS/NC 1232

1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The structural effects of the monoamine oxidase inhibitor Pargyline on the nerve endings of rat vas deferens

Previous Serial Number: Same

Principal Investigator: Keith C. Richardson

Other Investigators: I. A. Michaelson

Cooperating Units: Laboratory of Chemical Pharmacology,
National Heart Institute

This project is being held in abeyance as the work will have to be repeated with permanganate fixation.

1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Pinocytosis in the Brains of dead rats

Previous Serial Number: None

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.55
Professional:	.80
Others:	.75

Project Description:

Objectives: To determine the minimal amount of structural integrity commensurate with pinocytosis.

Methods: The carcasses of exsanguinated rats were kept at about 5 C for 1, 5, and 13 hours before immersion in water at 37 C for an additional hour, during which ferritin was perfused through the cerebral ventricles. Attempts to inhibit pinocytosis with 10^{-3} M solutions of iodoacetate or n-ethylmaleimide were made in several live animals. The cerebral tissue was examined electronmicroscopically.

Major Findings: Somatic death did not abolish pinocytosis. Many ependymal cells were not only structurally intact, but viable as manifested by their very large number of ferritin-laden pinocytotic vesicles and vacuoles. Glial and neuronal processes also contained such vesicles. The metabolic poisons did not abolish pinocytosis but did severely damage the ependymal cells. In these cells and in those undergoing autolytic changes, some of the ferritin-containing vacuoles may not be pinocytotic but artifactual, having been formed de novo from membrane fragments.

Significance: Anoxic cells maintained at low temperature and in situ are still capable of the vital function of pinocytosis.

Proposed Course: To ascertain in this material and in pure membrane systems the origin of membrane-bounded structures enclosing ferritin and to so define the minimal morphological requirements for pinocytosis.

Honors and Awards: None

Publications: None

1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Normal fine structure of nerve endings in the nucleus of the trapezoid body and ventral cochlear nucleus

Previous Serial Number: None

Principal Investigator: Nicholas J. Lenn

Other Investigators: T. S. Reese

Cooperating Units: None

Man Years

Total:	.25
Professional:	.25
Other:	0.0

Project Description:

Objectives: The nucleus of the trapezoid body and the anterior ventral cochlear nucleus, and to a much lesser extent certain other limited portions of the bulbar auditory system of mammals, are distinguished by the presence of large, calyciform axonal endings, as well as other smaller nerve endings. The fine structure of these unusual synaptic structures had not previously been investigated.

Methods Employed: Normal rats, chinchillas, and a cat were fixed by perfusion with osmium tetroxide. The regions of interest were obtained by dissection, and embedded in epoxy resin. Thicker sections were studied with the light microscope after alkaline toluidine blue staining. Thin sections from selected regions were examined with the electron microscope after staining with heavy metal salts.

Major Findings: The processes of the calyciform endings were readily identified by their large size and the multiple synaptic contacts they formed with proximal dendrites and perikarya of neurons. The individual synaptic contacts were typical of neurochemically mediated synapses of the mammalian nervous system. However, when compared to the other nerve

endings of the region, the calyciform process was found to have larger synaptic vesicles.

Significance: Although the physiological significance is not completely clear, the unusual arrangement of a single axon making neurochemical contact with a post-synaptic neuron at a large number of points, as described above, associated with large diameter of this axon preterminally, undoubtedly represents an important specialization of the mammalian auditory system. That these afferent axons contain larger synaptic vesicles than the smaller, presumably efferent, endings suggests that facilitation and inhibition are mediated by different neuro-transmitters in this system.

Proposed Course of Project: This project is completed and is to be published.

Honors and Awards: None

Publications:

Lenn, N. J. and Reese, T. S.: The fine structure of nerve endings in the nucleus of the trapezoid body and the ventral cochlear nucleus. Am. J. Anat. 00:000,000 1966.

1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Morphogenesis of the inner ear.

Previous Serial Number: SAME

Principal Investigator: Robert J. Ruben

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.3
Professional:	1.0
Others:	0.3

Project Description:

Objectives: To determine the distribution in time and space of the terminal mitoses in populations of 18 cell types in the developing inner ear of the mouse.

Methods employed:

1. Single pulses of tritiated thymidine were injected into pregnant mice at known times during gestation. After they had matured the inner ears of the offspring were analysed autoradiographically.
2. For purposes of autoradiography the inner ears were embedded and sectioned serially by a technique especially adapted in this laboratory.
3. The mouse cochlea was reconstructed from serial sections in a two dimensional projection. On such projections the distribution of labeled and unlabeled cells was mapped.
4. A specially designed computer program was used to follow changes in cell population dynamics during embryological development of the inner ear.
5. Genetically inbred mice were maintained for this study. It is also planned to make use of a group of mutant strains of mice in which the inner ear is affected.

Major Findings:

1. The period of development during which the terminal mitoses occurred was shorter in the cochlea than in the vestibular apparatus.

2. In the cochlea receptor cells, supporting cells and ganglion cells did not divide after the third postpartum day. The connective tissue and Schwann cells continued to divide until at least 7 days after birth.

3. Two spatial patterns of terminal mitosis were discovered in the cochlea. The wave of terminal mitosis of the haircells and their supporting cells begins at the apex of the cochlea on the twelfth day of gestation and sweeps down the cochlea, reaching the base on the 16th day of gestation. The wave of terminal mitosis in the spiral ganglion cell population, in contrast, starts at the base on the 12th day of gestation and ceases at the apex on the 15th day of gestation.

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

1. Differences in the temporal and spatial patterns of terminal mitosis in the several cell populations of the inner ear during embryonic development raise interesting possibilities concerning the sequence in which these cell groups affect one another during morphogenesis of the inner ear. Some of these possibilities can be tested experimentally.

2. The difference in the distribution of terminal mitoses in time in the cochlea and vestibular apparatus suggest a much more circumscribed period during embryonic development of the cochlea during which teratologic agents may produce major auditory defects than is true for the vestibular apparatus in which terminal mitoses occur over a much longer period.

3. The fact that the wave of terminal mitoses in the populations of cochlear cells of several types proceeds along the basilar membrane in a direction opposite to the subsequent wave of cellular differentiation is an unexpected finding whose explanation will require further work.

4. The techniques developed, and the results obtained, during this study open the way for similar analyses of approximately 25 known point mutations in the mouse, each of which probably affects the inner ear in a slightly different way. Such studies may yield information concerning the paths of action of each of the genes.

Proposed Course of the Project:

1. This phase of the work has been completed, and the project has been successfully terminated. The results are currently being prepared for publication. Subsequent work with normal and mutant mice will be carried on by the principal investigator at a different institution.

2. The methods and information developed in this study will be applied to an analysis of the causal sequences which operate during the development of the inner ear of the mammal.

Honors and Awards: None

Publications: None

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Ocular Morphogenesis

Previous Serial Number: SAME

Principal Investigator: Alfred J. Coulombre

Other Investigators: Jane L. Coulombre, Paul N. Anderson and Edward R. Wolpow.

Cooperating Units: None

Man Years:

Total:	5.0
Professional:	4.0
Others:	1.0

Project Description:

Objectives: During the past year the objectives of this project have been expanded. This is reflected in the list of objectives below:

1. characterize the species specificity of the retina-lens factor;
2. determine the relative ability of neural retinae of different embryonic ages to support lens growth and differentiation and to maintain lens orientation;
3. test the ability of neural tissues other than retina to support the differentiation and growth of the embryonic lens of the chick;
4. use the in vitro test system which was developed in this laboratory to characterize and to attempt to isolate the retina-lens factor;
5. conduct a series of investigations similar to those listed above with respect to the retina-pigmented epithelial factor which was demonstrated in this laboratory during fiscal year 1965;
6. develop mathematical techniques for accurately describing the shape of the embryonic lens, and for quantitating changes in shape as development proceeds;

7. develop and apply accurate techniques for the measurement of changes in lens volume and cell number during the development of the lens of the chick embryo;
8. measure changes in total protein of the lens as a function of age.

Methods Employed:

1. Microsurgery of chick embryos will be used to remove, increase the amount of, or experimentally rearrange the relationships between, the several tissues of the developing eye.
2. Tissue and organ culture will be employed in assessing the interactions between pairs of intraocular tissues derived either from different parts of the eye, from different stages in development, or both.
3. Routine histological and histochemical procedures will be employed to determine the results of these procedures.
4. Autoradiographic analysis will be conducted on ocular tissues which have previously been pulse labeled with tritiated thymidine.
5. A combination of photographic and mathematical manipulations will be adapted to the accurate measurement of the volume of the lens as a function of age.
6. The Lowry technique will be used to determine the amount of protein in the embryonic lens as a function of its age.
7. Appropriate sampling techniques will be developed which will permit accurate estimates to be made of the numbers of cells in each of the lens cell populations (epithelium, Ringwulst and lens fiber mass), and to determine in what way these cell populations vary with embryonic age.

Major Findings:

1. The volume of the lens of the chick embryo increases exponentially throughout the period of incubation. From at least 5 days of incubation onward there is a progressive decrease in the rate of growth.
2. The increase in the volume of the lens during development is brought about by an increase in the number of its cells, and by an increase in the mean volume of the lens cells. The lens fiber mass contributes most importantly to the increase in lens volume. The lens fiber mass contains between 20,000 and 30,000 fibers on the fifth day of incubation. New lens fibers are recruited into the lens fiber mass from the Ringwulst, rapidly at first, and progressively more slowly at older ages. By the 20th day of incubation there are nearly 300,000 fibers in the lens fiber mass.

3. The enlargement of lens cells to become lens fibers is brought about, at least in part, by synthesis within them of increasing amounts of lens protein. Accurate measurements of the total amount of lens protein as a function of embryonic age reveal an exponential increase during development. The amount of lens protein per unit volume of lens increases with age. This increase in protein concentration in the lens is accompanied by a decrease in water content and an increase in specific gravity.

4. The increase in the volume of the growing lens is, in part, under the control of the neural retina. Lens growth ceases when the neural retina has been removed, and is sustained when the neural retina is removed and then replaced. It remains to be determined to what extent this control is exerted by controlling the number of lens cells which enter the lens fiber population, and to what extent growth is controlled by regulating the volume of the individual cells (e.g. by controlling such processes as protein synthesis).

5. It had previously been demonstrated that well formed lenses develop from the lens epithelium alone when this tissue is substituted for the lens. During the past year it was demonstrated that the ability of the lens epithelium to respond in this way to the eye environment decreases progressively with advancing age.

6. Previous investigations showed that both the size and orientation of the developing lens are under the control of other ocular tissues, especially the neural retina. The shape of the lens is also precisely controlled. During the past year it was possible for us to quantitate the shape of the lens in several ways using specially developed mathematical formulations. As a result it has been shown that the shape of the lens changes progressively and systematically with age in such a fashion that its posterior surface becomes more and more convex relative to the anterior curvature of the lens. This quantitative descriptive study of the development of lens shape is preliminary to an analysis of the factors which control lens shape during embryonic development.

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

1. A battery of descriptive and experimental techniques is being brought to bear on the developing lens so that, step by step, we are learning in some detail the mechanisms by which its size, shape and orientation are controlled during embryonic development.

2. While the present phase of this complex investigation has focused upon the lens considered as a target of influence of the other ocular tissues, it is our ultimate goal to consider each of the ocular tissues in turn as both a source of influence on other tissues and as a target of influence exerted by surrounding tissues. The flow sheet of ocular development which is resulting from such studies opens the way to elective control of specific phases of eye development, and provides a rational basis for understanding developmental abnormalities of this organ.

Proposed Course of the Project: To pursue the objectives defined above over a period of at least several years.

Honors and Awards: None

Publications:

Coulombre, A. J.: The Eye. In DeHaan, R. L. and Ursprung, H. (Eds.) Organogenesis. New York, Holt, Rinehart and Winston, Inc., 1965, Chap. 9, pp. 219-251.

DeLong, G. R. and Coulombre, A. J.: Development of the Retinotectal Topographic Projection in the Chick Embryo. Exp. Neurol. 13: 351-363, Dec. 1965.

Coulombre, A. J. and Herrmann, H.: Lens Development. III. Relationship between the Growth of the Lens and the Growth of the Outer Eye Coat. Exp. Eye Res. 4: 302-311, Dec. 1965.

Serial No. NDB(I)-65 LNS/EE 1235

1. Neuroanatomical Sciences
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Histogenesis of the neural portions of the visual system as related to the onset of function.

Previous Serial Number: SAME

Principal Investigator: Alfred J. Coulombre

Other Investigators: Paul N. Anderson and Edward R. Wolpov

Cooperating Units: None

Man Years:

Total:	0.0
Professional:	0.0
Others:	0.0

Project Description:

This project was outlined at the time of the last annual report in anticipation of the arrival of two Research Associates (Drs. Paul N. Anderson and Edward R. Wolpov) on 1 July 1965. Following the arrival of these two Public Health Service Officers it was decided not to undertake this project, but to have these two investigators devote their attention to more urgent and more promising undertakings related to the project on ocular morphogenesis (Serial No. NDB(I)-65LNS/EE 1234). Because of this decision, the present project was terminated without having been begun.

Annual Report
July 1, 1965 through June 30, 1966
Laboratory of Neuropathology
National Institute of Neurological Diseases and Blindness

The Laboratory of Neuropathology, represented by the Section on Experimental Neuropathology, has been engaged in four series of investigations as part of a long term program.

(1) Perfection of Method of Preservation. Since only perfectly fixed tissues are useful for microscopic investigation, current techniques were critically surveyed. In the course of routine preservation by the procedure of perfusion via the ascending aorta, an intracardial influx of air during the operative procedure was detected. Such air was prevented from entering the systemic circulation by filling the chest cavity and performing all operations with the heart submerged. These precautions ensure proper preservation of experimental material which otherwise might be lost. A further check of various steps disclosed that by this submerged heart method the time required for completion of the fixation can be reduced to about one hour, while four or more had been previously recommended.

(2) Identification of Artifactual Changes. Since in the course of histologic preparation a variety of artifactual changes are introduced, it is important to identify them so that they will not be erroneously interpreted as significant changes. A microscopic survey of paraffin sections disclosed that in otherwise well preserved tissues, the nucleoli were dislodged and displaced from their original sites in 1-2 per cent of all neurons. The affected neurons were situated along the surface of the microscopic sections and the nucleoli were always expelled in the direction of cutting; therefore, the mechanism was related to the mechanical action of the microtome blade. These results invalidate theories implicating such expulsion as a mechanism by which nucleoli can participate in cytoplasmic protein synthesis.

(3) Pathologic Neuronal Manifestations. Since clinical neuropathologic concepts are based on the study of material preserved by immersion of the brain and spinal cord in various fixatives, a series of experiments has been planned for the purpose of establishing in perfused fixed material the nature and specificity of pathologic neuronal changes. The study of serial sections of brain stem from animals with severed facial nerve disclosed in some species diminution of ribonucleic acid stainable material beginning at the periphery of the motor neuron, but such an acute retrograde change was found to be of different intensity and quality in various species. These results suggest that the reaction of neurons as manifested morphologically is influenced by several factors, among which degree of protein synthesis and

intensity of cell metabolism may play a role. These species variations in neuronal reaction must be taken into consideration before a diagnosis of acute retrograde neuronal changes can be made and before the cellular changes observed with other techniques can be correctly interpreted.

(4) Identification of Cell Types. As a basis for determining the morphologic criteria of physiologic and pathologic reactions of the central nervous system, the microglia cells and oligodendrocytes were scrutinized. The development of a quadruple staining method permitted a consistent and selective staining of these two cell types in their entirety, as well as of blood vessels, neuronal perikarya and myelinated fibers.

(a) A study of the appearance, distribution and spatial relationship of each cell type disclosed that there are considerable regional and species variations in the morphology of microglia cells, suggestive of differences in their function. (b) The extraordinary shape of microglia cells in such regions as the ventricular wall was, in accordance with a newly formulated hypothesis, associated with local histologic factors which affect the development of these cells. (c) The nature of cell changes around encephalitic foci suggests that current concepts about the reaction of microglia in pathologic material must be revised. (d) The permanence of these cells with aging indicates that whatever function they may serve is not significantly altered during senescence. - Although the function of microglia cells is still obscure, their affinity to neurons, myelinated fibers and ependymal cells suggests that they are concerned with the metabolic requirements of the normal central nervous system. A correct appreciation of the degree of interdependence must await the results of intensive investigations with more elaborate and sophisticated techniques.

1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Histochemical study of nerve cells

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To discern the distribution of glycogen-containing neurons.

Methods employed: Rabbit brains are fixed in situ and prepared according to methods developed in this Section.

Major findings: There are through the spinal cord and brain stem two types of motor nerve cells which are distinguished by varied histochemical composition.

Significance: The identification of biochemical differences between neurons may give a basis for understanding functional and pathologic differences in reaction.

Proposed Course of Project: Microscopic examination of the histologic material which has been prepared by various histochemical enzyme digestion methods.

Honors and Awards: No

Publications: No

1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The effect of aging on the central nervous system.

Previous Serial Number: SAME

Principal Investigator: Alden W. Dudley, Jr.

Other Investigators: Robert S. Ledley and Marilyn Belson,
National Biomedical Research Foundation

Cooperating Units: National Biomedical Research Foundation
Silver Spring, Maryland

Man Years

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To establish what effect aging may have on cerebral neurons of the rabbit.

Methods employed: Computer method for measurement of neuronal perikarya and nuclei.

Major findings: A computer procedure is developed for measuring diameter, area and volume of neuronal perikarya and nuclei.

No significant age differences are demonstrable in the size of neurons.

Significance: Aging does not affect size of neurons.

Proposed Course of Project: This project is completed. A manuscript with description of the computer method is ready for publication. Manuscripts on the results of microscopic examination and of measurements with the computer method are being prepared.

Honors and Awards: No

Publications: No

1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1964 through June 30, 1965

Project Title: Hematologic control of primates.

Previous Serial Number: SAME

Principal Investigator: Mrs. Margaret G. Johnson

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.6
Professional:	0.0
Other:	0.6

Project Description:

Objectives: To clarify the reason for poor health in primates.

Methods employed: Different primate species are regularly examined by various hematologic techniques; weight and dietary regimen are controlled.

Major findings: Many of the primates suffer from severe infections and aberrations of blood composition.

Significance: A careful hematologic examination is required prior to subjecting primates to experiments.

Proposed Course of Project: Regular check of primates and control of infectious condition.

A long term study of the hematologic status in healthy animals.

A long term study of the effect of varied dietary regimen and adjuvants on the hematologic status.

Honors and Awards: No

Publications: No

Serial No. NDB(I)-63 LN/EN 1063

1. Neuropathology
2. Section on Experimental
Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The reaction of mesodermal cells in the central nervous system during senescence.

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

This project is completed.

1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Perfection of the perfusion technique for fixation in situ.

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.4
Professional:	0.3
Other:	1.1

Project Description:

Objectives: To eliminate failures in fixation and to specify conditions under which perfect fixation can be obtained.

Methods employed: Deeply anesthetized animals were perfused via the heart, first with a saline solution and then with various coagulating fixatives. The operation was performed with the chest cavity filled with fluid and the heart submerged.

Major findings: By this procedure, air influx during the operation was prevented.

The interval between perfusion and dissection can be significantly shortened.

Significance: Consistent fixation of material is achieved, whereby the incidence of imperfect fixation is minimized and valuable histologic material is saved.

The time required for preparation of material is speeded up, whereby experimental material can be scrutinized microscopically within a short time.

Proposed Course of Project: To investigate the effect of varied conditions of perfusion on the adequacy of fixation of the brain.

Honors and Awards: No

Publications:

Cammermeyer, J.: Submerged heart method to prevent intracardial influx of air prior to perfusion fixation of the brain. Acta Anat. 000: 000-000, 1966.

1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Acute degenerative changes in the central nervous system.

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total: 0.0

Professional: 0.0

Other: 0.0

Project Description:

Objectives: To define the sequence of changes in well-fixed materials.

Methods employed: Experimental microembolization is carried out in various animal species which at different post-operative stages are sacrificed by the perfusion procedure developed in this Section.

Major findings: Minute lesions are distributed in a manner which varies for each animal species and the operative procedure.

Significance: A study of tissues undergoing acute degenerative changes in experimental material prepared according to strict scientific standards will form a basis for a correct appreciation of neuronal changes encountered in clinical neuropathologic material.

Proposed Course of Project: Microscopic examination is awaiting the histologic preparation of already completed experiments.

Honors and Awards: No

Publications: No

1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Cytologic characteristics of microglia cells.

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	2.0
Professional:	0.4
Other:	1.6

Project Description:

Objectives: To define the cytologic characteristics of microglia cells under normal and pathologic conditions.

Methods employed: Microscopic sections of perfused fixed brains are stained by a method for the simultaneous demonstration of blood vessels, neurons and myelin sheaths.

Various animal species were selected, and some of them were treated with different drugs.

Major findings: The microglia cells are not significantly influenced by the aging process, length of narcosis, or treatment with cortisone or reserpine.

They display severe changes around encephalitic foci.

There is a considerable regional and species variation in their appearance.

Significance: Since these cells occur profusely throughout the central nervous system it is expected that they play an important role in the normal function of the central nervous system. It is hoped that systematic studies may give some insight into the nature of such a role.

Proposed Course of Project: To analyze the reaction of these cells under varied functional and dietary conditions.

Honors and Awards: No

Publications:

Cammermeyer, J.: The hypendymal microglia cell. Z. Anat. Entwicklungsgesch. 124: 543-561, 1965.

Cammermeyer, J.: Morphologic distinctions between oligodendrocytes and microglia cells in the rabbit cerebral cortex. Amer. J. Anat. 118: 1-21, January 1966.

1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A comparative study of acute retrograde neuronal changes

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total: 0.7

Professional: 0.1

Other: 0.6

Project Description:

Objectives: To establish whether acute retrograde changes of motor neurons are similar in all species.

Methods employed: Cranial motor nerves were cut in different animal species. Serial sections of the brain stem were stained by a combined PAS-galloycyanin method.

Major findings: The distribution of Nissl substance is altered in the acute stage. According to already completed studies of both rabbits and mice, in motor neurons the initial loss of Nissl substance begins at the cell periphery.

The manner of alteration is different in various species.

The manner of alteration is the same in motor neurons of different regions within each species.

Significance: The varied pattern of alteration in different species suggests that factors involved in the cellular reaction are not the same in all. It appears to be influenced by the rate of cellular metabolism. These differences must be recognized when studies concerned with submicroscopical and histochemical changes in retrograde neuronal reaction are being investigated.

Proposed Course of Project: A microscopic analysis and photometric examination of the histologic material.

Honors and Awards: No

Publications: No

1. Neuropathology
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Extranuclear expulsion of nucleoli.

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objectives: To determine the cytologic characteristics of nucleoli situated outside neurons.

Methods employed: Paraffin embedded material of animals treated in different ways was cut and stained. The position of the nucleolus was determined in cell populations of 1000-2000 neurons.

Major findings: The nucleoli are expelled in the direction of cutting and the affected nuclei are situated along the surface of microscopic sections, suggesting that the blade of the microtome is the cause of this cytologic anomaly.

Significance: Only when a distinction is made between artificial and true expulsion of nucleoli may it be possible to determine whether such a mechanism is active in intracytoplasmic synthesis as postulated by many authors. According to present results, when paraffin material is studied, the artificial mechanical displacement of nucleoli introduces an error which invalidates the hypothesis of an intra-vitam expulsion.

Proposed Course of Project: Completed

Honors and Awards: No

Publications:

Cammermeyer, J.: Artifactual displacement of neuronal nucleoli in paraffin sections. J. Hirnforsch. 000: 000-000, 1966.

Annual Report of the
Laboratory of Neurophysiology, Intramural Research
National Institute of Neurological Diseases and Blindness

July 1, 1965 through June 30, 1966

Wade H. Marshall, Ph.D., Chief

Synaptic mechanisms continue to be a major subject of study in the Spinal Cord Section. The manner in which unitary or quantal synaptic events are combined to form the larger evoked synaptic potentials has been elucidated for spinal motoneurons. Afferent fibers from muscle spindles make monosynaptic connections with motoneurons which are widely distributed over the surface of the motoneurons. Different afferent nerves may have somewhat different patterns of termination and the interaction between synaptic activity produced by stimulation of different nerves is influenced by the dendritic distribution of their terminations. Each nerve ending may produce from one to several unitary, quantal synaptic events when the ending is activated by an action potential. Even synaptic contacts in the periphery of the dendritic tree provide a significant excitatory depolarizing effect in the cell soma near the spike trigger zone. A member of the Section on Mathematical Biophysics, NIAMD, has developed a quantitative theory of the role of neuronal dendrites and collaboration with him has contributed to this analysis of synaptic and dendritic physiology.

Experiments are beginning on the trophic effects of nerves and the long-term consequences of alteration in the activity in nerves. Chronic recording of muscle action potential has been achieved and the effects of muscle tenotomy on spinal reflex and muscle twitch time have been studied. The results are as yet preliminary.

Pacemaker activity in molluscan nerve cells has been studied using the voltage clamp technique. Pacemakers and non-pacemakers differ in their response to prolonged depolarizing potentials. The pacemaker potentials have been clearly shown to be endogenously generated in the pacemaker cell itself.

Sensory processing in the cochlear nucleus has been studied in anesthetized and in unanesthetized decerebrate cats. Relatively simple sensory mechanisms predominate in the cochlear nucleus in general, although we have demonstrated differences between the dorsal and ventral divisions of the nucleus. A marked alteration in response patterns of neurons in the primary auditory nucleus is produced by barbiturates, emphasizing the distortion in neural properties introduced by this class of drugs.

Summary, concl.

The primary events in photoreception in the Limulus eye have been analyzed by electrophysiologic means and described in terms of an electronic analogue. Possible relationships between the observed electrical events and various ions and photopigments have been hypothesized. Further experiments to test these hypotheses are planned.

Serial No.: NDE(I)-58 LNP/SC-501
1. Laboratory of Neurophysiology
2. Section on Spinal Cord
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Generation of impulses in nerve cells

Previous Serial Number: SAME

Principal Investigators: B.O. Alving and P.G. Nelson

Other Investigators: W.H. Fryegang, Jr.¹, S.I. Rappaport², and
A. Bak³

Cooperating Units: 1/2/: Section on Membrane Physiology,
LNP/NIMH
3/: Electronics Section, LNP/NIMH

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: The objective of this research was to examine in detail the differences in the properties of neurons which initiate spikes spontaneously (pacemakers) in contrast to those which generate spikes only in response to excitation from some external source.

Methods employed: Somata of pacemaker and non-pacemaker neurons from the visceral ganglion of *Aplysia* were isolated from the rest of the ganglion by ligation of axon hillocks, and transmembrane potentials were recorded with an intracellular microelectrode. Analysis of intracellular ion concentrations was attempted by removing the somata of pacemaker and non-pacemaker neurons from the ganglion and subjecting these isolated somata to activation analysis at Oak Ridge National Laboratories. Voltage-dependent changes in membrane permeability were determined using the voltage clamp technique. The inside-to-outside impedance of both types of cells was found as a function of frequency by passing sinusoidal currents into the cell with one intracellular electrode and measuring transmembrane potential changes with another intracellular electrode.

Major findings: The isolated somata of pacemaker neurons were demonstrated to be capable of spontaneous activity while the isolated somata of non-pacemakers generated spikes only in response to electrical or chemical excitation. The current-voltage relations of pacemaker and non-pacemaker neurons are different. The two types of neurons differ primarily in their response to long duration depolarizing clamping pulses. The pacemakers show a shift in the apparent equilibrium potential not shown by the non-pacemakers. We believe this shift in the equilibrium potential is the result of a time and voltage dependent difference in the membrane permeability of pacemaker neurons which can be demonstrated with depolarizing voltages of long duration. No difference was found in the inside-to-outside impedance of pacemakers and non-pacemakers.

Significance to Bio-Medical Research and the Program of the Institute: The question of what determines the ability of some neurons to initiate spikes spontaneously is of increasing importance in neurophysiology. The presence of pacemaker neurons in the mammalian central nervous system has not been conclusively demonstrated partly because of the lack of adequate criteria for distinguishing between pacemaker and non-pacemaker neurons. The demonstration that the isolated somata of pacemakers can initiate spikes spontaneously provides conclusive evidence that some neurons have an intrinsic pacemaking property. The finding that the current-voltage relation of pacemakers and non-pacemakers are different defines an additional difference in the membrane properties of pacemakers and non-pacemakers which may be related to the mechanism by which pacemaker neurons initiate spikes spontaneously.

As the inside-to-outside impedance of the two types of neurons is the same, the pacemaking property of some neurons cannot be explained on the basis of passive electrical characteristics of the membrane.

Proposed course of project: The attempt to determine intracellular ion concentrations of the two types of neurons will continue; both activation analysis of the whole cell bodies and microspectrophotometric analysis of the neuroplasm will be carried out. The ions concerned in the generation of the spike and in pacemaker activity of the neurons will be determined with the voltage clamp technique. The equilibrium potential will be measured when the activity of the pacemaker neurons has been altered by physical means (light and temperature) to determine whether the shift in the equilibrium potential can be correlated with the activity of the pacemakers.

Honors and awards: NONE

Publications:

Nelson, P.G.: Interaction between spinal motoneurons
of the cat. J. Neurophysiol. 29:275-287, Mar. 1966.

Serial No.: NDB(I)-62 LNP/SC-934
1. Laboratory of Neurophysiology
2. Section on Spinal Cord
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Basic mechanisms of synaptic transmission

Previous Serial Number: SAME

Principal Investigators: R. Burke, F.F. Hiltz¹, P.G. Nelson,
N. Robbins, K. Frank

Other Investigators: W. Rall², R. Wurtz, M. Chapman

Cooperating Units: 1/ Applied Physics Laboratory, Johns Hopkins
University

2/ Office of Director, Intramural Research,
NIAMD

Man Years:

Total:	4.5
Professional:	3.0
Other:	1.5

Project Description:

Objectives: Three aspects of synaptic transmission have received attention in the past year: (1) The elementary or unitary synaptic events which are the components of the evoked postsynaptic potentials studied earlier have been analyzed in some detail. The synaptic activity produced by single afferent fibers has been described, evidence for the location of different synapses on the cell has been obtained and statistical analysis made of the unitary synaptic activity evoked by natural sensory stimulation. (2) Experiments have been begun attempting to demonstrate and analyze some of the long-term trophic properties associated with the synapse. Tenotomy of muscle has been used as a means of producing long-term alteration in nerve and muscle activity. Spinal reflexes have been used to test the synaptic function of nerves from these tenotomized muscles. (3) In molluscan neurons, a specific hypothesis as to cellular mechanism for conditioning has been tested.

Methods employed: Conventional electrophysiologic techniques of intracellular and reflex recording have been used in these experiments.

Major findings: (1) The synaptic events produced by the firing of single group Ia muscle spindle afferents have been analyzed and this analysis has confirmed the concept of a quantal basis for excitatory synaptic transmission in the mammalian central nervous system. Examination of the waveforms of Ia afferent evoked unitary synaptic events has indicated that different Ia fibers terminate on a widely differing loci on the motoneuron surface including the remote dendrites. In contrast to some previously held views, the distally located synapses may be quite effective in depolarizing the neuron. These results have been analyzed in relation to a general theory of dendritic function developed by Rall. Quantitative interpretation of the data has been made possible with an explicit statement of all the assumptions that are necessary for such interpretation. The statistical analysis of naturally evoked summated synaptic activity is still incomplete, but preliminary results indicate that the discharge pattern is the pooled output of multiple rhythmic sources, a situation which is amenable to analytical determination of the number of sources involved. (2) The monosynaptic reflex from the nerves of tenotomized muscle is increased and the latency of the reflex is decreased. This is confirmatory of other results in the literature. (3) The occurrence or absence of postsynaptic cell firing has been found to have no influence on synaptic efficacy when spikes and synaptic potentials are produced together in time.

Significance to Bio-Medical Research and the Program of the Institute: (1) Dendritic location of synapses has great theoretical significance in the calculation of neuronal input-output relations, particularly when dealing with an asynchronous time-series input such as is represented by stretch-evoked synaptic activity. Quantitative determination of: (a) the number of afferent sources, (b) their average frequency and pattern of firing, and (c) the locus of synaptic input to a motoneuron will permit a much more informed theoretical model of neuronal integration of synaptic information than has been possible to date. (2) The reflex studies following muscle tenotomy indicate that the effectiveness of synapses may be affected by changes in the long-term activity of those synapses. What specific changes in afferent activity occur with tenotomy is not known at present, however, so detailed interpretation of the results is not possible.

Proposed course of project: (1) The relationship between various important aspects of the motor reflex arc will be investigated. The amplitude and apparent dendritic locus of miniature EPSPs, post-synaptic characteristics of the motoneuron, such as membrane

resistance, conduction velocity, and size of motor unit controlled, will be examined in order to approach the problem of cell size as a determinant of reflex behavior. Statistical studies of EPSP occurrence pattern to stretch will be continued, and eventually extended to a quantitative study of the relationship between the patterns of synaptic input activity and the spike output of the cell. Rhythmic EPSPs will be used as a test for quantitative study of the mechanisms of post-tetanic potentiation and presynaptic inhibition in the mammalian CNS. (2) The mechanism involved in the increased spinal reflexes which occur from tenotomized muscles will be analyzed. Changed motoneuron threshold, increased synaptic efficacy, or changed nerve properties might all be involved. Recordings of nerve and muscle activity in normal and tenotomized animals are planned in order to specify what the changes in activity are which produce the reflex changes.

Honors and awards: NONE

Publications:

Burke, R.E. and Nelson, P.G.: Synaptic activity during natural stimulation of muscle spindles. Science 151 (3714): 1088-1091, Mar.4, 1966.

Serial No.: NDB(I)-62 LNP/SC-973
1. Laboratory of Neurophysiology
2. Section on Spinal Cord
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Integrative mechanisms in the central auditory pathway

Previous Serial Number: SAME

Principal Investigators: P.G. Nelson, E.F. Evans, S.D. Erulkar¹,
and J.S. Bryan²

Other Investigators: None

Cooperating Units: 1/ Department of Pharmacology, University
of Pennsylvania
2/ Section on Technical Development,
NINDB/NIMH

Man Years:

Total:	2.0
Professional:	1.5
Other:	0.5

Project Description:

Objectives: The goal of the study is to characterize in functional terms the sensory processing that occurs at various levels of the auditory system.

Methods employed: The firing patterns of single cells in the auditory pathway have been analyzed with respect to a relatively wide variety of acoustic stimuli such as frequency and amplitude modulated tones. The effects that barbiturates and interruption of some neural pathways may have on the functioning of the system is also being analyzed. Control of stimuli and the analysis of unitary responses have been greatly facilitated by the on-line use of a LINC computer. Electronic models have been developed to simulate the behavior of cells in the auditory pathway in order to analyze some of the mechanisms involved in the function of the pathway and to aid in devising further physiologic experiments.

Major findings: Considerable difference exists between the response patterns of cells in the cochlear nucleus and cells in the inferior colliculus. In the former, modulated stimuli evoke res-

ponses which are usually easily predictable on the basis of the responses to steady tones. Within the various subdivisions of the cochlear nucleus, however, considerable differences of response characteristics occur. The dorsal cochlear nucleus appears to receive more inhibitory input than do either of the two ventral nucleus subdivisions. In the inferior colliculus, however, more complex responses are quite common and it would appear that considerable processing of the auditory signal has occurred at this level. Here, unitary responsiveness may be a function of various stimulus parameters such as depth of change or rate of change of tonal frequency rather than the occurrence of a particular tonal frequency.

The response patterns of units in the dorsal cochlear nucleus are profoundly altered by barbiturates. Inhibitory influences are markedly diminished and thresholds to acoustic stimuli increased when decerebrate animals are given Nembutal.

Significance to Bio-Medical Research and the Program of the Institute: These experiments define some of the functional properties of single cells at two levels of the auditory system. Some neural mechanisms such as convergence, divergence, accommodation and inhibition suggest themselves as being involved in the increased degree of response complexity at the higher levels of the system. Relatively simple models involving these mechanisms can produce quite complex behavior and exceedingly complex circuitry is not necessary to produce the behavior seen at cochlear nucleus and inferior colliculus. The profound effects of the barbiturates on even the primary sensory nucleus in the auditory system emphasizes the danger involved in interpreting complex integrative phenomenon obtained from barbituratized preparations. Differences in the functional properties of various subdivisions of the cochlear nucleus have been shown in the present work giving weight to the anatomical evidence in this regard.

Proposed course of project: Further experiments with anesthetics other than barbiturates or with chronic unanesthetized or denervated preparations are planned. We hope to develop and use an even wider repertoire of stimuli. We wish to explore the inferior colliculus further with the extended frequency range of stimuli which we now have available. Experiments in the medial geniculate body are also planned.

Honors and awards: None

Publications:

Nelson, P.G., Erulkar, S.D., and Bryan, J.: Responses of units of the inferior colliculus to time-varying acoustic stimuli. J. Neurophysiology 1966 (in press).

Serial No.: NDB(I)-65 LNP/SC-1239

1. Laboratory of Neurophysiology
2. Section on Spinal Cord
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Photoreceptors in the Limulus eye

Previous Serial Number: SAME

Principal Investigators: T. G. Smith, Jr.

Other Investigators: None

Cooperating Units: Section on Physiology, Ophthalmology Branch,
NINDB (See NDB(I)-60 O/OPS-700c)

Department of Biology, Massachusetts
Institute of Technology, Cambridge, Mass.

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description:

Objectives: The objective of this research is to gain insight into the means by which the photic energy in light is transduced to electricity in photoreceptors. The intracellularly recorded potentials of the transducer cells (the retinular cells) of the horseshoe crab, Limulus polyphemus, are the object of study.

Methods employed: The responses of the retinular cells to light and electrical stimulation are analyzed under a variety of experimental conditions. In this way, the role of visual pigments of the photoreceptor cell membrane and of the various ions in photo-reception can be studied.

Major findings: During the past year there have been two major findings. The first is that the current-voltage curve of the photoreceptor membrane, determined in the dark, is doubly-rectifying and thus, the membrane's equivalent circuit may be represented by two semiconductor-like diodes placed in series. Light leads to a short-circuiting of one of these diodes which acts as a "constant-current" source to the other diode. The apparent mag-

nitude of this light-generated current is linearly related to the light intensity. The resultant steady-state potential change is logarithmically related to current and to light intensity. Thus, it appears that the Weber-Fechner relationship in vision in Limulus is a consequence of the membrane properties of its photoreceptors.

The second finding is that very intense lights evoke a transmembrane potential change in photoreceptors of vanishingly short latency. This photoelectric potential (PEP) has many of the properties of the so-called early receptor potential (ERP) recorded extracellularly in vertebrate eyes. Studies with manipulation of the ionic composition of the extracellular fluid indicate that neither sodium, potassium nor chloride participate directly in the PEP. This, plus the fact that the PEP persists when the preparation is frozen, suggests that the PEP may have a protonic or electronic basis. In addition, the sign of the PEP reverses when the potential across the cell membrane is reversed (i.e., if the cell interior is made positive) by extrinsic currents. If, as has been suggested for the ERP, these short latency responses arise directly from the rhodopsin molecule itself, these results indicate that the rhodopsin molecule is an integral part of the photoreceptor cell membrane.

Significance to Bio-Medical Research and the Program of the Institute: These observations emphasize the importance of the photoreceptor membrane in the earliest steps of the visual process. They indicate that the visual pigment may be an integral constituent of that membrane and that the characteristics of the membrane underlie the Weber-Fechner relationship in vision.

Proposed course of project: It is proposed to continue the study of the effects of changing the ionic composition on the PEP. Such studies should provide clues as to whether the "minor" ions (e.g. calcium, magnesium and hydrogen) underly the PEP or whether it is an electronic phenomenon and, possibly, to the means by which light-activated rhodopsin leads to subsequent changes in the ionic permeability of the photoreceptor membrane.

Honors and awards: None

Publications:

Smith, T.G., Baumann, F., and Fuortes, M.G.F.: Electrical connections between visual cells in the Ommatidium of Limulus. Science 147(3664):1446-1448, Mar.19, 1965.

ANNUAL REPORT

July 1, 1965 through June 30, 1966

Laboratory of Biophysics, Intramural Research
National Institute of Neurological Diseases and Blindness

Robert E Taylor, Acting Chief

Work has continued in the Laboratory of Biophysics on essentially the same basis as reported for the last Fiscal Year with particular attention to problems concerning the basic mechanisms underlying the initiation, propagation and termination of excitation in nerve and similar irritable structures. Review of the literature makes it appear probable that the bulk of information processing in the central nervous system is related to local electrotonic interactions, instead of propagated activity. It would appear that the fundamental mechanisms are common and relate to the changes in the ionic permeabilities of membranes brought about by electrical potential changes and other physical and chemical agents such as mechanical displacement and synaptic transmitting substances.

Techniques for the replacement of the internal contents of the giant axon of the squid with continuous perfusion of known solutions coupled with voltage clamping procedures are being steadily improved. Some of the many possibilities for important investigations using these techniques have been worked on in Woods Hole, Massachusetts, Plymouth, England, and Vina del Mar, Chile. Of particular interest are the effect of calcium ions and the possible role of sulfhydryl groups on the sodium and potassium ion voltage dependent conductances, the degree to which other ions can pass through the sodium and potassium channels and the manner in which various substances selectively block these channels.

Evidence is accumulating that the ion selectivity and the voltage dependence are separate mechanisms. For example Chandler, Hodgkin and Meves measured the relative permeabilities of the fast channels which normally carry sodium to the alkaline earth metals and while there is a large range of relative permeabilities the time courses of the voltage dependencies are indistinguishable. Further evidence that the slow (normally potassium) and the fast (normally sodium) channels behave independently has been obtained in this laboratory with the observation that with voltage clamped squid axons perfused with ammonium and tetra-ethyl ammonium chloride only that part of the outward current carried by ammonium ions through the slow channels is blocked.

Experiments in Plymouth, England, in collaboration with Dr. Meves (Homburg, Saarland) have shown that the prolonged action potentials which occur with high concentrations of internal sodium ions are the result of an incomplete

inactivation of the sodium channels on depolarization for short times. This residual sodium conductance inactivates with a time constant of 1-2 seconds at room temperature. This slow inactivation process does not seem to be altered by changes in internal potassium, as suggested by others.

The pace of theoretical investigation in the laboratory is expected to increase with the acquisition of a full-time computer programmer to occupy the space which will become available on retirement of the EASE analog computer.

Pioneering work has been done in this laboratory in the use of film animation techniques for the presentation of computer output to demonstrate and study excitation and propagation of nerve impulses. Visual presentation of computer output, while not new, is yet in a rather primitive state of development. Understanding of and insight into the properties of complex systems can be increased enormously. One picture is worth at least 1024 lines of printed output. Many problems remain to be investigated in which these techniques can be very useful, such as transmission in tapered fibers, spatial summation of subthreshold phenomena and decremental conduction.

The rather general kinetic model of ionic conductances developed last year yielded good fits for the potassium ion currents and is being extended to the sodium conductance.

Studies on artificial systems and the application of newer physical techniques to natural membranes -- made possible by the acquisition of additional space last year -- have begun to yield valuable information. At present this program has three facets: (1) thermally (and possibly hydration state) induced mesomorphic phase changes in the hydrophobic part of natural myelins studied with broad-line nuclear magnetic resonances; (2) the effect of electric field strength and anionic charge density on the cationic binding to fixed negative sites on the surface of newly synthesized organic semiconductors which are also ion exchangers; and (3) projected measurements of conventional light scattering and low frequency dielectric dispersion on artificial films (air-water and water-water interfaces) of phospholipid-protein complexes for studies of fluctuations indicating possible domain type behavior.

A timely and important Conference on Physical and Mathematical Approaches to the Study of the Electrical Behavior of Excitable Membranes was held in Woods Hole, Massachusetts supported by a grant from the NIH to the University of Maryland with a considerable amount of space and services donated by MBL. Of 18 papers presented three were from this laboratory and two others by former members.

Serial No. NDB(I)-62 LB/CB 935

1. Biophysics
2. Biophysics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Ionic Permeabilities of Excitable
Membranes. Electrical Experiments and
Analyses.

Previous Serial No.: Same

Principal Investigator: Cole, Kenneth S. and Chandler,
W. Knox

Other Investigators: Hodgkin, A.L. (University of
Cambridge, Cambridge, England),
Meves, H. F. (Universität des
Saarlandes, Homburg-Saar, Germany)
and Guttman, R. (Brooklyn College,
Brooklyn, New York)

Cooperating Units: University of California, Berkeley,
California
Marine Biological Laboratory, Woods
Hole, Massachusetts
Marine Biological Association,
Plymouth, England

Man Years

Total:	2.4
Professional:	2.4
Other:	0.0

Project Description:

Objectives: The ionic current flow across the membrane of the squid giant axon has been measured, without the complications of excitation and propagation, after a sudden change of the membrane potential. The currents have been analyzed in terms of the membrane permeability to sodium and potassium ions. Much of classical nerve physiology is explained by these permeabilities which are themselves not understood. The long range objectives are the further interpretation of nerve function in terms of these fast ionic permeabilities and the elucidation of the structures and mechanisms

by which the permeabilities are controlled.

Methods Employed: Measurements of the ionic current flow across the membrane of the giant axon of the squid, without the complications of excitation and propagation have been improved by the simultaneous use of replacement of internal contents and continuous perfusion of known solutions. In addition experiments were conducted with a simpler system using a sucrose gap technique to measure ionic currents in the squid giant axon.

Major Findings: The development of our understanding of the ionic movements in relation to electrical activity in nerve membranes is being critically and extensively reviewed for a forthcoming volume on Membranes, Ions and Impulses.

The long lasting action potentials in axons perfused with sodium or choline solutions are probably due to an incomplete inactivation of the sodium carrying system during depolarization. Evidence for this was obtained from voltage clamp experiments on axons perfused with Na or Cs fluoride solutions. In these experiments in Plymouth, England the normal delayed potassium conductance did not return following perfusion with the sodium solution.

The temperature characteristics of the Woods Hole squid axons using sucrose gap techniques have been analyzed and compared to the theoretical calculations reported in Project 936.

Scientific Significance: A thorough understanding of the relationships between ionic concentrations, inside and outside of the cell and the dependence of the ionic currents on potential are necessary to understand the normal functioning of the excitable system and as part of the attempts to elucidate the molecular structure of the membrane.

Proposed Course of Project: Further work is planned using the techniques of voltage clamping and internal perfusion which have been developed.

Honors and Awards: None

Publications:

Guttman, R.: Temperature characteristics of excitation in space-clamped squid axons. J. Gen. Physiol. 49: 1007-1018, May 1966.

Author: Cole, K. S.: Membranes, Ions and Impulses.
University of California Press, 1967 (In Press).

Serial No. NDB(I)-62 LE/CB 939

1. Biophysics
2. Cellular Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Ionic Permeabilities of the Squid Giant Axon Membrane. Electrical Experiments and Analyses with Alterations of Environments.

Previous Serial No.: Same

Principal Investigator: Gilbert, D. L.

Other Investigators: Taylor, R. E, Ehrenstein, G. M., Lecar, H. and Rojas, E. (Universidad de Chile)

Cooperating Units: Marine Biological Laboratory, Woods Hole, Massachusetts. Laboratorio de Fisiologia Celular, Universidad de Chile, Montemar, Chile.

Man Years

Total:	2.5
Professional:	2.5
Other:	0.0

Project Description:

Objectives: To study the ionic current flow across the nerve membrane without the complications of excitation and propagation in terms of individual ion currents and to observe the effects of changes in normal internal and external ionic concentrations. The long range objectives are the interpretation of nerve function in terms of ion movements and the elucidation of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: Standard voltage clamp techniques are employed with the addition of a control of the internal environment by replacement and perfusion.

Major Findings: Further and more refined experiments were done to measure the current voltage curves of the

membrane of the squid giant axon with identical solutions on the inside and the outside. In the absence of calcium the membrane shows a non-time dependent, almost linear electrical behavior with either sodium or potassium as the only cations present. Addition of a small amount of calcium in the external solution restores the time dependent non-linear behavior.

Perfusion with a solution of potassium fluoride with artificial sea water outside results in normal electrical behavior although no active ion transport occurs. It has been found that addition of sulfhydryl containing compounds produces an increase in the normal resting potential of the membrane and addition of substances which combine with sulfhydryl groups produces depolarization and blocking of activity. The detailed results of these experiments are in the process of analysis.

Scientific Significance: That calcium is a key ion in the electrical and osmotic behavior of cell membranes has been considered to be the case for at least fifty years. With the present techniques of perfusion and voltage clamping the details of how calcium acts can be determined at the phenomenological level for the squid axon and should have wide implications.

Sulfhydryl groups are of importance in the maintenance of structure in proteins and particularly for many enzymes.

Proposed Course of Project: Only by continued and extensive investigation of the effects of changes in internal and external ion concentrations will the evidence be forthcoming which can provide the basis of a rational attack on the problem of molecular mechanisms in the membrane. These experiments will be conducted on the only available material which is suitable, vis., the axons of the squid obtained in Woods Hole, Massachusetts and in Chile.

Honors and Awards: None

Publications:

Taylor, R. E: Effects of anesthetics on membrane phenomena. Pharmacol. Rev. 17: 212-214, July 1965.

Rojas, E., and Ehrenstein, G.: Voltage clamp experiments on axons with potassium as the only internal and external cation. J. Cell. and Comp. Physiol. 66: 71-78, Dec. 1965.

Ehrenstein, G., and Gilbert, D. L.: Slow changes in potassium permeability in squid giant axon. Biophys. J. (In Press).

Serial No. NDB(I)-62 LB/I 940

1. Biophysics
2. Instrumentation
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Ionic Permeabilities of the Squid Giant Axon. Effects of Chemical Agents.

Previous Serial No.: Same

Principal Investigators: Binstock, L. and Lecar, H.

Other Investigators: Armstrong, C. M. (University College, London)

Cooperating Unit: Marine Biological Laboratory, Woods Hole, Massachusetts

Man Years

Total:	1.4
Professional:	1.4
Other:	0.0

Project Description:

Objectives: The voltage clamp technique allows measurements of ionic current flows across nerve membrane as a function of voltage and time. These parameters have been combined into a set of empirical equations capable of expressing nerve membrane activity, but the physical meaning of the equations is unknown. It is hoped that the use of various chemicals known to effect nervous activity may contribute to understanding the physical mechanisms of the membrane. Irrespective of the chemical properties of the substances, this may permit dissection of the lumped parameters of the equations as well as a correlation of known chemical properties with the observed effects.

Methods Employed: The voltage clamp technique used in these experiments were described previously. For the internal perfusion experiments, modifications to the Tasaki suction perfusion technique were made to allow the use of the voltage clamp technique. This required inserting the coaxial electrode, which consists of an axial current electrode and a potential measuring probe,

into the suction or outflow cannula without clogging the perfusion flow, a task much more difficult for the Woods Hole axon than the Chile axon, because of the smaller size of the Woods Hole axons.

Major Findings: Substitution of ammonium ions for internal potassium produces an outward ammonium current under voltage clamp. The permeability ratio of ammonium to potassium in the potassium or slow channel is almost a half. In the sodium or fast channel, the permeability ratio of ammonium to sodium is about a third. From this data it seems clear that the ammonium ion can pass through either channel and is either a "sodium-like" ion or "potassium-like" ion being a partial substitute for both ions. Internal perfusion of TEA with ammonium blocks the outward movement of ammonium ions in the same way it does potassium. It seems that TEA is operating on the slow channel of the membrane to block the outward movement of "potassium-like" ions.

Substitution of ammonium ions for external sodium produced a depolarization of 15-20 mv, ammonium being a strong depolarizer.

Scientific Significance: The ammonium ion acting as a "sodium-like" or "potassium-like" ion lends credence to the idea that there are separate channels in through which sodium and potassium ions pass. These findings should also give some insight into the membrane structure itself.

Proposed Course of Action: Further experimental work is planned using the internal perfusing technique for different concentrations of ammonium both internal and external. Of particular interest are the effect of temperature and pH and the use of Tetrodotoxin to block current through the fast channel, and perhaps some more experiments with TEA. It might also be of interest to study some other ions which partially substitute for both sodium and potassium.

Honors and Awards: None

Publications:

Armstrong, C. M.: Time course of TEA⁺-induced anomalous rectification in squid giant axons. J. Gen. Physiol.
(In Press).

Armstrong, C. M.: Interference of tetra n-propylammonium chloride with outward Na^+ current flow in squid giant axons. Nature (In Press).

1. Biophysics
2. Mathematical Biophysics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Ionic Permeabilities of Nerve Membranes:
Theoretical Investigations.

Previous Serial No.: Same

Principal Investigator: FitzHugh, R.

Other Investigators: Cole, K. S. and Lecar, H.

Cooperating Unit: Division of Computer Research and
Technology, NIH

Man Years

Total:	1.2
Professional:	1.2
Other:	0.0

Project Description:

Objectives: To investigate the bases, consequences, and extensions of ionic permeability concepts by mathematical analysis and computation.

Methods Employed: Mathematical analysis, with particular emphasis on theories of nonlinear differential equations, stability and cable equations, has been used in conjunction with the digital computers at the NIH.

Major Findings: Investigation of the kinetic model of ionic conductances in the nerve for which curves have already been fitted for potassium membrane is being extended to the sodium conductance. Curves for this model have been fitted, as a first step, to those obtained from the Hodgkin-Huxley model, which are a fairly accurate representation of experimental curves. Fits have been obtained with two different sets of values of the parameters. In each case one of the positive parameters of the original model have had to be made negative in order to obtain a fit, and the next step is to decide how best to modify the model so as to account for at least one of these sign changes.

The preliminary film made from computer solution of the partial differential equations of an axon showing a number of properties of the excitation and propagation of nerve impulses, was shown to participants at a Gordon Conference on Biomathematics in July, 1965, and many useful comments and criticisms received. The film is being remade, with improvements in film technique and presentation. In order not to be so limited by the small memory capacity of the LINC computer, the actual solution of the equations has been reprogrammed for the Honeywell 800 central computer, and the resulting data punched on paper tape, which is then fed into the LINC for display and filming. The difficulties in handling bulky reels of paper tape will be eliminated when the new IBM 360 central computer is used in conjunction with the newly acquired magnetic tape reader connected to the LINC.

Electrical noise and fluctuation phenomena in nerves are of interest both as a means of learning about the fundamental ionic conduction processes and for characterizing nerves as information-transmitting devices. The study of noise fluctuations in nerve, begun last year, has been continued. Conductance fluctuations predicted by the various kinetic models have been determined theoretically and the results of these calculations have been used as a guide in the design of a noise experiment.

Scientific Significance: The concept and the measurements of the sodium and potassium ion movements across the squid axon membrane offer a highly specific and general approach to the factors underlying normal and pathological nervous processes which cannot as yet be investigated directly in higher animals and man. An analysis of the mathematical properties of the Hodgkin-Huxley equations makes possible a better understanding of their possible modifications and their limitations than is obtainable by a purely physical interpretation.

The extension of mathematical models of the nerve membrane to chemically nonspecific kinetic models is a logical step beyond the largely empirical model of Hodgkin and Huxley, and leads the way to more specific molecular models. Experimental data on which to base molecular models are lacking, but kinetic models may help to plan experiments for obtaining such data.

The contrasts between calculations and the array of experimental facts give a basis to conclude that the simple process of electro diffusion is not a principal factor in the behavior of the squid axon membrane.

Proposed Course of Project: Studies on impulse propagation, trains of impulses, and adaptation, as outlined in last year's annual report, are still needed.

The film animation technique being used now to present the basic properties of impulse excitation and propagation in nerve fibers promises to be a valuable research tool for studying space clamp stability under various conditions of chamber length and electrode configuration, propagation in tapered fibers such as dendrites, and decremental conduction in drug-treated fibers.

Honors and Awards: None

Publications:

FitzHugh, R.: A kinetic model of the conductance changes in nerve membrane. J. Cell. Comp. Physiol. 66: 111-117, Dec. 1965.

FitzHugh, R.: An electronic model of the nerve membrane for demonstration purposes. J. of Appl. Physiol. 21: 305-308, Jan. 1966.

FitzHugh, R.: Theoretical effect of temperature on threshold in the Hodgkin-Huxley nerve model. J. Gen. Physiol. 49: 989-1005, May 1966.

Cole, K. S.: The nerve impulse. In Rodhal, K. (Ed.): Nerve as a Tissue. New York, New York, Hoeber-Hanper, 1966.

Cole, K. S.: The melding of membrane models. Ann. N. Y. Acad. Sci. (In Press).

Serial No. NDB(I)-62 LB/ME 938

1. Biophysics
2. Membrane Biophysics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Alternating Current Bridge Measurements of
Squid Axon Membrane Dielectric Properties.

Previous Serial No.: Same

Principal Investigator: Taylor, R. E

Other Investigators: None

Cooperating Unit: Marine Biological Laboratory, Woods Hole,
Massachusetts

Man Years

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: The need for more and detailed measurements of membrane dielectric properties remains, particularly in the low frequency range and the effects of temperature and possible drug and ion effects.

Methods Employed: The presently available methods for space and voltage clamping the squid giant axon are required as well as the developing techniques for internal replacement and perfusion and the use of accurate bridge networks.

Major Findings: The theoretical work done last year on data obtained previously has been completed and published. The best possible fit of the data to a single relaxation time model yielded the result that the temperature dependence seemed to be occurring in the non-lossy part of the membrane. The characteristic frequency of about 28 kilocycles did not change with temperature.

Scientific Significance: Detailed information on the impedance of the membrane, particularly the voltage and temperature dependence relations, imposes constraints on any molecular model which is proposed. One example is that the membrane does not have the same dielectric properties as the simple bilipid layers which have been worked on.

Proposed Course of Project: Further experiments are planned and will be done when the possibility presents itself. The techniques are available but the squid are to be had only at Marine Biological Stations. Transportation of squid appears to be feasible but the development of the necessary facilities are beyond the budgetary and manpower resources of the Laboratory.

Honors and Awards: None

Publications:

Taylor, R. E: Impedance of the squid axon membrane.
J. Cell. and Comp. Physiol. 66: 21-27, Dec. 1965.

Serial No. NDB(I)-65 LB/ME 1240

1. Biophysics
2. Membrane Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Molecular Biophysics - Physical Properties
of Membranes and Simple Membrane-like
Systems.

Previous Serial No.: Same

Principal Investigators: Ehrenstein, G., Lecar, H. and
Stillman, I.

Other Investigators: Rojas, E., Lettvin, J. Y. and Pickard,
W. F. (MIT)

Cooperating Unit: NIAMD and MIT

Man Years

Total:	1.5
Professional:	1.5
Other:	0.0

Project Description:

Objectives: To study physical properties and molecular
structure of well-defined synthetic systems related to
natural membranes. To examine related molecular
properties of excitable membranes with newer physical
techniques.

Methods Employed: Wide line nuclear magnetic resonance
spectra are obtained to determine phase changes in
myelin (human, guinea pig, bovine) induced thermally
and by changes in hydration state. Examination of
fluctuations in phospholipid mono and bilayers by light
scattering and low frequency dielectric dispersion
measurements. Determination of cationic binding to
ion-exchange groups on newly synthesized organic semi-
conductors by careful measurement of pH at the semi-
conductor solution interface and the effects of anionic
charge density and electric field strength on the
binding.

Major Findings: The nuclear magnetic resonance studies indicate that the hydrocarbon part of the lipid in natural myelin is a rather liquid state and that thermally induced phase changes do occur. Fixed oxyanionic sites on the surface of organic semiconductors have been found experimentally to select between cations in a manner which is altered by the surrounding electrostatic field. This field is in turn a function of the number of charges injected or changes in the electric field strength induced in the semiconductor. It was found that this latter effect saturated at about 80,000 volts per centimeter.

Scientific Significance: Study of the structure of cell membranes generally and possible phase changes being involved in function is of great importance. Not only are membranes responsible for electrical activity and transfer of materials into and out of cells but it is progressively being found that most if not all macromolecular synthesis in cells goes on in association with membranes as well as most if not all enzymatic processes.

Since ion-exchange phenomena are presumed to be explicitly involved in the ion selectivity and voltage dependent permeability changes in excitable membranes quantitative studies of the molecular mechanisms involved in model systems are clearly needed.

Future Course of Project: In addition to further studies on monomolecular and bimolecular films and studies of thermal noise fluctuations in natural and artificial membranes, feasibility studies are being conducted concerning the possible use of newer physical techniques such as (1) low energy electron diffraction as a tool for studying surface ordering, (2) microwave dielectric relaxation measurements for studying bound water in polymer solutions and monolayers and (3) conductance measurements on organic semiconductors in relation to the possible role of electronic conducting mechanisms in membranes.

Honors and Awards: None

Publications:

Rojas, E., Lettvin, J. Y., and Pickard, W. F.: A demonstration of ion-exchange phenomena in phospholipid mono-molecular films. Nature 209: 886-887, Feb. 1966.

ANNUAL REPORT

July 1, 1965 through June 30, 1966

Laboratory of Neurochemistry, Intramural Research
National Institute of Neurological Diseases and Blindness

Dr. D. B. Tower, Chief

This year the complement of Laboratory personnel is again at full strength, including four new research associates (one for each Section) and a visiting scientist from Sweden. Despite the taxing of available space, we think this a most salutary situation. Working visits from several colleagues, three summer students, and continuing collaboration with many NIH and extramural colleagues (as noted in individual project reports) have greatly enriched our programs. We have just lost one of our active collaborators, Prof. Heinrich Waelisch. He was a close friend and valued advisor to the staff of this Laboratory, and he was a giant in neurochemistry. He will be sorely missed.

Research progress continues, as indicated in the attached, individual project reports. The work by the Enzyme Chemistry Section on the Na-K-activated, Mg-dependent ATPase of neural tissues remains both informative and challenging. Essentially complete uncoupling of the initial transphosphorylation (kinase) activity from succeeding steps has been achieved by treatment of the enzyme with oligomycin plus N-ethyl maleimide (NEM). These studies provide strong support for a multistep reaction sequence in which there is initial transphosphorylation from ATP to a macromolecular acceptor on the enzyme protein activated at low Mg^{++} concentrations by Na^+ , the stoichiometry requiring 2 Na^+ per ATP. This reversible exchange reaction, which can be so clearly demonstrated in NEM-treated enzyme, is normally inhibited at higher levels of Mg^{++} which convert the high-energy phosphorylated intermediate to a form unable to react with ADP (and hence unable to reverse). This conversion apparently involves a second site of Mg^{++} activation and may represent either a conformational change of the macromolecule or an intramolecular migration of phosphate. The effect of NEM, presumably mediated via sulfhydryl groups, is to prevent such transformations even at relatively high concentrations of Mg^{++} . Normally the final stage of the reaction sequence is the K^+ -activated dephosphorylation of the enzyme, a process so fast that new rapid-flow techniques may be necessary for studies of its stoichiometry and thermo-dynamics. Thus these studies are gradually unravelling the complexities of the enzyme system apparently responsible for monovalent cation transport across cell membranes. As these studies progress in various centers around the world, it is clear that the work by the Enzyme Chemistry Section remains in the forefront of this field of research.

In the Section on Physiology and Metabolism the multipronged approach to the problem of functional organization of bioelectrogenic plasma membranes continues. A central facet relates to the question of protein-lipid interactions and complexing and is being approached both in terms of lipoprotein structure, synthesis and specificity as well as in terms of attempted reconstitutions of membranes from lipid bilayers (lecithin plus cholesterol) plus functional proteins (such as Na-K-ATPase). For the former, serum lipoprotein synthesis has been taken as a model system and is yielding data on

lipid specificity and the nature of the protein "core" or "carrier", data which may serve as analogies to intramembranal organization. The other set of studies involve attempts to effectively solubilize various proteins destined for study in reconstituted membranes and parallel work aimed at altering in situ membrane composition (of *Nitella*) while monitoring impulse conduction, along the membrane. It is likely to be some time before meaningful syntheses can be expected but the data obtained enroute are both valuable and intriguing.

Another area of promise is represented by studies on fluid distribution and electrolytes from the Section on Amino Acids and Electrolytes. Data from ontogenetic and comparative studies of incubated tissue slices in vitro make it possible to construct a fairly detailed schema of compartmentation of fluids (and electrolytes) in cerebral tissues from both mature and developing brain. These studies have established that the swelling of cortical slices in the presence of added K^+ or glutamate or after circulatory arrest is clearly neuronal in locus, whereas most of the so-called "preparative" swelling (accessible in vitro to chloride but not inulin) is clearly glial (probably astrocytic) in locus. Furthermore glial cells in subcortical white matter behave differently than glia in cerebral cortex in such respects, and there seems to be a strong indication that cortical glia normally exclude chloride in vivo but lose this ability under usual in vitro conditions. A number of interesting directions for future research are indicated by these analyses, but of immediate importance is the fact that these studies, together with those from several other groups, have contributed significantly to correction of serious misconceptions present in the field. The factor of artifacts of fixation and processing of neural tissues for electron microscopy is finally being recognized after having long been ignored or minimized by electron microscopers, and consequently the interpretations of little or no interstitial space in brain based on such electron micrographs can no longer be considered valid. Acceptance of the presence in the central nervous system of significant degrees of extracellular spaces is finally being achieved. One result of these developments is the firm establishment of the importance of transport processes in determining or influencing blood-brain barrier functions and the distribution of solutes across the barriers and across neural cell membranes.

Customarily in these reports the importance of concentrating on basic aspects of neurochemistry has been emphasized with the expectation that clinical relevance and applications would naturally follow. This philosophy is no better exemplified than by the clear-cut demonstrations (by Dr. Brady's group in the Section on Lipid Chemistry) of the nature of the metabolic derangements responsible for Gaucher's disease and Niemann-Pick disease. The program of the Section on Lipid Chemistry has been directed to a large extent to studies of structure, biosynthesis, maintenance, catabolism and functional significance of neural sphingolipids. Accumulations of glucocerebroside in reticulo-endothelial cells in tissues of patients with Gaucher's disease and of sphingomyelin in cells of various tissue of patients with Niemann-Pick disease have long been recognized, but largely as a result of work in the Section on Lipid Chemistry, the various possible metabolic errors could be narrowed to defects of catabolism. In order to attack these problems, it was essential to have available the appropriate, isotopically-labelled substrates

and intermediates for the relevant reaction sequences. The PL-480 project with Dr. David Shapiro (at the Weizmann Institute, Rehovot, Israel) has proved to be invaluable in providing a number of the necessary key compounds. Consequently the presence in normal animal and human tissues of glucocerebrosidase (splitting glucocerebroside to ceramide + glucose) and sphingomyelinase (splitting sphingomyelin to ceramide + phosphorylcholine) has now been demonstrated. When spleens from patients with Gaucher's disease were examined, glucocerebrosidase activity was found to be markedly attenuated (15% or less of normal). This finding has been independently confirmed and extended to brain-tissue of patients with the infantile form of the disease. Similarly when livers from patients with Niemann-Pick disease were tested, sphingomyelinase activity was either absent or greatly attenuated, again a finding confirmed independently. These metabolic blocks in the catabolism of the respective sphingolipids appear to account specifically for the accumulation of the excess lipids in these diseases. From the nature of the defect in Gaucher's disease, it is postulated that the normal source of the glucocerebroside is as a degradation product of either ganglioside (in the central nervous system) or globoside (systemically). Globoside is the major stromal glycolipid released from senescent erythrocytes and structural considerations suggest that other, analogous diseases may arise along the degradative pathway of globoside. In fact Fabry's disease seems to be just such an example. The achievements summarized briefly here have been recognized in the recent Superior Service Award to Dr. Brady. It is anticipated that the much more difficult problem of Tay-Sachs disease will prove amenable to similar assaults and that therapeutic approaches to these conditions will prove more rationally feasible.

The Laboratory of Neurochemistry, IR, NINDB has now been in existence for 5 years. In retrospect these have been productive years and have witnessed a considerable degree of maturation especially in terms of training neurochemists. The immediate future involves some uncertainties. A successor to the present Associate Director for Intramural Research remains to be found so that any proposals for future moves of the Laboratory to Bldg. 36 and hopefully more adequate facilities must await the programs of the new director. The present limitations on CORD deferments for Research Associates pose problems for continuities in the training program, for the size of the available recruitment pool, and eventually for output of trained personnel in the field. It is to be hoped that consideration will be given to the eventual nationwide impact of any prolonged hiatus in the influx and outflux of research trainees. Despite such problems of the moment, the Laboratory seems to be well found and embarked on a number of important and rewarding ventures.

1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Metabolism of free and protein-bound amino acids in neural tissues.

Previous Serial Number: Same

Principal Investigator: Dr. D. B. Tower.

Other Investigators: Dr. H. Knizley, Jr.; Mr. E. L. Peters.

Cooperating Units: Laboratory of Kidney & Electrolyte Metabolism, NHI.

Man Years

Total:	2.3
Professional:	2.1
Others:	0.2

Project Description:

Objectives: To investigate the metabolic interrelationships (and factors affecting them) of amino acids, especially of the glutamate and aspartate group, in the free and protein-bound pools of neural tissues, with emphasis on cerebral cortex studied in vitro.

Methods: See previous reports and below.

Major Findings: (1) Amino acid analyses on acid hydrolysates of sheep erythrocyte ghost proteins (see 1964-65 report) have been completed. Analyses on the first 5 samples were repeated and 5 additional samples were analyzed. With the exception of the seryl residues, no differences in analyses could be demonstrated between the proteins from low K⁺ erythrocytes and those from high K⁺ erythrocytes. Thus for over 95% of the total protein N, the coefficients of variation for means of the 10 samples did not exceed 5-7%. Two samples, one from low K⁺, the other from high K⁺ ghosts, were analyzed in quadruplicate with similar results. Marked variability of seryl residues was encountered (range 1.96 to 4.95% of total N) and reproducibility was poor on repeat analyses. Studies on samples hydrolyzed in evacuated sealed tubes for varying lengths of time suggested greater lability of seryl residues in proteins from low K⁺ ghosts but some lability was evident for both types. It has been recently reported (Schaffer & Balakir: Anal. Biochem. 14:167, 1966) that phosphoseryl residues are much more labile to acid hydrolysis, exhibiting 3x the destruction observed for seryl residues under identical

conditions. This factor may account for the lability encountered in our samples. No further work on this problem is contemplated in view of Dr. Hoffman's move to Yale late in 1965.

(2) Studies by Mr. Peters on the in vitro metabolism of methionine by cerebral cortex slices have been hampered by infrequent access to the amino acid analyzer and especially to the combined analyzer and isotope counting flow cell assembly as consequences of increased demands on instrument time. Thus, the anticipated specific activity data has not yet been acquired in sufficient amount to permit conclusions to be drawn. Adaptations of the column separation procedure for keto-acids developed by Dr. Knizley (see below) have provided good separations from amino-acid-free (Dowex-treated) tissue extracts of pyruvate, α -ketoglutarate, α -ketobutyrate, etc. These procedures plus the use of D-amino acid oxidase have permitted preparation of C^{14} -keto acids from various C^{14} -amino acids in good yield and purity for use with incubated slices. Conventional analyses indicate that additions of α -amino or α -ketobutyric acids (5 mM) to incubating slices of cortex from methionine sulfoximine - intoxicated cats will promote normal levels of slice glutamic acid just as additions of methionine (5 mM) have been shown to do. These findings are consistent with the methionine \rightarrow homoserine \rightarrow α -ketobutyrate \rightarrow ? pyruvate \rightarrow α -ketoglutarate \rightarrow glutamic acid source for the carbon skeleton of glutamate under these conditions.

(3) Investigations of various aspects of aspartic acid metabolism have been initiated by Dr. Knizley. Procedures based on the method of Busch (J. Biol. Chem. 196:717, 1952) were developed to permit column chromatographic separation in less than 8 hrs. of relevant amino and keto acids from TCA-soluble extracts of incubated cortical slices. A battery of columns was set up so that simultaneous runs of 6-8 samples could be effected. Quantitation of aspartate and of N-acetyl-aspartate (after separation and acid hydrolysis) has been carried out by specific enzymatic assays. Under the conditions employed clear-cut evidence has been obtained for conversion of C^{14} -aspartic acid to N-acetyl aspartic acid by slices of cat cerebral cortex incubated aerobically at 37° in bicarbonate-saline-glucose media for 30 min. The S.A. of labelled N-acetyl aspartic acid was 5-12% of the S.A. of L-aspartic acid in the system. Since previous attempts by others to demonstrate such a reaction have been equivocal, these observations are most helpful in accounting for the source of the large amounts of N-acetyl aspartate present in cerebral cortex. Further metabolic studies and purification of the acetylating enzyme(s) are in progress.

Significance: This project bears directly upon the fundamental problem of delineating some of the interrelationships between cerebral structure and metabolism, and between metabolism and functional activity, so that an eventual integration of these facets of cellular neurochemistry can be realized.

Proposed Course: To continue these studies along lines indicated by present results.

Publications:

1. Tower, D. B.: Arrest of seizure activity: biochemical aspects and pharmacology. Epilepsia 6: 141-155, June 1965.
2. Tower, D. B.: Enzymatic determination of glutamine and asparagine. In: Hirs, C. H. W. (Ed.): Enzyme Structure (vol. 7 of Methods in Enzymology), New York, Academic Press, in press.

1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Electrolytes and energy metabolism in cerebral cortex
in vitro.

Previous Serial Number: Same.

Principal Investigator: Dr. D. B. Tower.

Other Investigators: Mr. E. L. Peters.

Cooperating Units: Dr. I. Klatzo, Sect. on Clin. Neuropath, SN, NINDB;
Mr. B. Lloyd, Sect. on Cytol., OB, NINDB.

Man Years:

Total: 1.1
Professional: 0.9
Others: 0.2

Project Description:

Objectives: To study the in vitro metabolism of electrolytes and of energy-producing cycles and compounds thereof in incubated slices of cerebral cortex.

Methods: See previous reports and below.

Major Findings: (1) Completion of studies on fluid spaces and electrolytes in neonatal and developing kitten cerebral cortex and on adult cat corpus callosum provided considerable insight into the general problem of fluid and electrolyte distribution in cerebral tissues:

(a) In kitten cortex, three distinct periods could be discerned: at birth (1-4 days of age), incubated cortical slices exhibited no swelling, chloride and sucrose-inulin spaces of similar magnitudes and no effect of time of addition or of K^+ on spaces accessible to sucrose or inulin; by age 1 month, incubated cortical slices exhibited swelling in presence of extra (27 mM) K^+ but no other changes from neonatal characteristics; after age 2-3 months, additional slice swelling associated with increased chloride spaces and sucrose spaces dependent upon time of addition or on K^+ could be demonstrated. There is coincidence of these changes with morphological maturation of neurons at age 1 month and with proliferation of glia (? astrocytes) at and after age 3 months, after completion of myelination of cortical axons. Gross confirmation of these morphological stages was provided by light microscopy (Dr. Klatzo) and some

electron micrographs of neonatal cortex were obtained (Mr. Lloyd) that showed no recognizable astrocytes. Prof. George Pappas of Columbia confirmed to us the lack of recognizable astrocytes in significant numbers up through 1 month of age.

(b) For studies on corpus callosum, a technique was developed to permit use of unsliced tissue. Corpus callosum was dissected out bluntly and cut free from surrounding tissues by two lateral cuts and then divided into two sections by a mid-line cut. Thus cut surfaces were minimized and two sections about 150 mg. in weight and less than 0.6 mm thick could be obtained from one cat brain in less than 10 min. from moment of sacrifice. Sections were incubated under conditions used for cerebral cortex and handled in identical fashion except for recovery on Millipore filter circles (in order to minimize sticking). Results with corpus callosum were in distinct contrast to cerebral cortex: swelling of incubated sections was minimal (7% vs. 32% for cortex), chloride spaces were significantly smaller (47 vs 64%) and equal to sucrose spaces, and inulin spaces were approximately half the size of sucrose spaces (whereas in cortex the two are equal in size). In most parameters, corpus callosum sections behave much more like slices of liver than slices of cerebral cortex. If a major compartment in cerebral cortex for swelling and access of chloride but not sucrose is a glial (astrocyte) compartment, then the behavior of glia (astrocytes) in white matter must be very different under the same in vitro conditions. This conclusion receives additional emphasis from the fact that in cut brain it is known that the total number of glial cells is the same in both cortex and subcortical white (corpus callosum) (cf. Heller & Elliott: Canad. J. Biochem. Physiol. 32: 584, 1954).

(2) The data obtained in the studies on fluid and electrolyte distribution over the past several years have been subjected to extensive analysis in order to delineate as much as possible the details of fluid compartmentation as well as to point up the most appropriate routes of further investigation. The results of analyses of fluid compartmentation are in press in three papers (see below). Promising areas for future investigation appear to include additional studies on circulatory arrest (see 1964-65 report), more detailed studies on K and Na fluxes \pm ouabain, attempts to evaluate fluid spaces in fetal and neonatal cortex in vivo, and studies on distribution of water per se. Such approaches are in progress.

Significance: Energy-yielding metabolism is the basic factor underlying neuronal function and activity, and electrolyte metabolism (which clearly depends upon it) provides a fundamental link between cellular chemistry and the functional activity of impulse conduction. The understanding of factors involved is essential for the elucidation of both normal functioning of neural tissues as well as deranged function of hyperactivity states and of states associated with concussion and edema.

Proposed Course: To pursue investigations along lines indicated by present results.

Publications:

1. Tower, D. B.: Problems associated with studies of electrolyte metabolism in normal and epileptogenic cerebral cortex. Epilepsia 6: 183-197, Sept. 1965.
2. Tower, D. B.: Distribution of cerebral fluids and electrolyte in vivo and in vitro. In: Seitelberger, F. and Klatzo, I. (Eds.): Brain Edema, Vienna, Springer-Verlag, in press.
3. Bourke, R. S. and Tower, D. B.: Fluid Compartmentation and electrolytes of cat cerebral cortex in vitro. I. Swelling and solute distribution in mature cerebral cortex. J. Neurochem., in press.
4. Bourke, R. S. and Tower, D. B.: Fluid compartmentation and electrolytes of cat cerebral cortex in vitro. II. Sodium, potassium and chloride of mature cerebral cortex. J. Neurochem., in press.
5. Tower, D. B. and Bourke, R. S.: Fluid compartmentation and electrolytes of cat cerebral cortex in vitro. III. Ontogenetic and comparative aspects. J. Neurochem., in press.
6. Tower, D. B.: Commotio cerebri from a neurochemical standpoint. In: Caveness, W. F. et al. (Eds.): Head Injury, Philadelphia, J. B. Lippincott, 1966, in press.

1. Neurochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Enzymological Aspects of Neural Function.

Previous Serial Number: Same.

Principal Investigator: Dr. R. W. Albers

Other Investigators: Mr. G. J. Koval, Dr. G. J. Siegel, and Miss M. Hurley.

Cooperating Units: Dr. L. Guth, Section on Experimental Neurology,
Laboratory of Neuroanatomical Sciences, NINDB

Man Years

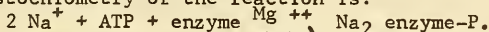
Total: 4.6
Professional: 3.0
Other: 1.6

Project Description:

Objectives: To assess the functional roles and interrelations of enzyme systems which characterize neural tissue.

Methods: Isotope studies; classical techniques of enzyme purification and characterization; cytological fractionations.

Major Findings: (1) The initial stage of the $\text{Na}^+ - \text{K}^+$ -ATPase reaction catalysed by the cell membranes of Electrophorus electric organ is a phosphorylation of the active site by ATP. We had previously established that sodium ions participate in this step. We have now established that the stoichiometry of the reaction is:



We have also measured the equilibrium constant for this reaction. This permits us to calculate that about 90% of the free energy of the γ -phosphate of ATP is transferred to the enzyme.

Another phase of this study has focused on the transformation of this high energy enzyme-phosphate to a lower energy configuration. This second stage of the $\text{Na}^+ - \text{K}^+$ -ATPase reaction now appears to involve an enzyme sulfhydryl adjacent to a disulfide bond. This might indicate a sulfide-disulfide interchange. However the fully reduced enzyme is still active in hydrolysing ATP.

(2) The continuing studies on the trophic aspects of the nerve-muscle relationship will be reported by Dr. Guth (Ser. No. NDB(I)-63 LNS/EN 1054).

Significance: The $\text{Na}^+ - \text{K}^+$ ATPase is an enzyme system which is either the actual transport mechanism for exchanging internal sodium for external potassium across the cell membrane or it is the metabolic "engine" for driving this pump. The sodium ion pump is the "generator" of the potential energy which is used in the transmission of nerve impulses. In addition several other cellular concentrative mechanisms appear to be dependent upon this same potential energy source: the ability of cells to concentrate amino acids and sugars now also appears to be dependent upon a parallel influx of sodium ions. Protein synthesis is also dependent upon maintaining a high intracellular ratio of K^+/Na^+ . Conversely, defects in this system undoubtedly account for a number of pathological conditions and the response of the system to drugs such as cardiac glycosides and aldosterone is of therapeutic importance.

Proposed Course: We have obtained considerable insight into the first two stages of the $\text{Na}^+ - \text{K}^+$ ATPase reaction. Many details remain which may be investigated by our present methodology. Preliminary studies of the last stage of the reaction indicate that it is a K^+ activated dephosphorylation of the enzyme. Studies are in progress concerning the stoichiometry and thermodynamics of this reaction. However the rate of this last stage is so fast that new rapid flow techniques may be necessary.

Discussions with Dr. Berger of the NHI Laboratory of Technical Development indicate that we may be able to use existing instrumentation in their laboratories for this work.

Some of the results of the work in progress lead to speculation about the general evolutionary development of membrane specialization. The ability of cell membranes to (1) selectively exclude sodium ions and (2) to capture the potential energy released by subsequent influxes of sodium ions appears to underlie both the nerve impulses and the whole spectrum of transducers which mediate sensation and synaptic transmission. Thus it seems of the highest importance to pursue the molecular biology of these two processes for controlling sodium ion flux. The modulation of these processes would appear to be the fundamental operation of the brain.

Publications:

1. Fahn, S., Koval, G. J., and Albers, R. W.: Sodium-Potassium-activated Adenosine Triphosphatase of Electrophorus Electric Organ I. An Associated sodium-activated transphosphorylation. J. Biol. Chem. 241: in press.
2. Fahn, S., Hurley, M. R., Koval, G. J. and Albers, R. W.: Sodium-Potassium-activated Adenosine Triphosphatase of Electrophorus Electric Organ II. Effects of N-Ethyl maleimide and other sulphydryl Reagents. J. Biol. Chem. 241: in press.
3. Albers, R. W. and Koval, G. J.: Sodium-Potassium-activated Adenosine Triphosphatase of Electrophorus Electric Organ III. An associated potassium-activated neutral phosphatase. J. Biol. Chem. 241: in press.

1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Metabolism of Complex Lipids of Nervous Tissue.

Previous Serial Number: Same.

Principal Investigator: Dr. R. O. Brady, Jr.

Other Investigators: Drs. J. N. Kanfer, A. Gal, and J. P. Kampine;
Mr. R. M. Bradley and Mr. O. Young.

Cooperating Units: Dr. David Shapiro, Dept. of Organic Chemistry,
Weizmann Institute of Science, Rehovoth, Israel.

Man Years:

Total: 4.8
Professional: 3.8
Other: 1.0

Project Description:

Objectives: (1) To elucidate the biosynthetic pathways for the formation of long chain fatty acids, cerebroside, gangliosides, and sphingomyelin; (2) to study the control mechanisms which regulate these processes; and (3) to study the metabolic fate of sphingolipids in normal and lipodystrophic disease states.

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. The metabolism of these labeled materials has been investigated in vivo and in vitro.

Major Findings: (1) We have extended the series of experiments with spleen tissue obtained from 3 Gaucher's patients reported in a preliminary communication (Brady, R. O., Kanfer, J. N., and Shapiro, D.: The metabolism of glucocerebrosides. II Evidence of an enzymatic deficiency in Gaucher's Disease, Biochem. Biophys. Res. Comm., 18: 221 (1965)). In the additional 8 cases investigated, there was consistent confirmatory evidence for the existence of a marked decrease in glucocerebroside-cleaving enzyme activity in patients with Gaucher's disease. The most striking deficiency was seen in a spleen sample obtained from a child with the infantile form of Gaucher's disease in which essentially no glucocerebroside could be detected. A report of these studies is in

press. In personal communications, Dr. Lars Svennerholm in Gothenberg, Sweden and Dr. A. D. Patrick in London have indicated that they have confirmed our finding of diminished glucocerebrosidase activity in Gaucher's patients.

(2) With the use of labeled sphingomyelin, we have been able to demonstrate the presence of an enzyme in rat and human liver tissue which catalyzes the hydrolysis of sphingomyelin. The products of the reaction are phosphorylcholine and ceramide (N-acyl sphingosine). The enzyme was partially purified and its characteristics determined. Since sphingomyelin has been shown to accumulate in excessive amounts in various tissues of patients with Niemann-Pick disease, it was of prime importance to determine the level of this sphingomyelin-cleaving enzyme in tissues obtained from patients with this disease. A very drastic attenuation of sphingomyelinase was observed in liver and kidney tissues from patients with Niemann-Pick disease. It is believed that the specific metabolic lesion in Niemann-Pick disease is a deficiency of this sphingomyelin-cleaving enzyme.

(3) Studies have also been carried on the biosynthesis of sphingomyelin. The results of these experiments indicate that sphingomyelin synthesis can occur in brain tissue by the enzymatic acylation of sphingosyl-phosphorylcholine. This finding indicates that there is an alternative pathway for sphingomyelin formation in brain differing from a previously reported pathway in liver tissue in which sphingomyelin formation occurs from ceramide and cytidine diphosphate choline.

(4) An enzyme has been discovered in intestinal tissue which catalyzes the hydrolysis of both gluco- and galactocerebroside. This enzyme is different from the spleen glucocerebrosidase which is inactive with galactocerebroside as substrate. Further studies on the level and distribution of glucocerebroside and galactocerebroside hydrolyzing enzymes are being carried out in demyelinating nerve preparations.

Significance: The initial experiments demonstrating the nature of the enzymatic deficiency in Gaucher's disease have been extended and confirmed. The nature of the metabolic lesion in Niemann-Pick disease has been demonstrated. Analogous studies are underway to attempt to discover the metabolic aberrations in Fabry's and Tay-Sachs' diseases. The understanding of the specific metabolic abnormality in the various sphingolipodystrophies is an absolute prerequisite for attempts to formulate logical therapeutic procedures in these diseases.

Proposed Course: We propose to attempt the chemical synthesis of labeled ceramide-trihexoside, the compound which accumulates in various tissues of patients with Fabry's disease. Work is also in progress to devise procedures for the chemical synthesis of gangliosides. Once this has been accomplished, these techniques will be used for the preparation of labeled gangliosides in order to attempt to demonstrate the nature of the metabolic lesion in Tay-Sachs' disease. Attempts will be made to obtain highly purified enzymes from normal human sources which catalyze the hydrolysis of glucocerebroside and sphingomyelin. The detailed molecular structures of these enzymes will be investigated and the

possibility of enzyme replacement trials will be explored.

Honors and Awards:

Superior Service Award, Department of Health, Education and Welfare,
April 11, 1966 (Dr. Brady).

Publications:

1. Brady, R. O., Scow, R. O., Urgoitti, E., and Bradley, R. M.: The effect of pancreatectomy on fatty acid synthesis in liver of fasted rats. Biochim. Biophys. Acta 106: 241-247, Oct. 1965.
2. Bradley, R. M.: An improved method for the determination of long-chain fatty acid amides. Biochim. Biophys. Acta 106: 417-418, Oct. 1965.
3. Brady, R. O., Gal, A., Kanfer, J. N., and Bradley, R. M.: The metabolism of glucocerebrosides. III. Purification and properties of a glucosyl- and galactosylceramide-cleaving enzyme from rat intestinal tissue. J. Biol. Chem. 240: 3766-3770, Aug. 1965.
4. Brady, R. O., Bradley, R. M., Young, O. M. and Kaller, H.: An alternative pathway for the enzymatic synthesis of sphingomyelin. J. Biol. Chem. 240: PC 3693-4, Sept. 1965.
5. Kanfer, J. N., Young, O. M., Shapiro, D., and Brady, R. O.: The metabolism of sphingomyelin. I. Purification and properties of a sphingomyelin-cleaving enzyme from rat liver tissue. J. Biol. Chem. 241: 1081-1084, March 1966.
6. Brady, R. O., Kanfer, J. N., Mock, M. B., and Fredrickson, D. S.: The metabolism of sphingomyelin. II. Evidence of an enzymatic deficiency in Niemann-Pick disease. Proc. Nat. Acad. Sci. U. S. 55: 366-369, Feb. 1966.
7. Brady, R. O., and Kanfer, J. N.: Cerebroside glycosidases. In Colowick, S. P. and Kaplan, N. O. (Eds.): Methods in Enzymology, New York, N. Y. Academic Press, Inc., in press.
8. Brady, R. O., and Kanfer, J. N.: Rat liver sphingomyelinase. In Colowick, S. P. and Kaplan, N. O. (Eds.): Methods in Enzymology, New York, N. Y., Academic Press, Inc., in press.
9. Brady, R. O.: Sphingolipidoses. A medical progress report. New England Journal of Medicine, in press.

10. Brady, R. O., and Shapiro, D.: Recent studies in the sphingolipidoses. Israel Journal of Medical Science, in press.
11. Brady, R. O., Kanfer, J. N., Bradley, R. M., and Shapiro, D.: Demonstration of a deficiency of glucocerebroside-cleaving enzyme in Gaucher's disease. J. Clin. Invest., in press.

1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Structural and Metabolic Studies of Gangliosides

Previous Serial Number: Same.

Principal Investigator: Dr. J. N. Kanfer

Other Investigators: Drs. R. O. Brady, A. Gal, and E. Martensson;
Mr. O. Young and Miss R. Richards.

Cooperating Units: None.

Man Years

Total: 3.1
Professional: 2.1
Other: 1.0

Project Description:

Objectives: To investigate the structure and metabolism of gangliosides.

Methods: Structural studies are performed by chemical syntheses and by enzymatic degradation of gangliosides. Attempts are being made to induce enzymes in soil bacteria by enrichment culture and to extract the enzymes from these organisms for such studies. Potential precursors of ganglioside are labeled by chemical syntheses. These materials are examined in various brain enzyme preparations with regard to their role in ganglioside synthesis.

Major Findings: Three additional potential radioactive precursors of gangliosides have been synthesized: 1, erythro-³H-sphingosine, threo-³H-sphingosine, and ceramide-lactoside-¹⁴C. The formation of sphingolipids has been investigated in vivo using labeled erythro or threo sphingosine. These studies were undertaken because previous work on sphingomyelin formation indicated that a threo-ceramide was a much better precursor of sphingomyelin than erythro-ceramide. This was an unexpected finding in view of the fact that only erythro sphingosine derivatives have been isolated from mammalian tissue. Our present findings indicate that both the erythro and threo forms of sphingosine are metabolically active and can be converted to complex sphingolipids.

Significance: Studies in this laboratory indicated that the so-called

"Tay-Sachs" ganglioside probably is a normal intermediate in ganglioside formation (Kanfer, J. N., Blacklow, R. S., Warren, L., and Brady, R. O.: Biochem. Biophys. Res. Comm. 14: 287-291, Jan. 1964). This finding helps to pinpoint the metabolic abnormality which occurs in Tay-Sachs' disease, i.e., it may be either a failure to complete the normal ganglioside molecule by the addition of a final molecule of galactose or a defect in ganglioside catabolism. Investigations are underway in order to distinguish between these alternatives.

Proposed Course: The catabolism of gangliosides will be investigated using enzyme systems from brain tissue of various sources. Further precursors of gangliosides will be synthesized chemically.

Publications:

1. Young, O. M., and Kanfer, J. N.: An improved separation of sphingolipids by thin-layer chromatography. J. Chromatography 19: 611-613, Feb., 1965.
2. Kanfer, J. N., and Gal, A. E.: In vivo conversion of erythro and threo DL-sphingosine-³H to ceramide and sphingomyelin. Biochem. Biophys. Res. Comm. 22: 442-446, Jan., 1966.
3. Kanfer, J. N., and Brady, R. O.: Studies on the metabolism of gangliosides. In Volk, B. W. (Ed.): Third International Symposium on Cerebral Sphingolipidoses, Pergamon Press, in press.

1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Immunochemical Studies in Multiple Sclerosis.

Previous Serial Number: Same.

Principal Investigators: Drs. R. O. Brady and J. N. Kanfer

Cooperating Units: Dr. John E. Somers, Division of Neurology,
University of Missouri Medical Center,
Columbia, Missouri

Man Years

Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: To determine whether auto-immune phenomena participate in the pathogenesis of neurological diseases.

Methods: Examinations are made of the sera from patients with multiple sclerosis and animals with experimental allergic or viral encephalomyelitis for the presence of antibodies to gangliosides, asialogangliosides, myelin sheath lipids, etc. Animals are immunized by several procedures with various ganglioside preparations.

Major Findings: Research efforts are continuing to determine if there is a relationship between anti-sphingolipid antibodies which appear in some of these sera and various demyelinating disease states. Data obtained in the course of this study indicated that anti-ganglioside antibodies appear in the serum in approximately 20% of patients with demyelinating diseases such as multiple sclerosis and viral encephalitis. It has been found that various preparations of gangliosides exhibit distinct differences in antigenic potency.

Significance: Since ganglioside preparations vary in antigenic potency, it is important to try to discover the reason for this finding since it may be an important clue to the cause of the difference in levels of anti-ganglioside antibody in these conditions.

Proposed Course: Gangliosides will be prepared and exact analyses performed including experiments to determine if they contain peptides. Traces of the latter material may be quite important as far as the antigenicity of the gangliosides is concerned. When these tests have been completed, the immunology of the rigorously characterized materials will be investigated.

Publications:

1. Brady, R. O.: Immunochemical properties of glycolipids. J. Am. Oil Chem. Soc., 43: 67-69, Jan. 1966.

1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Investigation of the Anti-cancer Activity of Derivatives of Tetrolic Acid.

Previous Serial Number: Same.

Principal Investigator: Dr. R. O. Brady.

Other Investigators: None.

Proposed Course: This project is terminated. No significant anti-cancer activity was observed using varying dose of tetrolylpantetheine.

1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Sphingolipid Metabolism in Tissue Culture Cells.

Previous Serial Number: None.

Principal Investigator: Dr. J. N. Kanfer.

Other Investigators: Mr. O. Young.

Cooperating Units: Drs. M. Mock and D. S. Fredrickson, NHI, Laboratory of Metabolism; Dr. W. B. Uhlendorf, Laboratory of Viral Immunology, Div. Biologic Standards.

Man Years

Total: 0

Project Description:

Objectives: To initiate a program designed to study the metabolism of complex sphingolipids in homogeneous mammalian cell populations. The cell lines available at present are principally from peripheral organs. The ultimate goal will be to work with neural cells.

Methods: Examination of the metabolism of various radioactive sphingolipids in certain cell lines.

Major Findings: Preliminary studies utilizing cell lines derived from patients with Niemann-Pick disease indicate that the level of the sphingomyelin-cleaving enzyme is depressed when compared with similar cells from normal individuals. In one case, cells from a patient originally diagnosed as a non-Niemann-Pick case showed a lowered enzyme level. Subsequent chemical assay indicated this individual was afflicted with the disease.

Significance: This observation corroborates evidence of our recent demonstrations of attenuated levels of sphingomyelinase in solid tissue derived from patients with Niemann-Pick disease. Since cultures derived from skin tissue also have lowered enzyme level, this may become a convenient diagnostic tool. These initial studies have also demonstrated that the cells possess the ability to metabolize certain other sphingolipids.

Proposed Course: To further document these preliminary observations concerning Niemann-Pick disease. To inaugurate a long-range program using tissue culture methods in order to learn exactly how cells synthesize and utilize sphingolipids.

1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on the metabolism of sphingolipids in tumor tissue.

Previous Serial Number: None.

Principal Investigator: Dr. R. O. Brady

Other Investigators: Drs. C. Tal (Jerusalem); and D. Shapiro (Rehovoth).

Cooperating Units: Laboratory of Experimental Neurology, Hadassah University Hospital, Jerusalem; Department of Organic Chemistry, Weizmann Institute of Science, Rehovoth, Israel.

Man Years

Total: 0

Project Description:

Objectives: To determine the metabolic pathways of sphingolipids in neoplastic tissues.

Methods: Various complex sphingoglycolipids will be synthesized chemically and labeled with ^{14}C . The metabolic fate of these materials will be studied in vivo and at subcellular levels.

Major Findings: It has been recognized for some time that an abnormal β -globulin occurs in sera of patients with cancer and of pregnant women at term. The cell surface receptor for this agglutination factor in HeLa and other cancer cells appears to be identical with ceramide lactoside (Tal, C.: Proc. Nat. Acad. Sci. U.S. 54: 1318, 1965).

Significance: These observations would seem to indicate some fundamental difference in sphingolipid metabolism in rapidly growing and neoplastic tissue when compared with non-neoplastic tissue. The elucidation of the nature of this metabolic alternation may shed considerable insight into the processes which occur in oncogenesis.

Proposed Course: The enzymes which catalyze the catabolism of sphingoglycolipids will be investigated in tissue culture preparations obtained from normal and neoplastic tissue sources. This project is being initiated at the present time.

1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on Bioelectrogenesis.

Previous Serial Number: Same.

Principal Investigator: Dr. E. G. Trams.

Other Investigators: Dr. W. Stahl; Mr. C. J. Lauter.

Cooperating Units: None.

Man Years

Total: 1.5
Professional: 1.3
Other: 0.2

Project Description:

Objectives: This is a combination of related studies dealing with investigations into the mode of generation and propagation of bioelectric potentials and currents. The immediate aim of the research is to elucidate the mechanisms by which ionic gradients are maintained selectively; the phenomena which are involved in chemical transmission of impulses; and the lipid-protein structure of the plasma membrane.

Methods: Several approaches to this problem are currently under investigation: 1) the use of reconstituted lipid bilayer membranes in vitro including elucidation of the lipid components which can make such bilayers excitable. 2) studies on soluble and particulate fractions of brain and electric organ of Electrophorus in order to establish the characteristics of the enzymes involved in active sodium transport; 3) the use of more primitive organisms, such as Nitella, in which bioelectric potentials are propagated.

Major Findings: Experiments have been continued in which crude and defined lipid fractions have been studied as to their ability to form bimolecular lipid leaflets. The lipid fractions were obtained from mammalian brain or from the electric organ of Electrophorus electricus. Although the exact lipid composition of excitable bilayers has not been established adequately, it has been observed that cholesterol and phosphatidyl choline in certain proportions are necessary to maintain mechanical and electrical stability of the bilayer.

In studies of the Na^+ and K^+ activated ATPases of brain and electroplax, the major emphasis was placed upon investigations of the characteristics of some of the enzymes involved in the various steps which in effect constitute the sodium pump. One of the principal aims was to solubilize the particulate elements of the enzyme systems which are active in the sodium transport system. Solubilization of these sub-cellular particles would make the involved systems much more amenable to an analysis of their structure and function. Various approaches have been tried, such as floatation and sedimentation procedures under varying conditions, using density gradients; use of a variety of organic solvents at different concentrations and in a number of combinations; and degradation of certain constituents of these particles by enzymic attack. (Cf. studies by Stahl et al: Biochem. J., in press). So far, the solubilization studies have not met with success. One interesting aspect of these experiments was the effect of dimethylsulfoxide (DMSO) which tended to activate the $\text{Na}^+ - \text{K}^+$ ATPase at relatively high concentrations. Analytical studies, still in progress, indicated that certain lipid elements, which could be inhibitory, apparently are solubilized and partially removed from the particulate enzyme(s).

Studies on Nitella have only recently been initiated. They are designed to follow in a single cell the effects of chemical alteration of the plasma membrane while the impulse propagation is being monitored. The lipid composition of plasma membrane of Nitella will be investigated and attempts will be made to utilize Nitella lipids in reconstituted membranes.

Significance: This investigation is principally concerned with the function and structure of the plasma membrane in relation to the bioelectric process: maintenance of the resting potential, initiation of the impulse and its propagation and the problem of chemical transmission. The elements involved in these phenomena are essentially unknown, although it is generally accepted that the plasma membrane (a bimolecular lipid leaflet) plays the dominant role in bioelectrogenesis. Identification of the chemical constituents of the plasma membrane, their topical organization and their alterations are basic to the study of the fine structure and function of nerves and electric tissue.

Publications:

None.

1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The mechanism of lipoprotein synthesis.

Previous Serial Number: Same.

Principal Investigator: Dr. E. G. Trams

Other Investigators: Mr. Carl J. Lauter

Cooperating Units: Dr. E. A. Brown, Lab. Chem. Pharmacol., NHI and
Dr. M. Heimberg, Dept. of Pharmacol., Vanderbilt
Univ., Nashville, Tenn.

Man Years

Total:	1.4
Professional:	1.2
Other:	0.2

Project Description:

Objectives: To investigate the mechanisms of lipoprotein synthesis as potential models for the coding of lipid-protein interphases.

Methods: Lipoprotein synthesis is studied in the rat by means of isotopic precursors: Radioactive lipid- and protein precursors are administered to the rat and the absorptive mechanisms and systems involved in lipoprotein synthesis are challenged with diet loads. Plasma and tissue lipoprotein synthesis is followed over various time periods by studying the chemical composition of the protein and lipid moieties and their relative utilization of administered isotope. Use is made of chromatographic techniques to analyse the constituent parts of the lipoproteins. In some cases use has been made of the isolated perfused liver in order to follow lipoprotein synthesis.

Major Findings: Plasma lipoprotein synthesis was followed using labeled triglycerides, fatty acids and a variety of amino acids. It was found that in the intact animal and in the perfused liver, considerable net synthesis of plasma lipoproteins had taken place, as determined by chemical analysis. The turnover times of the low density lipoproteins were extremely fast. Both lipid and protein precursors were utilized at a higher rate in the low density lipoproteins than in the higher density fractions. Amino acid analyses of lipoprotein

fractions, in a comparison of fed and fasted rats, showed that the amino acid composition of the low density lipoproteins changed in response to triglyceride absorption. Marked changes were observed in the percent content of a number of amino acids, although the significance of these changes is not presently understood. A phenomenon which has been investigated in more detail was the efficient utilization of radioactive methionine. This amino acid has long been regarded as a lipotropic factor. It has been assumed, that the lipotropic effect of methionine was due to its ability to act as a donor of labile methyl groups for phospholipid synthesis. The present investigations indicate that methionine may play an essential role in lipoprotein biosynthesis, other than that of a methyl donor. Methionine is one of the more lipophilic amino acids and can be considered as a link between the hydrophilic protein moiety and the hydrophobic lipid moiety of the lipid-protein interphase. Even though methionine occurs in lipoproteins only in small amounts, it was found that methionine content (and isotope utilization from labeled methionine) seemed to increase proportionally to triglyceride or fatty acid utilization in lipoprotein synthesis. On the basis of some of these findings, a number of models have been proposed which might explain the mechanisms by which lipid-protein interphases are assembled.

Significance: The investigations outlined above not only will yield a better insight into the structure and the metabolism of the plasma lipoproteins but should also indicate by what mechanisms peptides and lipids aggregate into definite complexes of biological significance. This project has evolved from some experimental observations and through some of our current thinking on the structure and function of the plasma membrane. We have postulated that the membrane is composed of a lipid-bilayer with protein covering certain portions of the bilayer and also interdigitating with the lipid phase in some areas. The concept was developed, that a template mechanism or a coding principle exists which will determine the composition of the lipid phase. It was reasoned that a similar, if not identical, problem exists in the formation of the plasma lipoproteins. The study of lipoprotein synthesis appeared a logical approach to this problem and was selected since, a facile experimental approach seemed feasible.

Proposed Course: It is proposed to continue this investigation along the lines described above. Further studies will be made of the in vivo and in vitro incorporation of isotopic precursors. It is proposed to fractionate some of the lipoproteins after their isolation into their lipid and peptide portions and to investigate the metabolic fate of the latter. Since the methodology for the study of lipoproteins leaves much to be desired it is proposed spend considerable effort in improving the available methods and to search for new approaches for lipoprotein isolation or separation.

Publications:

1. Trams, E. G. and Brown, E. A.: Models for lipoprotein synthesis.

J. Theor. Biol., (in press).

2. Trams, E. G., Brown, E. A. and Lauter, C. J.: Lipoprotein synthesis I. Rat plasma lipoprotein composition and synthesis from radioactive precursors. Lipids, (in press).

1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Pilot studies and consulting services in neurochemistry

Previous Serial Number: Same.

Principal Investigator: Dr. E. G. Trams

Other Investigators: Mr. C. J. Lauter

Cooperating Units: (see below)

Man Years

Total:	0.6
Professional:	0.5
Other:	0.1

Project Description:

Objectives: To provide for various NINDB-IR programs and investigators a focal point for consultation, advice and assistance on neurochemical and biochemical aspects of research problems. To develop new approaches and test new methods; to study non-recurring problems amenable to current biochemical approaches; and as appropriate, to train and/or supervise technical or sub-professional personnel and research associated in such areas.

Methods: As required (see below).

Projects Prosecuted during FY 66:

1. For the Medical Neurology Branch (Dr. Carmichael): An extensive study on the purification of isotopic d-tubocurarine was initiated. The d-tubocurarine had been obtained from commercial sources and was then labeled with tritium by catalytic exchange methods. It was found that the labeled as well as the unlabeled curare occurred in the samples in a number of isomeric forms which could be separated by chromatographic techniques. It was observed that isotopic exchange partially destroyed d-tubocurarine and also labeled substances which had curare-like activity but were not d-tubocurarine. At the present time we are mainly concerned with the isolation of d-tubocurarine on a preparative scale for the Medical Neurology Branch. Attempts are under way to characterize and identify some of the curariform products in commercial d-tubo preparations. Chemical assays, radio-

assays and bioassays are used throughout in combination.

2. For the Laboratory of Biophysics (Dr. K. C. Cole): Choline fluoride was prepared. This substance apparently had not been prepared elsewhere and was not listed in any of the standard reference works. Preparation of choline fluoride was achieved by titration of choline carbonate with HF, lyophilization and recrystallization of the product.

3. For the Surgical Neurology Branch (Dr. Ommaya): Facilities have been made available and instruction provided to Dr. Faass for electrolyte analyses and space indicator analyses on cerebral tissues from monkeys subjected to experimental concussion.

4. For the Section on Limbic Integration, NIMH (Dr. P. McLean): A study was initiated with the aim to develop adequate staining methods for non-myelinated nerves in the CNS. As starting material bovine splenic nerve was obtained and various empirical approaches were investigated. At the present time it appears as if a more thorough study of the chemistry of the constituent elements of non-myelinated nerve are necessary before an adequate and specific staining method can be developed. It was suggested that in the interim, ultraviolet fluorescence microscopy (for catecholamines) might be used.

5. For Laboratory of Chemical Pharmacology, NHI (Dr. E. A. Brown): The occurrence and metabolism of catecholamines in elasmobranchs was investigated. In the larger elasmobranchs, the liver contributes about 20-25% of the weight of the animal. Approximately 20-25% of the liver weight is accounted for by lipids. It had been suggested that lipid metabolism is closely regulated by the autonomic nervous system and specifically by catecholamine levels. In the studies carried out so far, the metabolism of epinephrine and nor-epinephrine has been investigated. It was found that catechol-O-methyltransferase systems are quite active in elasmobranch fishes. In contrast, a number of other enzyme systems concerned with the inactivation of natural and foreign products showed very weak activity only.

6. The metabolic fate of hydrocarbons of the paraffin series was studied by the use of n-hexadecane- 2-H^3 . These hydrocarbons are major constituents of mineral oil which has been used as a simulated bulk vehicle for absorption studies. Considerable controversy exists as to the amount of hydrocarbon absorbed in the mammalian intestinal tract. It was found that in rats only minute amounts of radioisotope from n-hexadecane- H^3 could be recovered from tissues. Some of the metabolic products are simple oxidation products of n-hexadecane, such as n-hexadecanol and palmitic acid. A major metabolite has not been identified and the structure of this product is presently under investigation.

7. For Metabolism Service, NCI (Staff Members): Instruction was provided in the use of automatic amino acid analyzers.

Publications:

1. Fredrickson, D. S. and Trams, E. G.: Ganglioside Lipidosis: Tay-Sachs Disease. In Stanbury, Wyngaarden, J. A., and Fredrickson, D. S. (Eds.) The Metabolic Basis of Inherited Disease, McGraw Hill, New York, 1965, pp. 523-538.

ANNUAL REPORT
July 1, 1965 through June 30, 1966
Laboratory of Molecular Biology
National Institute of Neurological Diseases and Blindness
Intramural Research

Ernst Freese, Chief

A. Structure and Alteration of Nucleic Acids

1. Use of Transforming DNA for the Classification of Mutagenic versus Inactivating DNA Alterations.

Mutagenic DNA alterations are defined as those changes of DNA which do not prevent replication but occasionally or always give rise to a change in the sequence of bases in some of the progeny DNA. Inactivating DNA alterations, in contrast, do block nucleic acid replication, except when they are repaired or occasionally overcome otherwise. They either involve drastic changes of one or more bases, rendering impossible the pairing with a complementary base, or they interrupt the continuity of the information by crosslinking or breakage of the sugar phosphate backbone. These alterations are rarely mutagenic but lead to chromosomal breaks, large chromosomal alterations, or induced recombination. In transforming DNA, mutagenic alterations are measured by the induction of mutations, using the method of linked mutation induction, whereas inactivating alterations are measured by the inactivation of a particular transforming marker and the simultaneous absence of a significant mutagenic effect. These quantitative determinations permit one to explain the biological effects of different agents and enable one to predict the relative frequency of pointmutations versus large chromosomal alterations.

In the past the distinction between mutagenic and inactivating DNA alterations has not been possible, because an observed lethal effect could be caused either by an alteration of DNA itself or by a reaction of some other cellular component. Only one of the two types of alterations could therefore be measured at a time, either mutagenic alterations by the induction of mutations or large chromosomal alterations by cytological observations. Many agents such as peroxides which exhibited a weak mutagenic effect actually are strong inactivating agents with pronounced ability to induce chromosomal breaks. It is noteworthy that all strong carcinogens which can chemically alter DNA are inactivating rather than mutagenic agents.

The possibility to distinguish experimentally mutagenic and inactivating alterations in transforming DNA was first realized by the use of hydroxylamines. At a 1 M concentration hydroxylamine is predominantly mutagenic, by attacking cytosine. At concentrations of 10^{-2} M and smaller, however, hydroxylamine exhibits only an inactivating effect on transforming DNA and is no longer mutagenic. This inactivating effect depends on the presence of oxygen and it has now been shown that the reaction of hydroxylamine with

oxygen produces hydrogen peroxide. The hydrogen peroxide is destroyed again by a second reaction with hydroxylamine; its concentration is thus kept at a constant level. This finding explains the initially strange observation that high concentrations of hydroxylamine were less inactivating than low concentrations: at high concentrations of hydroxylamine the oxygen becomes limiting in the solution so that hydrogen peroxide is much faster destroyed than produced and consequently the inactivating effect is virtually absent at a concentration of 1 M hydroxylamine.

A strong inactivating effect on transforming DNA has been observed for several other compounds containing a free NOH group as well as by some hydrazines. Since these reactions depend on oxygen and give rise to hydrogen peroxide, it seems likely that all chemicals having these reactive groups will produce hydrogen peroxide and inactivate DNA. Most of these agents are carcinogenic.

2. The Effect of Hydrogen Peroxide on DNA and its Components.

Having observed that many inactivating agents give rise to hydrogen peroxide or the actually reactive OH radical, a systematic study of the reaction of hydrogen peroxide with DNA and its components was launched. It was found indeed that hydrogen peroxide has a drastic effect on DNA. It decreases the melting temperature of DNA and after prolonged treatment causes the complete separation of the two DNA strands at temperatures which are far below the ordinary melting temperature of DNA. When the reaction with the DNA nucleotides is measured, one observes the destruction of thymine, cytosine and guanine bases, which is noticed by a rapid decrease of the UV absorption of these nucleotides. The adenine deoxynucleotide releases the free base adenine which subsequently can further react, giving rise to a new UV absorbing compound. Experiments with radioactive compounds are under way which allow one to determine the decay products of the above reactions.

3. The Effect of Combining Mutagenic and Inactivating DNA Alterations.

When DNA harbors only mutagenic DNA alterations, its progeny produces mixed clones, which consist half of mutant and half of non-mutant organisms. This phenomenon can best be observed in phage T₄, in which the mutagenic alteration, by 1 M hydroxylamine, gives rise to mottled r-plaques. If this mutagenic effect is superimposed by an inactivating effect, either low concentrations of hydroxylamine or ultraviolet light, many pure mutant clones are produced. This conversion of mixed into pure clones is not observed in a mutant of phage T₄, which is deficient in its ability to repair ultraviolet induced lesions. Therefore, the conversion apparently is caused by a repair mechanism by which the inactivating alteration is cut out of DNA and the repair process copies the other mutated DNA strand, thus giving rise to a DNA molecule in which both strands are mutant.

4. Genetic Analysis of Virus Induced Mutants of Escherichia coli.

The properties of virus μ 1, which lysogenizes E. coli bacteria and simultaneously mutates them, has been further investigated, using lactose non-

fermenting mutants. Linkage tests have suggested that in such mutants the prophage is linearly inserted into the specific structural gene for either β -galactosidase or galactoside permease. The inserted phage apparently is under the control of the β -galactosidase operon, because addition of the inducer of β -galactosidase, isopropylthiogalactoside, increases the release of phage by a factor of 20.

5. Studies on the Structure of Chromosomes.

The DNA pieces which one isolates from cells must have been attached lengthwise to one another in chromosomes because genetic markers are one-dimensionally arranged. It is not known, however, whether this attachment involves only DNA itself or whether there exist any non-DNA links. The following experimental observation suggests ways in which this problem can be approached biochemically. When DNA was isolated from calf thymus nuclei in a glycerol-phosphate buffer, it showed a certain molecular weight distribution. But when the nuclei were first treated by saline EDTA at 4°C, before the DNA was isolated, the molecular weight of the subsequently obtained DNA increased with the time of treatment. It will now be attempted to obtain similar results in extracts of thymus nuclei and to identify the components required for the apparent polymerization of DNA.

B. Control Mechanisms and Differentiation.

Differentiation in higher organisms comes about by a multitude of individual biochemical reactions, each of which is subject to a specific control mechanism. The way in which a particular cell develops depends on its previous history, on its environment, and on its genetic constitution. In order to find out how these different factors interact and which molecular principles are involved, one has to investigate a simple system of differentiation for which both biochemical and genetic studies are feasible. *Bacillus subtilis* provides such a system for which mutants can be easily isolated and genetically characterized. One can study the induction and repression of individual enzymes such as alanine dehydrogenase and one can follow sporulation and germination. The sequence of the developmental processes during sporulation corresponds to a well-controlled sequence of functional steps by which the expression of individual genes is switched on; mutants blocked at different stages of sporulation also seem to be unable to perform any of the enzymatic reactions that occur later in the normal development. Specific results obtained in the last year with this bacterial system will be described below.

1. Derepression of Alanine Dehydrogenase in *Bacillus Subtilis* by Internal Induction.

Alanine dehydrogenase (Ald) is induced by L- or D-alanine and several alanine analogs. This enzyme is also increased by starvation of nicotinic acid, riboflavin, and pantothenate in mutants lacking the capability to synthesize these vitamins. Initially, this effect suggested a decrease in the concentration of an internal repressor whose synthesis or function might require the product of the vitamins as cofactors. It has been found, however,

that in all three mutants the internal concentration of L-alanine increases severalfold during the vitamin starvation. This increase is not caused by the increased alanine dehydrogenase itself because it has also been found in a mutant unable to produce alanine dehydrogenase. The apparent derepression of ALD by vitamin starvation seems to be therefore actually caused by an internal induction with L-alanine. This finding raises the question whether in other systems in which an apparent derepression has been observed the effect is not also caused by an actual internal induction. Experiments are planned to check this possibility.

2. Lack of Sporulation in Cytochrome a-Deficient Mutants of B. subtilis.

Cytochrome a is normally present in vegetative and sporulating cultures of *B. subtilis*. By mutagenic treatment 11 cytochrome a-deficient strains have been obtained. The cytochrome a concentration in these mutants is less than 15% of the parent strain. All of the mutants are either completely asporogenic or oligosporogenic, whereas the rates of exponential growth in sporulation medium are generally similar to the wild type. Revertants from the sporulation-minus character to a sporulation-plus character appear occasionally and are always accompanied by the restoration of cytochrome a activity to normal. These results show that in *B. subtilis* cytochrome a is not required for rapid exponential growth but seems to play an essential role in sporulation. The gene(s) for cytochrome a formation seems to be therefore a typical developmental gene, required only during differentiation.

3. A New Lysyl sRNA Produced During Sporulation.

Sporal transfer t-RNA can function as a substrate for amino acid activating enzymes from vegetative cells. Whereas the ability to accept several amino acids is significantly smaller in sporal than in vegetative RNA, the acceptance of lysine is more than twice that observed with vegetative RNA. Comparison of the MAK column elution profiles of lysyl transfer RNA from spores and vegetative cells shows that the spore preparation contains a species of lysyl t-RNA that is either absent in vegetative RNA or present only in very small amounts. This new lysine RNA appears late in sporulation, at the time at which the spore wall is synthesized and the prespore becomes refractile. The physiological role of this new species of lysine t-RNA will be examined.

4. Initiation of Germination.

The germination of *Bacillus* spores is specifically initiated by L-alanine. This process occurs in the presence of actinomycin D or chloramphenicol and therefore does not seem to require either nucleic acid or protein synthesis. In order to elucidate the mechanism of spore germination, mutants have been isolated which can no longer germinate in the presence of alanine alone. One of these mutants could germinate when, in addition to alanine, autoclaved glucose was added to the medium. It was eventually found that the germination of this mutant could occur when both fructose and glucose were added in addition to L-alanine. This finding indicates that germination is not merely

caused by the allosteric change of a protein but requires a series of enzymic reactions. During sporulation sucrose presumably accumulates and can subsequently be broken down to glucose and fructose during germination. These compounds are then further utilized for energy-producing reactions. In the deficient mutant, the enzyme which breaks down sucrose presumably is no longer present. This possibility is under investigation. It can now be understood why different sporulating organisms isolated in nature have different germination requirements. They are blocked at different steps of a complex network of reactions.

Serial No. NDB(I)-62 LMB/OC 947
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

FHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Structure and Alteration of DNA and Chromosomes.

Previous Serial Number: SAME

Principal Investigators: Elisabeth Bautz Freese, Ph.D., Ernst Freese, Ph.D.,
and Richard Welsh, Ph.D. (Guest Worker)

Other Investigators: Marvin S. Melzer, Ph.D. and Hans-Jürgen Rhaese, Ph.D.

Cooperating Units: None

Man Years:

Total:	4.7
Professional:	3.0
Other:	1.7

Project Description:

Objectives: 1. It is now possible to distinguish experimentally between mutagenic and inactivating alterations of DNA. Whereas the molecular mechanism of agents which induce mutagenic DNA alterations has been extensively studied, the chemical investigation of inactivating DNA alterations has been disregarded, presumably because many scientists were preoccupied with the determination of the genetic code. It is now clear, however, that inactivating alterations are responsible for chromosomal breaks and large chromosomal alterations, and thus give rise to diseases such as cancer. We have investigated the biological and chemical effects on DNA of typical inactivating agents such as low concentrations of hydroxylamine and hydrogen peroxide. We have further shown that inactivating DNA alterations, which do not induce pointmutations, can nevertheless alter the genetic expression of a mutation which has been induced by a mutagenic agent. 2. It is not known whether the DNA pieces in a chromosome are longitudinally held together by non-DNA links or whether DNA exists as one continuous double strand of which one chain is occasionally interrupted. Attempts are under way to decide between these alternatives.

Methods Employed: The production of hydrogen peroxide from hydroxylamine has been measured by the titanium sulfate method. For DNA melting and long term alterations of bases a Gilford recording spectrophotometer was employed. Spectra were measured in the Cary recording spectrophotometer. Reaction products of hydroxylamine and hydrogen peroxide were separated by paper chromatography eluted and then measured spectrophotometrically. T4 phages were mutated by treatment with hydroxylamine and subsequently inactivated by UV irradiation. Phages were plated on bacteria B for the detection of r- and mottled mutant plaques as well as for the determination of survival. Mutation in transforming DNA was measured by the observation of fluorescent mutant colonies and inactivation by the survival of the tryptophan marker. Di- and trinucleotides of deoxy-AMP were chemically prepared. The methods for chromosomal studies were the same as in the previous report.

Major Findings: 1. The reaction of dilute concentrations of hydroxylamine with DNA. a) We have shown that hydroxylamine reacts with oxygen to produce hydrogen peroxide. The produced hydrogen peroxide, or any hydrogen peroxide added to the reaction mixture, reacts further with the hydroxylamine to produce higher oxidized nitrogenous compounds. All of these compounds are inactive on transforming DNA, except perhaps nitroxyl, which is a very unstable intermediate of the reaction. The predominant compound responsible for the reaction of dilute concentrations of hydroxylamine with DNA apparently is the OH radical. The oxygen dependence of the reaction has been established by showing that no reaction occurs in nitrogen, and the radical involvement has been proven by the fact that addition of chelating agents which eliminate trace metal elements or are radical quenchers prevent the reaction with DNA. b) All four DNA nucleotides react with dilute concentrations of hydroxylamine, the effect on dGMP and dTMP being most pronounced. dAMP mostly reacts by the separation of adenine from the deoxyribose. Di- and trinucleotides of dAMP also showed the removal of A and the kinetic evaluation of the data revealed that any backbone breakage must be the indirect product of the removal of adenine followed by the breakage of the sugar phosphate backbone. c) Dilute concentrations of hydroxylamine have a very pronounced effect on DNA, especially at pH 9. During this reaction the extinction coefficient of DNA continues to rise at a rate which rapidly increases with the temperature. Eventually the two DNA strands have separated completely, as is shown by the subsequent decrease of the extinction coefficient. Similarly, very small amounts of hydrogen peroxide have a very pronounced effect on DNA.

2. The reaction of hydrogen peroxide with DNA nucleotides and their components. a) Hydrogen peroxide causes the removal of all four bases from their nucleotides. In the case of dTMP and dGMP, the bases are further destroyed so that the extinction rapidly decreases. In the case of dAMP the base A is removed and slowly reacts further with hydrogen peroxide, H_2O_2 . This reaction cannot occur on the nucleotide itself, but only after the base has been removed from the nucleotide, because the frequency of free A increases linearly with the time, whereas the frequency of the further reaction

product increases with the square of the time. dCMP reacts only very little with H_2O_2 . b) The reaction of H_2O_2 with the DNA bases and nucleosides verifies the findings made on the nucleotides and allows the identification of further reaction products.

3. Induction of Pure Mutant Clones by Repair of Inactivating DNA Alterations. When T4 phages are treated by high concentrations of hydroxylamine only mutagenic DNA alterations are induced which give rise to mixed mutant clones that can be seen as mottled r-mutant plaques. Inactivating DNA alterations, which can be induced, e.g. by low concentrations of hydroxylamine or UV, do not induce point mutations but nevertheless have a strong effect upon the mutant induction in T4 phages: the fraction of mixed mutant clones decreases during this treatment, whereas the fraction of pure r-mutant clones increases. If a phage mutant is used which cannot repair UV damage, no such preferential decrease in the frequency of mixed mutant clones has been observed. The results show that during the repair of a UV-induced lesion, the complementary DNA strand is copied and any mutagenic DNA alteration which might occur in this region is copied as a mutation. In this way, phage DNA which produces mixed mutant clones is converted into DNA which produces pure mutant clones.

When the inactivating DNA alterations were induced by low concentrations of hydroxylamine, the same decrease in the fraction of mixed mutant clones was observed in both the standard and the mutant strains. This result indicates that inactivating DNA alterations can be repaired in both strains. Hence the mutant strain, which cannot repair UV damage, is apparently unable to cut DNA near a UV-induced thymine dimer, whereas it is still able to repair DNA which has already been cut by the action of hydroxylamine.

4. Possible Peptide Links between DNA Molecules in Chromosomes. (Welsh)

DNA was purified from calf thymus nuclei in a glycerol phosphate buffer after the nuclei were treated by saline EDTA at $4^{\circ}C$ for different times. The longer the nuclei remained in saline EDTA the larger was the molecular weight of the isolated DNA. These results show that on standing in saline EDTA, the DNA in nuclei undergoes a change which renders it more resistant to the shear which is exerted during the subsequent isolation procedures. It is possible that special components which are present in the cell sap are used by the cell to link separate DNA strands together. The identification of such linking units will be attempted.

Significance of Program to the Institute: The drastic effects which low concentrations of hydroxylamine or hydrogen peroxide have on DNA explain why similar compounds have a strong chromosomal breaking action in vivo and why they are carcinogenic. Since both of these agents act via OH radicals, other compounds such as hydrazines or radiation, which produce such radicals, will give rise to the same effect on chromosomes. The observed reactions of hydrogen peroxide with DNA characterize therefore a large class of chromo-

somal breaking agents which induce only few pointmutations.

The combination of mutagenic and inactivating DNA alterations has shown that repair mechanisms are important also for the induction of mutations and that such repair mechanisms involve the copying of the complementary DNA strands. Undoubtedly chromosomal repair mechanisms are the major feature which preserves hereditary information also in higher organisms. If such repair mechanisms break down, either because the genes making the corresponding enzymes are missing or because a particular agent inhibiting such enzymes is contained in the food, minor doses of radiation or chemicals which induce inactivating DNA alterations can have drastic effects for the organisms.

The knowledge of the structure of chromosomes is important for the understanding of mutations, chromosomal duplication and function. If peptide links between DNA pieces should exist, they would provide an explanation for the extensive repair of chromosomal damage, which has been observed cytologically.

Proposed Course of Project: 1. Continue by radioactive methods to analyze the reactions of hydrogen peroxides and alkylating agents with oligonucleotides and DNA. 2. Examine the effect of a mutant altered in DNA polymerase on the mutation induction by different chemicals.

Honors and Awards: None

Publications:

Freese, E. and Freese, E. B.: The oxygen effect on deoxyribonucleic acid inactivation of hydroxylamines. Biochemistry 4: 2419-2433, Nov. 1965.

Freese, E.: Hereditary consequences of different DNA alterations. In Proc. of Mendel Centennial Symp. Prague (Aug. 1965). In press.

Freese, E. and Freese, E. B.: Mutagenic and inactivating DNA alterations. Proc. of Conf. on Radiation Microbiology, Chicago (Oct. 1965). Radiation Res. Suppl. In press.

Freese, E., Freese, E. B., and Graham, S.: The oxygen dependent reaction of hydroxylamine with nucleotides and DNA. Biochem. Biophys. Acta. In press.

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Genetic Analysis of Virus-Induced Mutants of Escherichia coli.

Previous Serial Number: SAME

Principal Investigator: Austin L. Taylor, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 0.32

Professional: 0.16

Other: 0.16

Project Description:

Objectives: The long term objective of this study has been to analyze, through a combined genetic and enzymological approach, the mechanism by which temperate bacteriophage Mu-1 induces mutations in bacteria. The two month period covered by this terminal report was devoted primarily to an investigation of virus-induced mutations in the lactose operon of E. coli.

Methods Employed: Conventional methods of genetic analysis and of enzyme assay were used throughout this work.

Major Findings: Previous work had shown that lactose non-fermenting (lac⁻) mutants obtained after infecting wild type lactose-fermenting E. coli with phage Mu-1 all possessed a Mu-1 prophage closely linked to the lactose genes. Moreover, linkage tests suggested that the prophages might be linearly inserted into the specific structural genes for either β -galactosidase (lac Z) or galactoside permease (lac Y), both of which are required for normal lactose metabolism. The insertion of foreign viral DNA (i.e., the prophage) into the continuity of a structural gene could well account for the mutational loss of the gene's function. Further evidence to support this insertion model was obtained in the following experiments.

Phage-induced galactoside permease deficient mutants (lac Z⁻Y⁻) were grown in the presence of isopropyl- β -thiogalactoside (IPTG), a gratuitous inducer of β -galactosidase and permease synthesis. The specific activity of β -galactosidase increased in normal fashion in such cultures. The amount of free Mu-1 phage released by these cultures was also determined. In the presence of IPTG, the amount of phage released by induction of the prophage was 20 to 40 times greater than the amount produced in the absence of IPTG. Thus, induction of enzyme synthesis by the lactose operon (to which Mu-1 prophage is genetically linked) led to a concomitant partial induction of vegetative phage development.

It is possible that IPTG might induce prophage in some non-specific manner totally unrelated to the induction of the lac operon. To test this possibility, a mutant regulator gene (lac i⁻) was placed into the phage-induced lac Y⁻ mutant strain. This gene permits constitutive (fully induced) synthesis of β -galactosidase at all times, even in the absence of inducers such as IPTG. The maximum yield of free Mu-1 phage from cultures of this strain was again 40 times greater than the yield from non-induced lac Y⁻i⁺ strains. Partial induction of the prophage is therefore not dependent on the presence of IPTG, but is instead a consequence of β -galactosidase synthesis. One might ask whether β -galactosidase (or one of the metabolites produced by it) is the actual inducer of prophage. This seems unlikely because 1) induction of the lactose operon with IPTG has no inducing effect on Mu-1 prophages located outside of the lac operon and 2) IPTG does partially induce Mu-1 prophage in lac Z⁻Y⁺ mutants (phage-induced mutants that are unable to synthesize any detectable amount of β -galactosidase).

It is well established that one of the first steps in enzyme induction is a rapid acceleration of messenger RNA (m-RNA) synthesis on the DNA template of the structural genes undergoing induction. On the other hand, little is known about the initial steps in the induction of prophage development. One of these steps must, however, involve synthesis of viral m-RNA bearing information for structural and possibly also catalytic proteins of the phage.

The prophage insertion model offers a plausible explanation for the phage- β -galactosidase coinfection observed in these experiments. It is possible that once m-RNA synthesis has been initiated in the lac operon, it may occasionally continue onward to transcribe the viral DNA which is inserted into DNA of the lac structural genes. This fortuitous formation of viral m-RNA might then trigger those events which ultimately lead to vegetative growth and release of mature virus particles.

Proposed Course of Project: This project is complete as of August 28, 1965.

Honors and Awards: None

Publications: None

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Participation of Ribonucleic Acid in the Regulation of Cellular Metabolism.

Previous Serial Number: SAME

Principal Investigator: Robert A. Lazzarini, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 2.1

Professional: 1.0

Other: 1.1

Project Description:

Objectives: Currently a body of evidence is developing that indicates that the control of protein synthesis is exerted at the level of both messenger RNA and transfer RNA. Although the role of transfer RNA in synthetic reactions is well established, the details of its role in regulatory mechanisms are unknown. The primary objective of this project is to study the participation of RNA, particularly transfer RNA, in the regulation of protein synthesis and gross morphogenic changes in *Bacillus subtilis*.

Methods Employed: Standard biochemical techniques are employed for the purification of RNA and enzymes from microbial spores and vegetative cells. Transfer RNA is separated from ribosomal RNA and DNA by column chromatography on Sephadex G-100. The amount of transfer RNA capable of accepting a specific amino acid is estimated by enzymic esterification of the t-RNA with radioactive amino acids of known specific activities. Chromatography on methylated albumin-kieselguhr and enzymic esterification of t-RNA with heterologous enzymes capable of recognizing only some of the species are employed in examining the heterogeneity of t-RNA that accept a particular amino acid. Coding properties of t-RNA from spores and vegetative cells are measured by their binding to ribosomes in the presence of polyribonucleotides.

Major Findings: Sporal t-RNA can function as a substrate for amino acid activating enzymes from vegetative cells. The ability of spore RNA to accept several amino acids is significantly less than vegetative RNA. In contrast, the acceptance by sporal RNA of lysine was more than twice that observed with vegetative RNA. Comparison of the elution profiles of lysyl t-RNA from spores and vegetative cells shows that the sporal preparation contains a species of lysine t-RNA that is either absent in vegetative RNA or present only in very small amounts. The appearance of this new lysine RNA occurs late in sporulation, at the time when the spore wall is synthesized and the prespore becomes refractile.

Significance to Program of the Institute: Control of protein synthesis and of the grosser morphogenic changes (sporulation and germination) in bacterial systems represents examples of regulatory mechanisms which are at this juncture more amenable to experimental investigation than the more specialized mammalian systems by virtue of the number of parameters which can be varied and controlled easily. It is anticipated that information obtained from these simpler systems will help form a basis upon which to investigate the more complex regulatory mechanisms operative in higher organisms and eventually to understand and control disease states of these systems.

Proposed Course of Project: The principal objective for the coming year is to examine the physiological role of the new species of lysine t-RNA. In attaining this objective two approaches will be employed: 1) further characterization of the sporal lysine t-RNA and comparison to its vegetative counterpart, and 2) a study of the biochemical events taking place late in sporulation when the new species of lysine t-RNA appears.

Honors and Awards: None

Publications: None

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Control and Differentiation of Protein Synthesis and Function

Previous Serial Number: SAME

Principal Investigator: Ernst Freese, Ph.D.

Other Investigators: L. Robert Berberich, M.D., Michael Kaback, M.D.,
Rüdiger Schmitt, Ph.D., Harry Taber, Ph.D. (Guest
Worker), and Richard Wax, Ph.D.

Cooperating Units: None

Man Years:

Total:	5.9
Professional:	4.3
Others:	1.6

Project Description:

Objectives: Differentiation in higher organisms comes about by a multitude of individual biochemical reactions, each of which is subject to a specific control mechanism. In order to learn the major principles, we are studying such control mechanisms in simple biological systems in which biochemical and genetic methods can be combined. We have concentrated on three related aspects which will be described: 1. The control of the synthesis of alanine dehydrogenase (ALD). 2. The control of sporulation. 3. The control of germination.

Methods Employed: Special methods have been developed for the measurement of L- or D-alanine in cells and their surrounding media. In particular, the concentration of L-alanine is measured enzymatically by the use of alanine dehydrogenase, ALD. Spores have been isolated and purified by a cesium chloride centrifugation and their germination has been measured spectrophotometrically. For the isolation of cytochrome deficient mutants, staining by benzidine chloride and subsequent characterization of cytochromes in a specially designed spectrometer were used.

Major Findings: 1. Derepression of alanine dehydrogenase synthesis by internal induction (Kaback, Berberich and Freese). The synthesis of ALD is induced by addition of alanine or several L- or D-amino acid analogs of alanine to growing *Bacillus subtilis*. The synthesis of this enzyme is also increased by a factor of 100 when mutants of *B. subtilis*, which are deficient in nicotinic acid, riboflavin or pantothenic acid synthesis are starved for the required vitamins. The enzyme increase found during starvation of a particular compound has been called "derepression", because presumably an internal repressor of the specific enzyme synthesis is no longer made. However, in our system as well as in other systems in which derepression has been observed, the increased synthesis of enzyme could equally well have been caused by an increased amount of some other compound which accumulated as the result of the particular starvation used. We have tested this possibility for ALD and have found indeed that in all three vitamin-requiring mutants the amount of L-alanine greatly increases during vitamin starvation. This increase is not caused by the concomitant increase of ALD, because it has also been found in a mutant unable to produce ALD. We therefore conclude that the apparent derepression of ALD in these mutants is actually caused by an internal induction of ALD owing to the greatly increased amount of L-alanine.

2. Control of germination (Wax and Freese). The germination of spores of *Bacillus subtilis* is initiated specifically by the addition of L-alanine. Using our ALD⁻ mutants we have shown that this induction depends only to a minor extent on the presence of an active ALD, because the ALD⁻ mutants can still germinate. The mechanism of initiation by L-alanine is still obscure. However, we have found among our mutants one which could no longer germinate in the presence of alanine and not even in a very rich medium. The germination could be obtained only if the rich medium had been autoclaved. After many attempts we eventually identified the compounds needed for the germination of this mutant: they are both glucose and fructose in addition to L-alanine. This finding shows that the initiation of spores is not as simple a process as had been envisaged by some scientists, but requires a whole series of enzymic reactions which can now be studied. Spores normally accumulate a compound, presumably sucrose, during sporulation, which they convert during germination into glucose and fructose. We are currently attempting to show that an enzyme (invertase or sucrase) is missing in the mutant spores. Since the vegetative cells of the mutant can grow perfectly well in normal minimal medium, the particular mutant enzyme is only of developmental significance to *B. subtilis*.

3. The effect of cytochrome a-deficient mutants on sporulation (Taber). A further example for the mutation of a developmental gene has been observed in cytochrome a-deficient mutants. All mutants which lack cytochrome a either completely lack the ability to sporulate or form fewer than 5% spores (the parent strain ordinarily forms 80-90% spores). The vegetative growth of these mutants, however, is completely normal. The cytochrome a deficiency is directly responsible for the inability to sporulate, because all revertants to sporulation have at the same time regained the ability to form cytochrome a.

Significance to Program of the Institute: The molecular understanding of differentiation is more amenable to analysis in microorganisms than in higher organisms because of speed of experimentation and the possibility to isolate many mutants. Nevertheless, fundamental biological processes are essentially the same in both types of organisms. Any findings in microorganisms can therefore be immediately extrapolated to postulates about the mechanism of differentiation in more complex systems. Sporulation and germination of *Bacillus subtilis* is a particularly simple model system for the study of differentiation. The vegetatively growing cells may be compared to the early embryonal cells which do not yet show much differentiation. The transition to a cell which no longer grows but changes its type to that of a sporulating cell and eventually gives rise to spores can be compared to the differentiation of a particular cell type in higher organisms. Already from our limited study involving several different mutants, it is clear that the simple process of sporulation involves many different biochemical steps and many different developmental genes and enzymes. The mechanism by which these genes are sequentially switched on during development presumably is undoubtedly similar in micro- and macroorganisms.

Proposed Course of Project: Using both mutants and biochemical methods, we shall attempt to further elucidate critical steps in developmental processes, in particular the trigger mechanisms which lead to sporulation and germination.

Honors and Awards: None

Publications:

Freese, E. and Yoshida, A.: The Role of Mutations in Evolution. In Bryson, V. and Vogel, H. J. (Eds.): Evolving Genes and Proteins. New York, Academic Press, 1965, pp. 341-355.

SUMMARY REPORT

July 1, 1965 through June 30, 1966

Laboratory of Perinatal Physiology

National Institute of Neurological Diseases & Blindness

Ronald E. Myers, Chief

The Laboratory has acquired the island of Desecheo lying 12 miles off the west coast of Puerto Rico. This island, approximately 600 acres in size, is of a mountainous, forbidding terrain and is covered with thick vegetation. It is currently being developed as part of the primate ecology program. An integrated social group of approximately 70 monkeys will be transferred from Cayo Santiago to Desecheo. This group of monkeys is currently under intensive study on Cayo Santiago with reference to social organization, dominance hierarchy, and reproductive behavior. After transfer to Desecheo, this group again will be observed for changes in social structuring which may result from the sudden transport of the group into an alien environment. Particular attention will be paid to whether the group's integrity will be sustained or whether the group will fracture off into smaller subgroups. Attention will be paid to the reproductive periodicity and to the dominance hierarchy to ascertain possible effects of the disruption in life pattern brought about by the transplant.

Detailed accounting of the pattern of reproductive activity and of birth on the islands of Cayo Santiago, La Cueva, and Guayacán has continued in an effort to characterize the pattern of cyclic reproductive activity as expressed in these three separate island circumstances. Of particular importance is the fact that La Cueva and Guayacán share the same climatic and ecological environment although they remain entirely separate as ecological niches. In contrast, the island of Cayo Santiago, located at the opposite end of Puerto Rico, represents a different environment, having an annual rainfall of 60 to 80 inches per year, in contrast to the dry, arid climate of La Cueva and Guayacán which enjoy only 20 inches of rain per year. The seasons of reproductive activity and reproductive yields are settling down to a more stable pattern on a year by year basis in La Cueva and Guayacán as the monkey populations there achieve a greater degree of stability. In the past year the breeding and birth seasons on La Cueva and Guayacán have become closely similar in their timing and in the characteristics of their normal distribution. This is in contrast to the breeding and birth seasons on Cayo Santiago where the peaks of activity occurred three months earlier. Continuing statistics of this sort over the next three to five years are required in order to more definitely determine the validity of the proposition which is suggested that breeding cyclicality in the primate may relate to environmental factors. The addition of Desecheo as a facility for free-ranging monkey studies will add further dimensions to the analysis of the factors defining breeding periodicity.

Earlier studies carried out on Cayo Santiago have indicated that the testes of the male rhesus monkey undergo cycles of hypertrophy and atrophy paralleling the cyclicity inherent in the breeding season. Currently, studies are being initiated of the ovaries and uterus of the female to determine whether menstrual cycling occurs throughout the year in the free-ranging circumstance as it does in the caged colony and whether the ovaries undergo changes in morphology in relation to the breeding season.

In the experimental enclosures on La Cueva studies involving manipulation of social structure are in progress with replacement of the dominant male by another strange male for specified periods of time. Induced changes in the social rank of the adult females quickly occur under this circumstance with loss of rank of the consort of the removed dominant male and increases in rank of less dominant females who initiate a consort relationship with the new dominant male. Reversal of these circumstances can be seen with replacement of the previously dominant male. Interestingly, the rank changes of the adult females are accompanied by changes in rank of their offspring. It is seen that the offspring participate actively in displays of aggression against others with increase in rank and share in the reception of aggression on fall in rank. Another study utilizing connecting tunnels between experimental enclosures has indicated that aggressive displays between animals of independent social groups occur primarily between dominant animals. Parallel studies on bands of free-ranging monkeys support the conception that significant interactions between bands occur primarily through the high-ranking members.

A wide range of studies has been carried out by guest workers on Cayo Santiago including studies of differences in behavior of young males of dominant mothers and those of subdominant mothers, studies of the importance of novelty in the expression of curiosity by the monkey, the dynamics of inter-band encounters, and long-term studies of social behavior in a specific monkey band.

In the central laboratory in San Juan, studies have been carried out of the mating behavior of caged Rhesus monkeys for the purpose of better understanding primate reproductive behavior itself, for ultimately investigating the neural mechanisms underlying various aspects of reproductive behavior, and finally as part of an effort to increase reproductive yields of the breeding caged colony. A count is taken of the menstrual cycle of individual females in the breeding colony. The individual females are then exposed for a two-day period to males starting on the 11th day after the beginning of the last menstrual flow. The behavior of the animals is observed through closed circuit television. Specific compatibilities or affinities exist between specific individuals while antagonisms occur between others. These compatibilities or antagonisms are so important that breeding succeeds or fails in relation to them alone. Exposure of specific individuals may yield only aggressive behavior. The maturity and experience of the individual males is another important factor in determining reproductive success and in expressing reproductive behavior. Males born in the laboratory and cage-

reared males exhibit impairments in the capability and the expression of reproductive behavior as compared to males of comparable age raised in the wild. Mating behavior is usually initiated by the male in the caged circumstance. However, initiative is occasionally taken by receptive females when paired with caged-reared males. Grooming activity is prominent between female and male in relation to reproductive activity. The great majority of grooming activity is female grooming male. In cages reproductive activity and conceptions occur throughout the year in contrast to the marked cyclicity observed in the free-ranging colonies.

In physiological psychology progress has been impaired by the lack of skilled technical personnel for the development of solid state control systems for the behavioral situations. However, progress has occurred in several areas where direct testing of animals was possible. The deficit following lesions of the prefrontal cortex in the monkey has been in delayed response performance. Classically, delayed response testing has been carried out with a pair of identically appearing visual objects with choice of correct response in relation to spatial rather than object cues. Considerable study and effort has been expended in an effort to determine the nature of this deficit in delayed response. In current studies the cue which tells the animal of the location of the reward is an object rather than spatial cue in that two objects, distinct and different in appearance, are utilized. One is rewarded depending upon the cue afforded the animal with each presentation. Lesions of prefrontal cortex disturb object-cued as much as spatial-cued delayed response performances. The essence of the deficit with prefrontal cortex lesions is one relating to delay in responding to an appropriate cue as such rather than a deficit having relation to spatial set as such.

In studies of commissure function it has been found that the localization of transfer of training between the hands of learned motor skill is co-extensive with that of the transfer of training between the hands of sensory discrimination tasks. This result indicates that the problem of learned motor skills is one of parietal lobe physiology primarily rather than one of physiology of frontal lobe and emphasizes the importance of sensory feedback from the extremities in relation to the motor act at the level of acquisition of motor skills. These findings fit with the suggestion from clinical experience that lesions of parietal lobe are more disturbing of the skilled motor act than are lesions elsewhere of the cerebrum.

Studies have been initiated attempting to define the neural mechanisms underlying vocalization in the monkey. Progress to date has confirmed the possibility of bringing vocalization of the monkey under environmental control by means of a behavioral test situation rewarding the animal with food upon vocalization. In these studies investigations will be carried out of cortical and subcortical mechanisms and their relation to vocalization.

In the area of comparative neurology studies have been completed on the ascending spinal projections in the lizard. The most interesting finding is that few fibers from the spinal cord terminate as far rostrally as the

thalamus. The great majority of fibers end in the lower brain stem and in cerebellum of the same side. Interestingly, no fibers end in the optic tectum as has commonly been ascribed for lower forms. Also, in carnivores and primates fibers from spinal cord fail to terminate in the superior colliculus. These findings cast doubt on the theory that superior colliculus is a nodal point for reception and interpretation of sensory input from diverse modalities in lower forms. These studies are currently being extended to other lower vertebrate types in an effort to define the phylogenetic evolution of the spinal projections to the brain. Other studies currently in progress include comparative studies of retinal projection in fish, amphibians, reptiles and mammals. In lower forms there is, in addition to projections to the optic tectum, multiple other projections leading to hypothalamus, ectomammillary nucleus, and to other foci. These latter projections, when fully analyzed for various forms, may prove of considerable importance to understanding of some of the reflex and autonomic aspects of light stimulation.

Efforts are being made in the area of neuroanatomical studies to develop further the techniques for investigation of fiber degeneration in the nervous system. Specifically, some success has already been obtained in using tinctorial stains for the demonstration of degenerating fibers. This technique may prove more successful than the silver techniques currently in use which are difficult to achieve uniform success with and which require careful individual handling of the tissue sections.

In the area of fetal physiology studies have been completed on the potential value of chemical analysis of amniotic fluid as an indicator of the fetal circumstance in utero. If the fetus in utero suffers embarrassment in relation to its acid-base status or if the fetus dies in utero, there may be critical alterations in chemistry of the amniotic fluid reflective of these alterations. The oxygen tension of amniotic fluid has been proposed as a possible indicator of fetal circumstance. However, on theoretical grounds, namely, because of the poor diffusibility of oxygen, it has suggested that oxygen tension may be a poor index of fetal circumstance. On the other hand, carbon dioxide, because of its good diffusibility, might be a more accurate indicator. For these reasons studies of amniotic fluid chemistry were carried out. The lactate concentrations in amniotic fluid show extreme variability in healthy fetuses at different gestational ages and also at the same gestational age. This degree of variability of lactate concentration in apparently healthy fetuses suggests that lactate levels in amniotic fluid bear a poor relationship to fetal circumstance. A separate study has determined and characterized as curves the pH, the bicarbonate concentration, and the partial pressures of CO₂ at different gestational ages. The variability of these values in different fetuses of the same gestational age has been relatively small. Furthermore, studies of concentrations of these substances following experimental fetal embarrassment and/or fetal death in utero has indicated these values shift in relation to fetal circumstance but with a time lag of 15 to 30 minutes. Hence, although determination of these substances in the amniotic fluid gives

a measure of fetal circumstance, the considerable time lag makes it of a lesser practical clinical importance. Of interest is the fact that the values of these substances in the amniotic fluid follows their values in the maternal bloodstream only after long time delays of up to 12 to 24 hours (in the presence of a dead fetus) . Hence, amniotic fluid is in slow equilibrium with the fetus. Alterations and adjustments of the acid-base status of the amniotic fluid occur through the fetus and in turn through the umbilical circulation. Some practitioners assert that high concentrations of oxygen given to the mother during parturition or under clinical stress have a deleterious effect on fetal welfare by virtue of vascular constriction within the utero-placental circulation. Such a circumstance is believed to occur in retrolental fibroplasia in relation to hyperoxygenation of the fetus. In a series of experiments involving chronic catheterization of fetal as well as maternal blood vessels it was determined that high concentrations of oxygen given to the mother in no circumstance produced deterioration of the fetus in terms of cardiovascular or acid-base status of the blood. It was interesting that babies in the best circumstances were the ones receiving the greatest effect on these functions whereas fetuses who have already undergone deterioration in acid-base and cardiovascular status benefit least from oxygen administration to the mother.

There has been increasing interest in the relation between birth weight and gestational age in the human. It has come to be recognized that there are babies who are too small for their gestational age at the time of delivery. These "small for dates" babies exhibit different mortality-morbidity statistics than do premature babies of the same weight. Generally speaking, such babies represent far better risks for survival but have medical problems of their own including increased incidence of pulmonary hemorrhage, tendency towards hypoglycemia during the perinatal period with consequent higher risk of brain damage, etc. It has been suggested that "small for dates" babies are associated with placental insufficiency. A study has been initiated on the consequences of placental insufficiency in the monkey by ligating fetal umbilical and placental vessels at different gestational ages. Such devascularization procedures result in considerable morphological changes in the placenta which are being analyzed by a placental pathologist within NINDB for correlation with placental pathology in the human. The results of this study have clearly indicated a relationship between the extent of placental insufficiency as produced by varying degrees of devascularization and the degree of growth retardation exhibited in the offspring. Growth retardation up to 45% has been exhibited by animals who have had ligation of fetal placental vessels. Lesser degrees of growth retardation have been seen with lesser degrees of devascularization. In addition, several instances of mild brain damage have been exhibited by animals in this series. No relation has been found between the degree of growth retardation and the occurrence of minimal brain damage. The conclusion of this study has been that "small for datesness" can be directly related to placental insufficiency.

Abruptio placentae is of major concern as a cause of fetal death or severe fetal brain damage. Little experimental work has been done on the problem of experimental abruption. A program of investigation of abruption as experimentally produced in the monkey has been initiated in the laboratory. Abruption of placentae beyond 25% to 40% of total volume results in immediate death of the fetus. Lesser degrees of abruption are tolerated and lead to brain damage. Interesting morphological changes occur as a result of the devascularization of the placenta from the maternal side. Within the study of placental abruption and placental insufficiency there are available pathological specimens of the placenta representing all varieties of devascularization from both the fetal and maternal sides. These studies together are yielding a better understanding of pathological changes of the placenta. It may be concluded that there exist only minor degrees of redundancy with regards to the placental tissue in relation to the support of fetal growth and well-being. The degree of redundancy diminishes with gestation.

An investigation of the pathology of the stillborn has been initiated with the focus of attention being primarily upon the problem of maceration. It is noted that stillborns can be divided roughly into those undergoing varying degrees of maceration or post mortem liquefaction and those in whom there is a relative preservation of morphology of organs despite prolonged death and incubation at body temperature in utero. The study of the pathology of the stillborn will be assessed primarily in relation to the thesis that maceration of the fetus occurs following sudden death of the fetus in utero whereas preservation of morphology with ultimate mummification relates to circumstances of gradual death of the fetus with gradual supervention of arterial hypotension. At a later time this thesis will be attacked experimentally. The pathology of the brain of the stillborn fetuses has yielded some interesting patterns of pathological change. Among these patterns the most prominent has been that of perivenular hemorrhage into the white matter. This pattern has also been seen in fetuses dying of asphyxiation at term.

Studies of the neuropathology of asphyxiation at birth have continued with the monkey. The studies have emphasized both the characterization of the morphological changes in the brain in relation to asphyxiation but particularly also the correlation of patterns of neuropathological change seen with patterns of functional derangement produced in the fetus during asphyxiation itself. Generally speaking, the pathology of perinatal asphyxia produces a reliably reproducible pattern of clinical and neuropathological change. Clinically the animals exhibit profound sensory changes over the distribution of trigeminal nerve and throughout the body. They show difficulty with sucking and swallowing, alterations in voice, and ataxic changes secondary to the sensory losses. Pathologically the animals exhibit destruction in specific brainstem centers such as the sensory trigeminal nucleus, the vestibular nuclei, the basal cerebellar nuclei, and in certain thalamic nuclei, most particularly the posterior and lateral ventral nuclei. The pathological changes vary from mild chromatolytic changes in neurons

to areas of focal necrosis and/or hemorrhage. However, other patterns of pathological changes are seen such as laminar necrosis, brain swelling, and perivenular hemorrhage in the white matter. Laminar necrosis has commonly been associated with brain swelling and has been seen in severely acidotic fetuses. A single case of severe cortical atrophy with ulegyria has been seen with neonatal asphyxia and was associated with severe bilateral retinal hemorrhages and increased venous pressure. Further studies utilizing asphyxia at term along with studies of blood pressure, heart rate, acid-base status, electrolyte distribution, lactate and other moieties of the blood will hopefully yield a greater insight into the relation between functional embarrassment during asphyxiation and patterns of neuropathological change.

During the course of studies with asphyxiation at term a remarkable difference was witnessed between babies delivered by cesarean section under local anesthesia versus those delivered by cesarean section under deep nembutal anesthesia. Those delivered under local appeared far more vulnerable to the onslaught of asphyxia and showed far greater incidence of neuropathological change with a given degree of asphyxiation. This important finding is being further investigated.

It was early recognized that the studies of brain damage should not be restricted to the perinatal period but should be extended into early and mid-gestational periods as well. Toward this end techniques of fetal surgery have been developed so the fetus may be removed from the uterus, vessels catheterized, studies of cardiovascular physiology and blood chemistry carried out along with other manipulations, the fetus returned to the uterus and brought to term. The first has involved compression of the cord for measured lengths of time at different gestational ages in the hopes of producing patterns of pathological change. The complete spectrum of alteration has been produced from no effect on cord compression for between 20 to 35 minutes depending upon gestational age to a circumstance of severe central nervous system damage with longer periods of compression. In between has been lesser degrees of brain damage with production of clinical neurological states resembling human cerebral palsy. The animals with lesser degrees of clinical and pathological involvement of the nervous system with cord compression have yielded disease states resembling those produced by asphyxiation at term. The most severe types of brain damage including destruction of all neurons in the neuraxis have not been seen with asphyxiation at term because of the difficulty of survival in the nursery. The most interesting point has been the similarity in distribution within the brain stem of the pathological changes with both cord clamp at different gestational ages and with asphyxiation at term.

Study of human infant brains exhibiting signs of severe brain damage has suggested that various types of pathology including porencephalic defects may relate to vascular occlusive phenomena occurring during the gestational period. Other pathological states such as hydranencephaly have been thought due to occlusion or compression of the major vessels in the neck. In an effort to experimentally study these phenomena the carotid arteries have been ligated in the neck at different gestational ages. Bilateral carotid artery ligation has failed in the majority of instances in producing CNS disease either clinically or pathologically. Examination of the vessels at the base of the brain has

indicated that anastomotic channels between the carotid and the basilar system have been adequate to carry the necessary volumes of blood to support development and maintenance of structure. However, when both the carotid arteries and the jugular veins have been ligated, pathological states are produced. In most instances infarction occurs with cyst formation in the region of distribution of the anterior and middle cerebral arteries. There remain only thin membranes where the anterior portions of the cerebrum had existed. In only one instance has the pathological appearance of hydranencephaly occurred in an animal with bilateral carotid and jugular ligation who had in addition profound fetal anemia and also possibly fetal compromise. A continuing effort is being made to reproduce this important and interesting pathological state.

Earlier work in the laboratory has indicated that the combination of perinatal asphyxia plus hyperbilirubinemia is required for the production of kernicterus. Infusion with bilirubin at high concentrations alone does not result in evidence for brain damage. It has become clear that the pattern of staining of the nuclei in the brain stem in experimental kernicterus in the monkey coincides with the pattern of neuropathological change occurring in relation to asphyxiation at the time of birth. Present studies are extending these investigations in an attempt to produce animals with chronic neurological syndromes. Present studies have attempted to investigate some of the binding assays which have been utilized in an effort to determine the extent of dissociated bilirubin present in the serum and available to produce CNS damage. Results of these studies indicate that the presently proposed laboratory tests for bilirubin dissociation are poor indicators of the production of kernicterus.

It has been possible to reproduce both the clinical and the neuropathological sequelae of lead intoxication in the newborn monkey. Newborn monkeys have been fed lead chloride in their milk formula. After a period of 1-3 weeks intake of lead the infants have exhibited increasing irritability, anorexia, vomiting, convulsions, ataxia, and finally depressed consciousness, and death. Pathologically the changes have included capillary endothelial hypertrophy and proliferation, reactive glial changes simulating the findings of lead intoxication in the human, granulomatous infiltrates with tumescence and petechial hemorrhages. A completely satisfactory paradigm for the study of lead encephalopathy is present in the monkey.

Studies have continued on the problem of experimental allergic encephalomyelitis as induced in the monkey. In the last year there has been a study of the susceptibility of all age groups to this disease. The most susceptible age groups are the 3 and 9 month olds whereas the year olds and adult animals are more delayed in the production of neurological symptomatology. The age group showing the most delayed symptomatology after inoculation with Freund's adjuvant and spinal cord material are those of the newborn period. Premature animals produce signs of CNS disease with a still longer incubation period. Thus, although animals of all ages have regularly produced severe and usually fatal CNS disease when charged with the spinal cord antigen, those which have been most delayed in development of disease have been those in the earliest age groups. There were interesting differences in distribution of lesions with a tendency for animals of the earlier age groups to develop lesions in

the brain stem and cerebellum, whereas animals of one year or older tend to produce lesions more prominently in the forebrain. The disease is fulminant with an incubation period of from two weeks to two to three months. The lesions develop over a 2-4 day period and tend to be granulomatous, hemorrhagic, and sometimes necrotizing. They are associated with infiltrations of both acute polymorphonuclear leukocytes and later with infiltrations of mononuclear leukocytes. One of the most interesting aspects was the associated extensive hemorrhagic retinopathy which resulted in severe changes in the optic fundus and was usually associated with pathological changes also in the primary optic pathways of a hemorrhagic, infiltrative, inflammatory nature.

In the past year studies have been initiated in a broad area relating to the regressive changes occurring chemically and morphologically within neural tissues in relation to circulatory failure and to death. These studies assume that these changes are similar to if not identical with the changes occurring in relation to circulatory and anoxic damage occurring in animals still surviving. Progress to date has indicated that with sudden cessation of circulation, neural tissue incubated at body temperature undergoes rapid dissolution with an obscuration of morphology, disintegration grossly of the tissues, and imbibition of fluid with leakage of electrolytes into the surrounding fluid. On the other hand, tissues similarly incubated and observed of animals who have undergone gradual death with gradual supervention of arterial hypotension have failed to show evidence for rapid dissolution of the tissue grossly or microscopically and have failed to imbibe fluid to the extent true of animals suffering sudden circulatory failure. The various functional parameters that relate to the presence or absence of these dissolutive changes and the biochemical alterations occurring within the tissues accompanying these alterations have yet to be determined.

Within the breeding colony and nursery 180 conceptions resulted from 1156 matings. The total number of deliveries was 181, 82 by cesarean section, and 99 by the vaginal route. Additional work within the breeding colony consisted of 1156 sperm tests, 1949 palpations for pregnancy, 530 blood counts, 96 urine analyses, and 152 fecal examinations. Forty animals asphyxiated at birth required intensive care in the nursery. Six animals with lead poisoning and 24 with experimental allergic encephalomyelitis created special feeding and handling problems for the nursery staff. Nine thousand routine dental, weight gain and lymph gland examinations were conducted on the infant and juvenile populations. The current inventory of animals is 65 adult males, 325 adult females and 66 juveniles within the caged colony in the central laboratory. Ninety new monkeys were acquired from various sources during the year.

Studies carried on within the veterinarian area have included studies on the pathogenesis and transmission of pulmonary acariasis. The availability within the laboratory of wild-reared animals of Indian origin, Cayo Santiago reared animals and laboratory cage-reared animals has made possible the study of the transmission of this common pulmonary disease of the Rhesus monkey. Animals brought in from India have manifested almost 100% infestation of the lungs. This results in chronic scarring and in some instances parenchymal fibrosis. The common use of the Rhesus monkey as laboratory animal makes it of concern

to know more about this important disease of the Rhesus monkey. Studies of laboratory reared animals up to 4 years of age has revealed no gross or microscopic evidence of infestation with pneumonyssus simicola. Cayo Santiago reared animals are variously infested but it is not possible to determine trends because of the smallness of the sample from this group. From these provisional findings it would appear that a close and prolonged association of infected and non-infected animals is required for the transmission of the disease.

Studies have been carried out of mycoplasma antibody titers in the sera of normal and clinically ill Rhesus monkeys in the caged colony. Mycoplasma has been incriminated as a cause of primary atypical pneumonia in man and also in certain other genital infections in man and animals. The studies of antibody titers in the monkey have been directed toward determining the possibility of infection of this species with this organism.

Studies are in progress of the birth weight of the Rhesus monkey in relation to gestational age and with reference to cesarean section versus vaginal delivery. One of the most important points emerging has been the great variability in the birth weight of animals delivered under a similar circumstance. There is a slight trend for the animals born by cesarean section to be slightly heavier than animals delivered vaginally.

The embryonic tooth development is being followed in the Rhesus monkey in cooperation with the National Institute of Dental Research and with the Cleft Palate Research Center. The anatomical materials are supplied by our laboratory to outside centers for morphological investigations.

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Social behavior, reproduction, and population dynamics in free-ranging Rhesus monkeys.

Principal Investigator: Dr. Carl B. Koford

Other Investigators: Dr. Halsey H. Marsden
Dr. John A. Morrison
Dr. John G. Vandenberg
Dr. Stephen Vessey

Cooperating Units: North Carolina Department of Mental Health. Raleigh, N.C.

Man Years:

Total:	14.3
Professional:	3.8
Other:	10.5

Project Description:

Objectives: 1. To investigate the daily, seasonal, annual, and lifelong behavior of free-ranging primates, principally Rhesus monkeys, with emphasis on reproduction, group relations, and population dynamics. 2. To determine the influences on behavior of internal and external environmental conditions.

Methods Employed: Information is obtained primarily by field observations of marked, free-ranging Rhesus monkeys on three islands. Selected animals are captured, examined, and released. Some are modified by castration, hysterectomy, or implants of hormones. Others are confined in enclosures for controlled studies.

Major Findings:

Cayo Santiago

The first birth in 1965 occurred on 7 January. Initial birth dates for each of the seven social bands extended over 73 days. All births occurred in a period of 139 days, which was 13 days longer than the 1964 birth season. In 1965 the peak of births occurred later in the year than in 1964. In 1964 there were 74 births (62% of total) in the 39 days between 1 February and 10 March. Ninety-three births (70% of total) occurred in the 40 days between 20 February and 31 March 1965. Maximum birth rates for a ten-day interval occurred during 1 to 10 February in 1964 and during 1 to 10 March in 1965.

During 1965 there were 132 births to the 156 adult females on Cayo Santiago. The birth rate was 85%, making the fourth consecutive year that it exceeded 80%. Only one precocious three-year-old female gave birth, whereas four three-year-olds did so in 1964. The birth rate for four-year-old females increased from 71% in 1964 to 85% in 1965. The sex ratio of infants born in 1965 was 62 males to 68 females; the sexes of two infants were unknown.

Infant mortality in 1965 was lower (9.8%) than in 1964 (11.8%). Eleven females and ten males one or more years old died during 1965. Evidence of tetanus has been found in one of three adult females which died in early 1966. In addition to these losses, twelve monkeys were shipped away to a research program in Tennessee.

At the end of 1965, the total population was 553 monkeys. Three of these were adult, solitary males. Seven social groups comprised 550 monkeys; the groups contained 177, 156, 64, 62, 50, 22, and 19 monkeys. To date the population has increased by 135 infants born since 17 January 1966. Sixty-nine are males, 64 are females, and 2 are not yet known. In addition to these, another eight infants born in 1966 have died. The total population on Cayo Santiago is now 685 monkeys. Approximately 15 infants are yet to be born in 1966.

The first mating observed in 1965 occurred on 17 July. By mid-August copulations or seminal plugs were seen frequently each day. Mating activity decreased rapidly in late November, and few copulations or plugs were observed in December. The latest copulations with evidence of ejaculations in 1965 were observed on 27 December. A female whose 12-day-old infant died on 27 February 1966 copulated several times on 1 April 1966.

Seventeen males changed social group attachment during the 1965 mating season. Five males were six or more years old, the others were three or four years old.

Five guest workers conducted research at Cayo Santiago in 1965 and early 1966. Mr. Duane Quiatt studied differences in behavior between young males of dominant mothers and those of subdominant mothers. Mr. Don Sade resumed a long-term study of social behavior in Group F in July, 1965, and is currently preparing a doctoral thesis from some of his data. Mr. Andrew Wilson observed Group J studying inter-troop encounters and the correlation of family relationships, social interactions, and dominance ranks. The interaction of monkeys with inanimate environmental objects and the importance of novelty in the exercise of curiosity was observed by Dr. Emil Menzel. Dr. William Draper has initiated a study on infant-mother relationships.

Long term studies of plant phenology were implemented with the marking of 20 representative trees to be examined monthly.

La Parguera

As of February, 1966, the populations on La Cueva Island and Guayacan Island have increased to 179 Rhesus monkeys. Twenty-three of these are kept permanently in enclosures. Forty births occurred in 1965 with a peak in late May and early June, as occurred in 1964 also. Twenty-eight of 32 free-ranging infants survived, a mortality rate of 12.5%. Four of the eight infants born in the enclosures survived. The total of 40 births was distributed among 54 adult females, giving a birth rate of 74% which was lower than in 1964 (87%).

Three heterosexual bands occupy La Cueva Island, consisting of 20, 36, and 60 individuals each. An all-male band reported in 1964 gradually fused with the largest band during the 1965 mating season. Guayacan Island is occupied by a single band of 39 monkeys. Two young males are solitary, one on each island.

Publications:

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Sade, D.S.: Some aspects of parent-offspring and sibling relations in a group of rhesus monkeys with a discussion of grooming. Am. J. Phys. Anthropol. 23: 1-17, Jan. 1965.

Vandenbergh, J.G.: Rhesus monkey bands. Nat. Hist. 75: 22-27, May 1966.

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1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Neuropathological Effects of Umbilical Cord Compression.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigator: Dr. Wendell H. Niemann

Cooperating Units: None

Man Years:

Total:	.7
Professional:	.2
Other:	.5

Project Description:

Objectives: To determine the pattern of clinical and neuropathological change produced by compression of the umbilical cord. To determine the length of cord compression required for production of neuropathological change at different gestational ages.

Methods Employed: Hysterotomy with sterile delivery of fetus; fetal electrocardiography; catheterization of fetal vessels with recording of blood pressures; blood gas and acid base studies of fetal blood; compression of the umbilical cord; return of fetus to uterus; fetal survival in utero; C-section on 158th day; assessment of clinical status of infant; extended survival into juvenile age; and finally, gross and histopathological study of CNS.

Major Findings: Depending upon length of cord compression and gestational age a variety of neuropathological changes are seen. The most severe is advanced cystic degeneration of the nervous system with destruction of neural parenchyma of brain and spinal cord. Surviving neurons are seen only in dorsal root ganglia, the myenteric plexus of the gut, and the bipolar cells of retina. Such brains also exhibit destructive changes of glial elements most severe in the forebrain with preservation of altered astrocytes in the brain stem and spinal cord. These animals do not survive beyond birth. A lesser degree of damage includes a clinical state compatible with survival through the newborn period but evidence clinically for severe CNS involvement including severe changes in postural tone and in motor ability. Neuropathologically such animals exhibit severe destruction of neurons symmetrically throughout large portions of the tegmentum of the brain stem, destruction of entire complexes such

as the superior olivary nucleus, the trigeminal sensory nucleus, vestibular nucleus and in inferior colliculus. These changes are associated with extensive capillary proliferation, connective tissue replacement, and astrocytosis. Still lesser degrees of damage produce a clinical status resembling spastic quadriplegia with a neuropathological picture associated with destructive changes in the posterior and lateral ventral nuclear complex and with changes in focal nuclear centers of the brain stem including inferior colliculus, vestibular nuclei, trigeminal nuclei, etc. Finally, cord compression as long as 35 minutes at earlier gestational ages and as long as 20 minutes at later gestational ages have resulted in no clinical abnormalities and no pathological changes in the brain.

Significance: These studies, it is hoped, will lead to a better understanding of the etiology and pathogenesis of some forms of cerebral palsy and mental retardation. Knowledge of the duration of cord compression required to produce signs of CNS damage is of clinical as well as basic importance to the understanding of brain metabolism.

Proposed Course of Project: More cases are required to establish more exact relationships between gestational age, length of cord clamp, and neuropathological alterations produced. Also, technical approaches more recently applied including cannulation of fetal vessels with cardiovascular and blood chemical studies will contribute significantly to understanding the pathogenesis of patterns of pathology in relation to anoxia.

Publications: No.

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A correlational study of brain lesion effects on a behavioral test battery.

Previous Serial Number: Same

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: Department of Psychology, University of Puerto Rico,
San Juan, Puerto Rico.

Man Years

Total:	.8
Professional:	.4
Other:	.4

Project Description:

Objective: To develop a method which permits a finer analysis of the consequences of particular brain lesions from a behavioral standpoint.

Methods Employed: Individual animal Ss (rhesus monkeys) will be subjected to a number of behavioral rests in automated apparatuses. In each of the tests, stimulus characteristics (e.g., hue, form, number of relevant dimensions, etc.) will be systematically varied.

By examining the performance of the same individual Ss on several different behavioral test situations, a better understanding of the effects of brain lesions, and, conversely, a better understanding of the nature of the test situations themselves may be attained.

Major Findings: The project is still in the stage of apparatus development.

Significance: Although behavioral tests have long been used to study the effects of brain lesions, progress toward finer understanding of the effects of particular brain lesions, or properties of behavioral tests has been slow and sporadic. The project will investigate effects of cortical lesions on performance by animals in various behavioral situations in a more systematic fashion.

Proposed Course of Project: To complete a workable design of apparatuses as soon as possible, and to continue on to the main part of the project.

Publications: No.

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The superior colliculus in the goat.

Previous Serial Number: Same

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: Department of Psychology, University of Puerto Rico,
San Juan, Puerto Rico.

Man Years

Total:	.2
Professional:	.1
Other:	.1

Project Description:

Objective: To investigate the anatomical projections to and from the superior colliculus in the goat. It is hoped that this will widen our understanding of the functional significance of this structure.

Methods Employed: Nauta-Gygax staining method after production of brain lesion. Modifications of the above method may also be used.

Major Findings: Histological preparations from the first two animals have been completed. Procurement of additional animals, interpretation of the slides already prepared has been postponed because higher priorities are assigned to other projects.

Significance: Though in mammals the superior colliculus has declined in its role as the primary way-station of the visual system, evidence indicates it is by no means a vestigial structure. In the goat, the superior colliculus undergoes massive development compared with other species, suggesting greater functional role played by the structure in this species.

Evidence suggests the goat may have panoramic vision as compared with central vision in the primates. Further, the oculomotor pattern of the goat differs from that of primates in that pursuing eye movements of moving objects are deficient and there is no tendency to fixate objects in the center of the visual field.

The study of the superior colliculus in the goat is, thus, expected to give a wider basis for the understanding of its role in the mammalian system.

Proposed Course of Project: Include studies of efferent projections from superior colliculus.

Publications: No.

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Reproduction Behavior of Caged Rhesus Monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Mrs. Carmen G. Ponce de Lugo
Mr. Luis R. del Río

Cooperating Units: None

Man Years

Total:	.8
Professional:	.1
Other:	.7

Project Description:

Objectives: To study in a detailed fashion the reproductive behavior of caged rhesus monkeys. This study seeks to determine the effects on behavior in the male of: (1) Age and experience of both male and female; (2) compatibility or affinity between particular animals; (3) receptivity of the female; (4) outcome of mating with respect to insemination; (5) possible seasonal influences.

The establishment of differences between the reproductive behavior of wild reared and cage reared animals is a minor parallel objective of the study.

Methods Employed: A group of 17 males representative of different levels of breeding performance and of different field backgrounds was selected to conduct this study. Depending on availability of estrous females, each male is mated once a week at the time of optimum female receptivity (10th-12th day of menstrual cycle). The female is brought into the observation cage where the male has been previously transferred. From this time, a 2-hour period of observation is conducted using closed-circuit television. Detailed records are kept of the duration and types of activity displayed including grooming, mountings and ejaculations. In addition, a descriptive impression of each of the animals' behavior during the observation period is recorded. At the end of the mating period (approximately 48 hours) a vaginal smear is taken for microscopic examination. The results of the sperm test and the final pregnancy outcome of the mating are recorded.

Serial No. NDB(I) 65-LPP 1263

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Ocular Pathology and Experimental Allergic Encephalomyelitis
in Young Monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Ludwig von Sallmann

Other Investigators: Dr. Ronald E. Myers, Dr. Edwin M. Lerner II,
Dr. Sanford H. Stone

Cooperating Units: Laboratory of Immunology, NIAID, Laboratory of
Ophthalmology, NINDB

Man Years:

Total:	1.2
Professional:	.4
Other:	.8

Project Description: For description of this project see NINDB(I) 65-0-Ch
1212C

Serial No. NDB(I) 65-LPP 1264

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on Auto-immune Diseases and Related Immunological Processes

Previous Serial Number: Same

Principal Investigators: Dr. Sanford H. Stone
Dr. Edwin M. Lerner II

Other Investigators: Dr. Ronald E. Myers, Dr. Wendell H. Niemann

Cooperating Units: Laboratory of Immunology, NIAID

Man Years:

Total:	.9
Professional:	.3
Other	.6

Project Description: For description of this project see NIAID-117.



1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Localization of Learned Motor Skill Functions in Corpus Callosum in the Monkey.

Previous Serial Number: None

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.7
Professional:	.1
Other:	.6

Project Description:

Objectives: To determine the localization within the forebrain commissure of information transmission related to learned motor skills in the primate.

Methods Employed: The series of animals have varying portions of the forebrain commissure sectioned. Transfer of learned motor skills between the hands is tested in those animals through utilization of a simple direct-confrontation behavioral situation in which the monkeys are taught various latchbox problems through one hand in the absence of visual guidance. Preservation of transfer indicates that the preserved portions of the commissure function within the task assigned whereas a failure of transfer indicates a noncontribution of the preserved commissural segments to the function.

Major Findings: Transfer of latchbox solving between the hands is preserved in monkeys who have significant portions of the posterior body region of the corpus callosum preserved. This localization is the same localization as that seen for tactual problem solving transfer between the hands. It is concluded that latchbox solving (learned motor skills) functions are related to those of sensory function and are subsumed by the parietal lobe and by the related portion of the commissure.

Significance: This study advances our understanding of the neural mechanism underlying the acquisition of learned motor skills.

Proposed Course of Study: Work with further animals is required to complete this study.

Publications: No.

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Pathology of the Stillborn.

Previous Serial Number: None

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.9
Professional:	.1
Other:	.8

Project Description:

Objective: To study the pathology of the stillborn monkey.

Methods Employed: All stillborn monkeys are subjected to post-mortem examination to determine the state of preservation of the tissues. In addition, the CNS of the stillborn monkeys will be examined and assessed for indications of brain damage.

Major Findings: The stillborn may be divided into two categories: fetuses showing advanced maceration of tissues with peeling of the epidermis, liquefaction or softening of internal organs and loss of normal tissue coloration and fetuses in which, despite prolonged incubation at body temperature in utero, there is preservation of intact morphology of internal organs. Intermediate circumstances are found the most common pattern being liquefaction of the contents of the cranial cavity with, however, preservation of intact morphology of somatic organs in general. Several patterns of neuropathological change have been seen. Perivenular hemorrhages in the white matter of the hemispheres is seen occasionally. Perivenular hemorrhages in the interlaminar and midline nuclei of the thalamus occurs in other instances. Most frequently there is autolysis of the tissues with obscuration of pathology.

Significance: Careful study of the pathology of the stillborn monkey may give clues to the cause for and nature of processes which led to fetal death.

Proposed Course of Project: Post mortem studies of the stillborn fetuses will continue. An attempt will be made to correlate the facts known about the type of fetal death with the occurrence of maceration. The theory will be

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Experimental Placental Insufficiency in the Rhesus Monkey.

Previous Serial Number: None

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Wendell H. Niemann

Cooperating Units: None

Man Years

Total:	1.2
Professional:	.4
Other:	.8

Project Description:

Objectives: The objectives of this study are threefold: (1) To determine the effects of ligation of the fetal placental vessels upon the morphology of the placenta. (2) To explore the relationship between placental insufficiency and "small for dates" babies. (3) To explore the possible relation between placental insufficiency and brain damage.

Methods Employed: Monkeys at different gestational ages are submitted to hysterotomy. The umbilical vessels emanating from the umbilical cord are approached as they spread out over the placental surface by retraction of the amnion from the surface of the placental cake. Larger or smaller radicles of the umbilical arteries and/or veins are ligated depending upon the degree of placental insufficiency desired. The incisions are closed and the fetus brought to term. On cesarean section at term the placental specimen is carefully preserved and the gross and microscopic morphology described. The newborn infant is weighed at birth and at periodic intervals during its survival. The newborns are evaluated for signs of brain damage.

Major Findings: For morphological changes of the placenta see project reports of Dr. Toshio Fujikura, PRB/NINDB. "Small for dates" babies have been regularly produced as a result of placental insufficiency in these monkeys. The highest degree of retardation in growth exhibited was to 55% of the normal. Lesser degrees of growth retardation have been seen depending upon gestational age at the time of vessel occlusion and upon extent of devascularization of the placenta. Several newborns have evidenced

mild brain damage clinically with motor retardation and postural abnormalities particularly in the hind extremities. Mild signs of cranial nerve abnormality also have occurred.

Significance: The findings are of interest to the problem of fetal growth retardation or "small for dateness" of babies from mothers with toxemia of pregnancy, diabetes, and other conditions. The findings concern the problem of cerebral palsy.

Proposed Course of Project: More cases are required to tie down the relationship between degree of retardation of growth and the gestational age and degree of placental devascularization. Particularly important is the effort to produce the maximum amount of placental insufficiency compatible with continued existence of the fetus with the hope of producing more clearcut evidence for brain abnormalities.

Publications: No.

Serial No. NDB(I) 66-LPP 1387

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Experimental Placental Abruption in the Rhesus Monkey
and its Relation to Brain Damage.

Previous Serial Number: None

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Wendell H. Niemann

Cooperating Units: None

Man Years

Total:	1.2
Professional:	.4
Other:	.8

Project Description:

Objectives: To develop a model of abruption placenta in the experimental animal and to study the brain pathology produced.

Methods Employed: Monkeys at various gestational ages are submitted to hysterotomy. Abruption of varying proportions of the placenta is produced at various gestational ages by finger dissection. The incisions are closed and the animals brought to term. At term placental specimens will be carefully studied for morphological changes and the fetus evaluated clinically and pathologically for brain damage.

Major Findings: In the exploratory studies thus far completed the abruptions were so extensive as to be incompatible with fetal survival in utero. In the first four animals studied, abruption of 40-50% of the total placental surface was produced. Fetal death followed in all four instances.

Significance: The proportion of the total placental surface which must be abrupted to produce brain damage of various types or to produce demise of the fetus can be determined at different gestational ages by using the techniques explored in the present experiment. These studies are important to understanding of the problem of spontaneous placental abruption in relation to fetal mortality and morbidity.

Proposed Course of Project: More extensive series of procedures are required particularly with lesser degrees of abruption to detail the relation between degree of placental abruption and extent of fetal compromise produced.

Publications: No.

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Perinatal Asphyxia in the MONkey and its CNS Consequences

Previous Serial Number: None

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Wendell H. Niemann

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	.4
Other:	.8

Project Description:

Objectives: To determine the patterns of clinical alteration and neuropathological change produced by asphyxiation at the time of birth. To attempt to correlate the patterns of neuropathological change produced with the functional changes occurring in the fetus during asphyxiation.

Methods Employed: Monkey fetuses are delivered by cesarean section at term and subjected to asphyxiation prior to the first gasp by the placement of a rubber sack over the head. The lengths of asphyxiation are determined by the degree of functional deterioration of the fetus. In this study an effort was made to produce the maximum degree of brain damage compatible with survival of the fetus. Studies were carried out on the cardiovascular, blood chemical (acid base status, partial pressures of blood gases, pH, lactate, etc.) and respiratory changes of the fetus during and after asphyxiation. Resuscitation of the fetuses was carried out using positive pressure oxygen ventilation.

Major Findings: With the varying lengths of asphyxiation carried out, there were varying degrees of brain damage clinically and neuropathologically apparent. The most common pattern of clinical alteration was one of sensory loss in the extremities and over the head of varying degrees, alteration in sucking and swallowing, alterations in the voice, and varying degrees of ataxia. In some instances the sensory alteration over the face and head resulted in persistent sores of a trophic or traumatic nature. Pathologically, destructive changes of varying extents were found in certain nuclei of the

brain stem including the vestibular nuclei, the sensory trigeminal nuclei, the inferior colliculi, and also the basal cerebellar nuclei. In addition, in many instances, there were similar changes in the posterior ventral and lateral ventral nuclei of the thalamus and infrequently in the putamen. The changes found in this distribution were variable in extent depending upon the severity of the insult. The least severe alteration was chromatolysis of neurons in the same distribution. More damaging circumstances included destruction of neurons and changes in the glia with microglial proliferation and astrocytic activation. More severe still was focal necrosis in certain loci, particularly in inferior colliculus and vestibular nuclei. In the latter circumstances connective tissue infiltration and capillary proliferation were commonly seen in association with the destructive changes in the neuronal elements. Finally, in some instances diffuse hemorrhagic changes were seen, again in the same topological distribution as above described. In general, the type and extent of neuropathological alteration could be correlated with various parameters of functional alteration in the status of the fetus occurring during the asphyxiation process. For example, hemorrhagic changes within the distribution pattern described occurred only in instances in which there had been profound and prolonged depression in blood pressure but with restitution of the cardiovascular system and survival for a period of time. Less commonly, other patterns of neuropathological change were seen, such as laminar necrosis, focal cortical atrophy with ulegyria, etc. These changes were seen respectively in circumstances of severe fetal acidosis and of elevated venous pressure in relation to the asphyxial process.

Significance: The major hope is to reproduce the patterns of neuropathological change seen in cases of human cerebral palsy and mental retardation. To a good extent the studies so far have been fruitful in this regard. More important still, however, is an effort to determine the critical parameters involved in the functional deterioration of the fetus during the asphyxial insult and to relate them to the various patterns of neuropathological change. Finally, efforts will be made to ascertain methods through which functional aberrations of the fetus may be corrected.

Proposed Course of Project: These studies will be continued both in terms of numbers of animals in various categories produced but particularly with extension of the numbers of physiological and blood chemical changes which are measured in relation to the asphyxial process. Efforts will be made to artificially produce alterations in functional status of the various factors determined to be important to production of brain damage in order to more clearly define relationships.

Publications: No.

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Experimental Hydranencephaly in the Monkey.

Previous Serial Number: None

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Karlis Adamsons, Jr., Dr. L. Stanley James,
Dr. Jerold F. Lucey, Dr. Philip Dodge

Cooperating Units: Columbia Presbyterian Medical Center, New York, N.Y.
The University of Vermont, Burlington, Vt.
The Massachusetts General Hospital, Boston, Mass.

Man Years

Total:	1.1
Professional:	.5
Other:	.6

Project Description:

Objectives: To experimentally produce hydranencephaly in the monkey.

Methods Employed: One theory suggests that obstruction to blood flow in the major vessels of the neck plays a major role in the pathogenesis of hydranencephaly in the human. In the present study monkey fetuses are surgically removed from the uterus preserving the umbilical circulation. The fetuses undergo bilateral ligation of carotid arteries and/or jugular veins. They are then replaced in the uterus and the incisions closed. On cesarean section delivery later at term the fetuses are carefully evaluated for signs of neurological abnormality. The brain specimens are removed and studied for pathological changes.

Major Findings: Bilateral ligation of the carotid artery in the neck at a variety of gestational ages does not result in cerebral pathology in the vast majority of cases. Ligation of both the carotid arteries and jugular veins bilaterally results in gross infarction in the region of supply of the anterior and middle cerebral arteries in the majority of instances. The classical neuropathological picture of hydranencephaly has been produced in only one instance in an animal in which there had been bilateral ligation of both the carotid arteries and the jugular veins and in addition production of fetal anemia.

Significance: The production of hydranencephaly in the monkey will greatly elucidate the pathogenesis of hydranencephaly in the human.

Proposed Course of Project: These studies require further cases in an effort to more closely determine the etiological factors underlying the production of hydranencephaly. At present it appears the fetus requires some further general medical insult beyond mere ligation of the carotid arteries and jugular veins to produce hydranencephaly.

Publications: No.

Serial No. NDB(I) 66-LPP 1390

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The effects of oxygen in high concentrations and vasopressor substances upon the fetus.

Previous Serial Number: None

Principal Investigator: Dr. Karlis Adamsons, Jr.

Other Investigators: Dr. Ronald E. Myers, Dr. Ingemar Joelsson,
Dr. Salha Daniel and Dr. Allen Hyman

Cooperating Units: College of Physicians and Surgeons, Columbia Presbyterian Medical Center, New York, N.Y.

Man Years

Total:	.8
Professional:	.2
Other:	.6

Project Description:

Objectives: To determine: (1) The effects of maternal breathing of oxygen in high concentrations ; and (2) administration of vasopressor substances upon the cardiovascular system and the acid-base state of the fetus.

Methods Employed: Hysterotomy is performed during the second half of gestation in the rhesus monkey. Indwelling catheters are placed in the carotid artery and the jugular vein of the fetus and electrocardiographic leads attached. The fetus is then returned to the uterine cavity and the animal allowed to recover. Arterial samples are then obtained from the mother and the fetus at intervals during air breathing and following exposure of the mother to 100% oxygen. Fetal blood pressure, heart rate and electrocardiograms are recorded continuously. The blood samples are analyzed for pH, pCO₂, total CO₂, and excess acid. Similar data are collected following administration of epinephrine and norepinephrine to the fetus and to the mother.

Major Findings: Under optimal conditions the acid-base state of the fetus closely resembles that of the mother. Oxygen tension in the blood supplying the brain in the vigorous fetus may exceed 40 mm/Hg. Administration of 100% oxygen to the mother increases oxygen tension in fetal arterial

blood, which, however, is associated with a small fall in fetal arterial pH. The increase in fetal pO_2 following maternal breathing of 100% oxygen varies depending upon the fetal condition. It may be minimal or even absent when fetal condition has considerably deteriorated due to impaired uterine perfusion. Responses to catecholamines in the fetuses up to 110 days are negligible in the presence of fetal acidosis. Marked and prolonged fetal acidosis can occur in the fetus with minimal change in blood pressure and heart rate.

Significance: No interpretation of the data as yet.

Proposed Course of Project: Additional studies will be required before the therapeutic value of maternal breathing of high oxygen mixtures during conditions of fetal distress can be ascertained. The same applies to evaluation of vasopressor substances administered to the mother in the presence of maternal hypotension.

Note: The project is still in a phase of overcoming considerable methodological difficulties. The ultimate aim is to provide preparations with indwelling fetal catheters that could be maintained for prolonged periods of time possibly through the completion of pregnancy.

Publications: No.

Serial No. NDB(I) 66-LPP 1391

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Rate of absorption of donor red cells following their administration into the peritoneal cavity of the fetus.

Previous Serial Number: None

Principal Investigator: Dr. Karlis Adamsons, Jr.

Other Investigators: Dr. Ronald E. Myers, Dr. Ingemar Joelsson,
and Dr. Allen Hyman

Cooperating Units: College of Physicians and Surgeons, Columbia Presbyterian Medical Center, New York, N. Y.

Man Years:

Total: .8

Professional: .2

Other: .6

Project Description:

Objective: Determination of the rate and extent of red cell uptake and final appearance in the intravascular space of the fetus following administration of donor cells into the peritoneal cavity.

Methods Employed: Catheters are inserted into the carotid artery and peritoneal cavity of the fetal rhesus monkey during the second stage of gestation. Fetus is then returned to the uterus and the maternal animal allowed to recover. Chromium labeled monkey red cells are then injected through the indwelling intraperitoneal catheter into the fetus and serial blood samples obtained at appropriate intervals from the carotid artery. Such observations are carried out with the fetus in good condition and following induction of fetal anemia by partial removal of fetal red cells.

Major Findings: Due to methodological difficulties, sufficiently long fetal survival following completion of the preparation has not yet been achieved to proceed with administration of donor cells.

Proposed Course of Project: Additional animals would be necessary for the achievement of the original objectives. Hopefully, refinement of the technique and improved post-operative management of the pregnant animal will yield preparations of sufficient stability for the evaluation of transperitoneal absorption of red cells by the fetus.

Publications: No.

Serial No. NDB(I) 66-LPP 1392

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Nembutal prophylaxis of brain damage.

Previous Serial Number: None

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Wendell H. Niemann
Dr. Geoffrey S. Dawes
Dr. Forrester Cockburn
Dr. Benjamin B. Ross
Dr. L. Stanley James
Dr. Salha Daniel

Cooperating Units: The Nuffield Institute for Medical Research, Oxford.
University of Oregon Medical School, Portland, Oregon.
Columbia Presbyterian Medical Center, New York, N.Y.

Man Years

Total:	1.0
Professional:	.2
Other:	.8

Project Description:

Objectives: In prior studies of neonatal asphyxia both local and general anesthetics were used in separate animals for the delivery of the fetus. It was noted that animals delivered under Nembutal anesthesia were far more refractory to asphyxiation than were the animals delivered under local in terms of the length of asphyxia sustained prior to deterioration of the functional indices of the fetus. It was not uncommon, for example, for fetuses delivered under Nembutal to sustain a heart beat up to 50 beats per minute for as long as 30 minutes of asphyxiation whereas animals delivered under local anesthesia regularly expire after 15-20 minutes of asphyxiation. The present study seeks to further investigate the significance of Nembutal in the prophylaxis of asphyxial brain damage.

Methods Employed: Two series of animals are delivered, one under local and one under Nembutal anesthesia. The animals of both series are asphyxiated for a specified length of time, measurements of various functional parameters of the fetus being carried on during and after asphyxiation. The animals are resuscitated with positive pressure oxygen ventilation and

observed clinically for the first two weeks of life. The animals are then sacrificed and studied neuropathologically.

Major Findings: With the animals asphyxiated twelve and a half minutes, all animals delivered under Nembutal general anesthesia exhibited no evidence for clinical abnormalities and on neuropathological examination showed no pathological change in the brain. All the animals delivered under local anesthesia exhibited evidence of clinical neurological deficit and on pathological examination revealed marked changes in the typical distribution. Other animals delivered under local but Nembutalized on delivery prior to asphyxiation responded similarly to those delivered under local anesthesia. It is inferred that adverse factors act generally on the mother producing fetal deterioration in utero prior to delivery. This deteriorated status of the fetus on delivery in animals under local anesthesia results in a higher incidence of brain damage and death.

Significance: These studies are of importance for the problem of parturition and brain damage in the human.

Proposed Course of Project: Delivery of the fetus by Cesarean section under local anesthesia produces a deterioration of the fetus in the uterus through unknown mechanisms. This fetal deterioration is interfered with or prevented by the use of Nembutal general anesthesia. Further steps in the investigation of this important effect will include determining whether other general anesthetic agents are similarly protective. More particularly, studies will be carried out in an effort to determine the adverse factors acting upon the fetus in utero. Possibilities include stress induced catecholamine production by the mother with consequent interference with uterine blood flow, possible cardiovascular changes in the mother herself affecting adversely the fetus either in his acid-base or blood gas availability.

Publications: No.

Serial No. NDB(I) 66-LPP 1393

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Acid Base Balance Studies of Amniotic Fluid in Rhesus Monkeys.

Previous Serial Number: None

Principal Investigators: Dr. André E. Hellegers, Dr. Henri C. Kock.

Other Investigators: Drs. L.A.M. Stolte, A. Elmore Seeds, Ronald E. Myers.

Cooperating Units: The Johns Hopkins University and Hospital, Baltimore, Md.

Man Years:

Total: 1.2
Professional: .4
Other: .8

Project Description:

Objectives: To determine the pH, bicarbonate concentration, and partial pressure of CO₂ in the amniotic fluid of monkeys at different gestational ages.

Methods Employed: Monkeys at various gestational ages from approximately 45 days until term were subjected to amniocentesis. Samples of amniotic fluid were tested for pH, bicarbonate concentration, and partial pressure of CO₂. Plots were then made of these values against gestational age.

Major Findings: Curves were derived characterizing the pH of amniotic fluid, its bicarbonate concentration and the partial pressures of CO₂ at different gestational ages in the Rhesus monkey. The values of these several determinations at specific gestational ages exhibited relatively small degrees of variation although at different gestational ages average values were quite different. The pH of amniotic fluid exhibited a straight line curve with a fairly sharp inclination becoming more acid later in gestation. The earliest determination showed average pH of 7.42 at 45 days gestation and average pH of 7.20 at 168 days or term. The bicarbonate concentration in the amniotic fluid exhibited a parabolic curve with a tendency for the bicarbonate concentration to drop from an average high value of approximately 28 millimols per liter at 45 days to a low of approximately 20 millimols per liter at 115 gestational days and then rising again to average levels of 21 1/2 millimols per liter at term. Again, the curve of partial pressures of CO₂

in the amniotic fluid was a straight line with the partial pressures increasing steadily through gestation from a low at 45 days of approximately 36 mm/Hg to one of 52 mm/Hg at term.

Significance: With the derivation of these curves of concentrations of these substances and taking into account the relatively small variability at particular gestational ages it is possible in the monkey utilizing these values to determine the presence or absence of fetal death.

Proposed Course of Study: This study has been completed.

Publications: Kock, H. C., Seeds, A. E., Myers, R. E., Stolte, L.A.M., and Hellegers, A.E.: Acid Base Balance Studies of Amniotic Fluid in Rhesus Monkeys. Accepted for publication in Am. J. Obst. & Gynec.

Serial No. NDB(I) 66-LPP 1394

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Acid Base Balance Study of Amniotic Fluid in Relation to Fetal Distress in Rhesus Monkeys.

Previous Serial Number: None

Principal Investigator: Dr. André E. Hellegers

Other Investigators: Dr. L.A.M. Stolte, Dr. Henri C. Kock, Dr. A. Elmore Seeds, Dr. Ronald E. Myers

Cooperating Units: The Johns Hopkins University and Hospital,
Baltimore, Maryland

Man Years:

Total:	1.1
Professional:	.3
Other:	.8

Project Description:

Objectives: To determine the rapidity and extent of alteration of acid base balance of the amniotic fluid in relation to induced fetal distress and fetal death in utero.

Methods Employed: Under deep nembatal anesthesia bridging vessels extending between placental cakes are cannulated along with the femoral vessels of the mother. Heart rates and blood pressures, as well as blood samples, may be obtained from both the fetus and the mother through these intravascular cannulae. Samples of amniotic fluid are taken by amniocentesis. Fetal distress of varying degrees and fetal death are induced through exposure of the mother to atmospheres of 10 per cent CO₂ for varying lengths of time. Rapidity of change of bicarbonate, of partial pressure of CO₂ and of pH could be determined in the amniotic fluid in relation to changes in levels of these values in the fetal and maternal blood streams.

Major Findings: Alterations in pH, concentrations of bicarbonate, and partial pressures of CO₂ in the amniotic fluid followed the alterations of these values in the blood stream of the fetus with a 15-30 minute time lag while failing to reflect changes of these values in the maternal blood over the period of time studied. It was concluded that the concentrations of

these components in the amniotic fluid were in more direct equilibrium with the fetal bloodstream. The interface for equilibrium establishment between amniotic fluid and the fetus is far greater than that between the amniotic fluid and the maternal circulation. Determination of pH, bicarbonate level and partial pressures of CO₂ in the amniotic fluid while failing to yield information of value in terms of immediate changes in fetal circumstance still allows a longer range assessment of the fetal circumstance in utero.

Significance: These studies are of theoretical interest in that they point up the close relationship between changes in acid base status in the fetus and amniotic fluid. However, they point out a significant lag in the amniotic fluid reflection of fetal circumstance. For the latter reason the close following of fetal circumstance by amniocentesis and determination of acid base status during parturition is not of practical clinical importance.

Proposed Course of Study: This study has been completed.

Publications: In preparation for publication.

Serial No. NDB(I) 66-LPP 1395

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Hyperbilirubinemia and Kernicterus in the Rhesus Monkey.

Previous Serial Number: None

Principal Investigator: Dr. Jerold F. Lucey

Other Investigators: Dr. Ronald E. Myers, Dr. Mario Ferreiro-Serrano.

Cooperating Units: University of Vermont College of Medicine,
Burlington, Vermont

Man Years:

Total:	.8
Professional:	.2
Other:	.6

Project Description:

Objectives: To develop an experimental clinical and neuropathological paradigm for kernicterus in relation to hyperbilirubinemia. In addition, to evaluate several dye binding assays in their predictive value for the development of kernicterus.

Methods Employed: Term fetuses are delivered by cesarean section and asphyxiated for twelve and a half minutes. They are then injected at 6-hour intervals with bilirubin. Six -hour samples of serum are taken and analyzed for levels of bilirubin in the serum. The neurological status of the newborn is frequently evaluated for evidence of developing CNS damage. On the death of the animal the brain is examined neuropathologically. Samples of the serum during the procedure are subjected to the H.A.B.A.dye binding assay and to the salicylate dye binding test, and the results compared with the clinical and pathological status of the infant.

Major Findings: A high incidence of bilirubin staining of the basal nuclei of the brain of the fetuses has been obtained in the present study when combined with newborn asphyxiation. The distribution of staining of the basal nuclei in the brain is co-extensive with distribution of brain damage produced by asphyxiation alone. The H.A.B.A. dye binding assay and the salicylate dye binding tests both have proven to be poor indicators of the development and degree of occurrence of kernicterus in the monkey.

Significance: This study was gauged primarily to evaluate the reliability of the two techniques of evaluating the proportion of bilirubin in such a form as it can produce kernicterus in vivo. The results suggest that the two techniques proposed for the chemical assessment of probability of brain damage with hyperbilirubinemia have a low degree of reliability.

Proposed Course of Project: Further studies are required to give a more firm basis for the above conclusion with regard to the value of the binding assay techniques.

Publications: No.

Serial No. NDB(I) 66-LPP 1396

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Neuropathology of Lead Encephalopathy in the Rhesus Monkey.

Previous Serial Number: None

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Angel Pentschew

Cooperating Units: State of Maryland Department of Mental Hygiene,
Baltimore, Maryland.

Man Years:

Total:	.5
Professional:	.1
Other:	.4

Project Description:

Objectives: To produce an experimental paradigm for the clinical and neuropathological study of lead encephalopathy.

Methods Employed: Lead chloride was dissolved in the milk fed to infants on a daily basis until the occurrence of clinical neurological symptomatology. In some instances lead was halted at that point, while, in others lead was continued intermittently until the death of the animal. The neurological status was evaluated regularly and notation made about the occurrence of and character of neurological symptoms. After death the animals were perfused and the CNS evaluated for the presence of gross and microscopic findings.

Major Findings: The monkey babies developed a clinical picture closely simulating lead encephalopathy as seen in the human. Vomiting with anorexia were early symptoms, usually associated with diarrhea. Later, the animals developed convulsions and evolved signs of ataxia, depressed consciousness with finally coma and death. Pathologically, the findings again closely resembled those seen in the human disease. The brain was often swollen, and was of a tough, rubbery consistency. Grossly there were areas of tumefaction or granuloma formation; areas of petechial hemorrhage were seen throughout the neural tissues. In one instance with chronic repeated exposure to the toxic agent there was a proteinaceous coagulum in the ventricular system associated with a gross granularity of the central nervous system substance.

Microscopically, again, the changes were similar to those of the human disease but more severe with reaction and proliferation of the glia, areas of hemorrhage, and capillary proliferation and hypertrophy. There were areas of chromatolytic and regressive changes in neurons in numerous loci. In addition, microscopically, there were areas of marked granuloma formation with mononuclear cell infiltration.

Significance: The demonstration of lead encephalopathy in the monkey infant leads to the possibility of studying the problem of lead encephalopathy experimentally in animals both in terms of etiology, pathogenesis, and therapy.

Proposed Course of Study: With completion of the present series the study will be terminated.

Publications: No.

Serial No. NDB(I) 66-LPP 1397

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Comparative Studies of Ascending Spinal Projections

Previous Serial Number: None

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.6
Professional:	.4
Other:	.2

Project Description:

Objectives: 1. To define patterns of central nervous system organization in vertebrates. 2. To make a survey of ascending spinal projections in several species from each vertebrate class.

Methods Employed: The animals are sacrificed 7 - 30 days after hemisection of the spinal cord and the brains are then processed according to the method of Nauta. The silver impregnated degenerating axons are identified and reconstructions are made of the course and termination of the degenerated fibers.

Major Findings: Of the five species so far examined only the lizard material has been worked up in detail. In this reptile, the most abundant preterminal and terminal degeneration is found in the lower brainstem and in the cerebellum. At medullary levels, fibers of spinal origin terminate throughout the reticular formation, particularly in its lateral portion. Only a few fibers cross the midline to terminate in the contralateral inferior reticular nucleus. Contralateral fibers are not found rostral to this nucleus except in the cerebellum. The terminating fibers in the medial reticular formation are sparse and diminish in number rostrally. In addition to the nuclei gracilis and cuneatus, which are well defined by profuse preterminal and terminal degeneration, the nucleus of the tractus solitarius, nucleus infima, and the vestibular nuclear complex receive fibers from the spinal cord. The majority of the spinocerebellar fibers reach the cerebellum

via the central spinocerebellar tract, while the dorsal tract contains but a few fibers. At mesencephalic levels degenerating fibers are most abundant in the periventricular gray substance and in the nucleus intercollicularis. A small portion of fibers passes through the latter nucleus to continue rostrally in a dorsomedial direction toward the periventricular gray of the tectum in the immediate vicinity of the mesencephalic nucleus of V. A few axons terminate among the small cells surrounding this nucleus, but the majority of this bundle continues rostrally in this dorsomedial position to terminate in the dorsal thalamus.

Significance: Comparative studies such as these are carried out in attempts to elucidate problems of human central nervous system organization by broadening our understanding of brain organization of lower vertebrate forms. These studies are the first comprehensive studies of this kind using the Nauta method. The findings in the lizard are, with few exceptions, similar to those observations made in all mammals so far examined. The discovery of the dorsal spinocerebellar tract in the dorsal funiculus may represent a more primitive arrangement. It is significant that the dorsal funiculus and the dorsal spinocerebellar tract in mammals carry similar proprioceptive and exteroceptive information. These observations on the morphological proximity of these systems therefore suggest a commonality of function. However, a great number of species of each vertebrate class must be examined before the generality of these observations can be ascertained.

Proposed Course of the Project: 1. To examine representative species of fish, amphibians, and reptiles for variation in ascending spinal projections using the Nauta method. 2. To examine these tracts and related nuclei using micro-electrode techniques in order to determine what kind of information the various tracts carry.

Publications: None

Serial No. NDB(I) 66-LPP 1398

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Development of neurohistological methods

Previous Serial Number: None

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: Mrs. Michie A. Vane

Cooperating Units: None

Man Years:

Total:	.3
Professional:	.1
Other:	.2

Project Description:

Objectives: 1. To develop more reliable neurohistological methods for the demonstration of degenerating axons, boutons terminaux, and neurons undergoing retrograde chromatolysis. 2. To improve existing methods for the demonstration of these neuronal structures, and to adapt existing neurohistological techniques for brains of submammalian vertebrates.

Methods Employed: The animals are sacrificed 7-30 days after enucleation of one eye and the brains are fixed in different fixatives. Variants of the Nauta method for the selective silver impregnation of degenerating axons are systematically investigated for improvement of staining characteristics and dependability. Investigations are underway for the development of a modification of this technique for paraffin imbedded material. The feasibility of employing fluorescent dyes for the demonstration of degenerating neurons and axons is currently studied.

Major Findings: Modifications of the Nauta method have been developed for the central nervous system of fishes, amphibians, and reptiles. Selective silver impregnation of degenerating fibers in paraffin imbedded material as well as by means of organic dyes has been accomplished in a few instances and the various steps in these procedures are now being scrutinized for the standardization of the techniques.

Significance: Progress in neurobiology has always been related to the development of new techniques. The introduction of the methods for the selective silver impregnation of degenerating axons by Nauta has greatly widened the scope of neuroanatomical research. The adaptation of the technique for studies of submammalian vertebrates has broadened the field of comparative neurology. The capriciousness of the method, however, necessitates improvement as well as development of new techniques for the demonstration of degenerating axons and axon terminals. The preliminary findings suggest the feasibility of staining these structures by other than silver stains, namely, organic dyes. Such techniques would, hopefully, be easier and more reliable.

Proposed Course of the Project: 1. To continue the systematic search for staining reactions that are selective for degenerating neurons and their processes. 2. To continue the improvement of existing methods.

Publications: None

Serial No. NDB(I) 66-LPP 1399

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Comparative Studies of Retinal Projections

Previous Serial Number: None

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.3
Professional:	.1
Other:	.2

Project Description:

Objectives: 1. To define patterns of central nervous system organization in primitive vertebrates. 2. To make a survey of the retinal connections with the diencephalon and mesencephalon in several species from each vertebrate class.

Methods Employed: Unilateral enucleations of an eye have been made in the following species: 1) toadfish (*Opsanus tau*); 2) frog (*Rana catesbiana*); 3) Tegu lizard (*Tupinambis nigropunctatus*); and 4) Moray eel (*Gymnothorax funebris*). The animals are sacrificed 20 to 35 days after surgery and the brains are then processed according to the method of Nauta. The silver impregnated degenerated axons are identified and three-dimensional reconstructions are made of the course and termination of the degenerated fibers.

Major Findings: Of the brains so far processed none has been analyzed in detail. The following general observations have been made: the fibers from the retina in the bull frog connect with the lateral geniculate nucleus, the hypothalamus, the ectomammillary nucleus and the optic tectum. The latter projection is characterized by terminations in four distinct layers. In the toadfish the same connections are noted with exception that there is no ectomammillary nucleus and the terminations in the optic tectum are confined to one layer.

Significance: The Nauta method provides us for the first time with an important tool for the tracing of thinly myelinated and non-myelinated axons, and since more primitive vertebrates have a great proportion of such fibers, we are now able to trace pathways that older methods have failed to elucidate. Our findings in the bull-frog confirm earlier studies on the retino-tectal projections employing micro-electrode techniques, in which four distinct layers in the tectum responded to different stimuli. Our information is still scanty or entirely wanting about the significance of the other projections from the retina. The study on the retinal projections in fish represent the first successful adaptation of the Nauta method to fish material. The significance of the absence of the basal optic roots in the specimens so far examined cannot be explained at this time.

Proposed Course of Project: 1. To examine representative species of fish, amphibians, and reptiles for variation in retinal projections using the Nauta method. 2. To examine these tracts and related nuclei using micro-electrode techniques in order to determine what kind of information the various tracts carry.

Publications: None

Serial No. NDB(I) 66-LPP 1400

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Functional properties of cells within the juxtastriate area 18 of the Rhesus monkey.

Previous Serial Number: None

Principal Investigator: Dr. Eugene C. Crichlow

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: Department of Physiology, School of Medicine,
University of Puerto Rico, San Juan, P.R.

Man Years:

Total:	.3
Professional:	.2
Other:	.1

Project Description:

Objectives: Anatomical findings have revealed that juxtastriate area 18 receives connections from the immediate adjoining portions of the striate cortex (Area 17). Juxtastriate area 18 sends fibers via the splenium of the corpus callosum into the homologous locus of the contralateral juxtastriate area 18, the ipsilateral "fingers" of area 18 proper and the homologous loci of the contralateral area 18 fingers. In this investigation an attempt will be made to deduce the functional characteristics of cells of this area.

Methods Employed: Extra-cellular unit recordings and intra-cellular unit recordings, when possible, will be used.

Major Findings: This project currently being initiated.

Significance: The juxtastriate area 18 lies in relation anatomically with that portion of the striate cortex corresponding to the vertical meridian. Since it is only these adjoining areas of striate cortex which project to juxtastriate area 18, the functional characteristics of cells of this area are particularly interesting inasmuch as they appear to be the only means of more directly conveying information from one striate cortex to another.

Serial No. NDB(I) 66-LPP 1400

Proposed Course of Project: To continue with project as planned.

Publications: No.

Serial No. NDB(I) 66-LPP 1401

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Investigations on the biochemistry of the necrobiosis of mouse brain.

Previous Serial Number: None

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.5
Professional:	.4
Other:	.1

Project Description:

Objectives: To study the biochemical alterations associated with post-mortem brain swelling observed in mice and the differences occurring between those which die rapidly versus those which die slowly.

Methods Employed: Rapid death of the mouse was accomplished by decapitation. Several methods were tried to produce slow death: 1) Mice were placed in a chamber into which the nitrogen flow was kept constant while oxygen flow was decreased; 2) They were placed in sealed flasks from 250 ml to 4000 ml. These two methods resulted in an increasing hypoxic state in the subjects. 3) They were treated with insulin to reduce blood sugar levels, and 4) with sodium nitrite to reduce oxygen carrying capacity of the blood. The proper conditions were not found which would maintain the mice near death for a minimum of 30 minutes.

The brains from the sacrificed mice were removed rapidly and incubated at 37°C in saline containing penicillin and streptomycin. The weights of the brains were recorded at intervals over a 50 hour period. Brains were frozen and lyophilized at intervals up to 50 hours.

Major Findings: All attempts to produce slow death in mice failed. However, data have been obtained on the wet and dry weights of the brains from decapitated mice incubated at 37°C in saline. The brain weight increases linearly with respect to time for a period of 24 hours when plotted

on log-log coordinates. The curve plateaus at the end of 48 hours when the weights of the brains are almost twice their original weight.

The dry weights of the brains decreased linearly with time when plotted on log-log coordinates.

Significance: The data show that during incubation in saline the brains gain in wet weight while decreasing in dry weight. The increase in wet weight can not be due to an increase of available low molecular weight (diffusible) substances due to the metabolic breakdown of substances in the cell since under the conditions of the experiment the tissue has no exogenous source of energy to maintain the integrity of its membranes. Consequently diffusible material must escape from the cell. This conclusion is substantiated by the net decrease in the dry weight of the brains during incubation. Therefore, other mechanisms must be sought to explain these observations.

Proposed Course of the Project: These studies will be continued with the cat and monkey where more careful control of physiological variables is possible. The cat and monkey brains will be studied not only for chemical changes occurring within the organ, but also will characterize the materials which diffuse from the tissues during their incubation in saline.

Publications: None

Serial No. NDB(I) 66-LPP 1402

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Investigations on the chemistry of necrobiosis of the cat brain.

Previous Serial Number: None

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:

Total:	.5
Professional:	.4
Other:	.1

Project Description:

Objectives: To establish the biochemical changes associated with post-mortem brain swelling observed in animals killed rapidly by decapitation versus animals killed slowly by exsanguination.

Methods Employed: Two cats were anesthetized with nembutal in each experiment. One cat was killed slowly by controlled exsanguination. EKG, heart rate, blood pressure, pulse pressure and respiration were monitored and recorded. The cat was bled until it reached arterial blood pressure levels of 15-20 mm of mercury. The animal was allowed to adjust to and maintain this hypotensive state for 30 minutes. Artificial ventilation was required. Blood was returned slowly as necessary to maintain the blood pressure. At 30 minute intervals thereafter blood pressure was adjusted to even lower levels. Large amounts of blood were eventually required to prevent circulatory collapse. The experiment was terminated when the original volume of blood had been returned to the animal. The control cat was sacrificed by decapitation at the time the experiment terminated.

Major Findings: When incubated in saline the brains from rapidly killed cats gained in weight and volume more rapidly than the brain from cats slowly killed by subjection to prolonged hypotension. The gains in weight were linear with respect to time when plotted on log-log coordinates. However, differences between the two types of brains were more dramatically illustrated when plotted on quadrangular coordinates. Little variation

in rate of weight gain was observed in the brains of rapid death cats. A wide variation in rates of weight gain was seen in slow death cats. Volume changes of the brains coincided with weight changes.

Significance: The information thus far accumulated has yielded quantitative data distinguishing between the brains of cats dying rapidly versus slowly. These data set the stage for subsequent studies of biochemical changes occurring in brains in relation to slow death versus rapid death.

Proposed Course of the Project: These experiments are to be continued and expanded to include the determination of biochemical processes responsible for inhibition of water and the differences observed between brains of subjects which die rapidly or slowly. Data obtained from these studies are believed applicable to investigation and understanding of similar phenomena which take place in vivo brain swelling. A knowledge and understanding of the mechanism of action of in vitro brain swelling may lead to ways and means of controlling brain swelling in vivo.

Publications: None

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Effect of a prefrontal cortical lesion on a non-spatial delayed response task.

Previous Serial Number: None

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigators: None

Cooperating Units: Department of Psychology, University of Puerto Rico, San Juan, Puerto Rico

Man Years

Total:	.9
Professional:	.4
Other:	.5

Project Description:

Objective: To investigate the extent of generality of behavioral deficit shown by the monkeys with prefrontal cortical lesions on delayed-response-type tasks.

Methods Employed: Individual monkeys are required to solve a delayed-response-type task in which spatial cues are replaced by object cues. Except this and consequent necessary control of spatial cues other features of the task are identical with the classic spatial delayed response task.

A pair of objects used differ from each other with respect to color and shape. An object consists of a "tray" part in which a bit of food is placed, and a "cover" part which conceals the food bit during delay and shows differential shape cue. The positions of the objects are varied from trial to trial according to a predetermined order.

An animal sees the process of an object being baited and the tray being covered. After delay it can obtain the food if it associates the object with the food and retains the association during the delay period.

To ascertain that the effect of the lesion is limited to the delayed-response-type task, all animals are trained also on two simultaneous visual discrimination tasks.

After original training of all the tasks, animals are tested for pre-operative retention, then undergo brain surgery except normal controls. After approximately a two-week recovery period, postoperative retention will be tested.

Major Findings: The behavioral test schedule has not been completed yet. However, all animals completed preoperative retention tests and underwent brain surgery except the control Ss.

Since all animals are still being tested, quantitative evaluation of results is not yet possible. However, a preliminary indication is that the prefrontal cortical lesion also impairs this type of non-spatial delayed-response task, whereas it has little effect on simultaneous visual discrimination tasks.

Significance: Although delayed response tasks and their varieties are used commonly to study effects of various kinds of brain lesion, curiously, to date, the task used in this project, in which spatial cues of classic delayed response task are replaced by visual cues in a straightforward manner, has not been known to be employed by other researchers.

The particular non-spatial delayed-response task should be useful in studying the generality of prefrontal cortical lesion effect on delayed response type tasks as it is carried out in this project.

Also, the particular task may become more useful in further analyses of the nature of prefrontal lesion effects on behavior, especially in view of recent evidence indicating that the prefrontal lobe may contain at least two functional areas, one relating to spatial features of behavioral tasks, and the other to visual cues.

Proposed Course of the Project: To continue the current plan and to add more experimental cases for clearer trends. Also, to add another group (with orbitofrontal lesion) and investigate the possible dual functional representation in the prefrontal lobe.

Publications: No.

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Effects of brain lesions on conditioned vocalization
in monkeys.

Previous Serial Number: None

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Shun-ichi Yamaguchi

Cooperating Units: None

Man Years

Total:	.2
Professional:	.1
Other:	.1

Project Description:

Objective: To observe effects of brain lesions on conditioned vocalization from the standpoint of understanding the relationship between the CNS and speech phenomena.

Methods Employed: Individual monkeys are placed in an operant conditioning situation to be conditioned to vocalize in order to obtain food reward. After conditioning is established, the animals will undergo brain surgery, and the resultant changes in vocalization behavior will be observed and measured.

Major Findings: The project has just begun (March, 1966). No findings are currently available.

Significance: Although speech disorders have been intensively studied in humans, experimental animal studies relevant to understanding speech functions are few, presumably because speech is considered unique to man. However, if speech is placed in a broader context of communication phenomena among living organisms, subhuman species also exhibit behavior with communication values.

It is still unknown if a rudiment of the CNS mechanism responsible for speech in man exists in any subhuman species, but if it does, studies

capitalizing on its existence should advance to a great extent the understanding of the speech function in man. The project is an attempt to explore the possibility that such a rudiment may exist in the monkey.

Proposed Course of Project: If initial exploration appears promising, further experiments defining neural mechanisms will be carried out. Attention will be directed to cortical and midbrain regions primarily.

Publications: No.

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Lactate concentration in amniotic fluid.

Previous Serial Number: None

Principal Investigator: Dr. Salha Daniel

Other Investigators: Dr. W. H. Niemann

Cooperating Units: Columbia Presbyterian Medical Center, New York, N. Y.

Man Years:

Total:

Professional:

Other:

Project Description:

Objectives: Determination of lactate concentration in amniotic fluid in relation to gestational age and possible relation to fetal distress.

Methods Employed: Five ml of amniotic fluid are aseptically collected at the time of cesarian section. The samples are quick-frozen and submitted for lactate analysis.

Major findings: Lactate determinations in the amniotic fluid of monkeys collected at various and also at the same gestational ages reveal a considerable (10 fold) degree of variability in concentration. Statistical methods have not yet been applied to more precisely characterize this variability statistically.

Significance: Lactate concentration in the amniotic fluid can not be used as an indicator of fetal distress because of its excessively wide range of occurrence in the normal fetus.

Proposed Course of Project: 1. Additional samples are needed in the lower gestational ages (40 to 80 day range). 2. Multiple samples from several individuals during various gestational ages would be valuable.

Publications: No.

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Title: Birth Weight and gestational age.

Previous Serial Number: None

Principal Investigator: Dr. Toshio Fujikura

Other Investigators: Dr. W.H. Niemann

Cooperating Units: Perinatal Research Branch, Section on Pathology, NINDB

Man Years:

Total:	.2
Professional:	.2
Other:	0

Project Description:

Objectives: A statistical study of the birth weights of over 600 animals of varying gestational ages. To develop a curve to predict birth weights at any gestational age.

Methods Employed: Commonly used statistical methods.

Major Findings: 1. High correlation of birth weight with gestational age is seen as expected. 2. Raw score difference between sexes is noted but statistical significance has not been determined. 3. The effects of multiparity, placental weight, bipartite or single placentas, and length of time in the colony have not been determined.

Significance: 1. Normative birth weight ranges at different gestational ages are needed to compare deviations from the normal in relation to current investigations within the laboratory. 2. A more uniform pathological classification of products of conception which do not come to term.

Proposed Course of the Project: Completion of statistical manipulations and plotting of curves.

Publications: None

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Title: The pathogenesis and transmission of pulmonary acariasis.

Previous Serial Number: None

Principal Investigator: Dr. Toshio Fujikura

Other Investigators: Dr. W.H. Niemann

Cooperating Units: Perinatal Research Branch, Section on Pathology, NINDB

Man Years:

Total:	.2
Professional:	.1
Other:	.1

Project Description:

Objectives: The mode of transmission of the species *Pneumonyssus simicola*.

Methods Employed: Three populations are under study, feral reared animals (India origin), Cayo Santiago reared animals and Laboratory reared animals. The lungs are examined grossly and microscopically for the presence of lung mites.

Major Findings: 1. Wild reared: 100% infestation of lungs examined. The intensity of involvement based on numbers of cysts seen on the pleural surface has been mild (2-3) to heavy (over 100). 2. Laboratory reared animals up to 4 years of age: no gross or microscopic evidence of *P. simicola*. 3. Cayo Santiago reared animals are variably infested. 4. Examination of the external nares, hair coat, tubinates and tracheal mucus has not revealed the presence of recognizable mite forms in a small number of samples taken from India reared animals. 5. Viable mites are seen at necropsy as long as 6 years in residence in the colony.

Significance: 1. The absences of lung mites in the Laboratory reared group suggests that a close physical relationship (maternal-infant) is necessary for the transmission of the mite. 2. Initial failure to recover mites externally on adults may be a result of periodicity of the parasite. 3. Viable mites after 6 years in cages suggests continued propagation within the host.

Proposed Course of the Project: 1. Additional samples to be collected for statistically significant numbers in all groups. 2. Continued sampling of external body parts for the presence of the mite at different times of the day. 3. Early morning stomach washing. Collection and examination to determine if the mites are coughed up and swallowed.

Publications: None

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Title: Mycoplasma antibody titers in the sera of normal and clinically ill M. Mulatta.

Previous Serial Number: None

Principal Investigator: Dr. Gilles Monif

Other Investigators: Dr. David L. Madden
Dr. W.H. Niemann

Cooperating Units: New York University School of Medicine, New York Medical Center,
Laboratory of Bacterial Diseases, NIAID

Man Years:

Total:	.3
Professional:	.2
Other:	.1

Project Description:

Objectives: Survey of the sera of clinically ill and normal individuals for antibody titer to mycoplasma spp.

Methods Employed: Collection of 5 ml whole blood samples from acute and convalescent animals and animals which show no clinical illness over a 6 months period. Submission of sera for a battery of serological tests to assay for the presence or absence of antibodies of mycoplasma species.

Major Findings: None. Sera is being collected at this time.

Significance: 1. The emergence of mycoplasma as a cause of primary atypical pneumonia in man and its incrimination in certain genital infections in man and animals (often associated with infertility in animals) raises the question of its presence in monkeys and possible public health significance. 2. Possible relationship to existing sterility within the breeding colony.

Proposed Course of the Project: Complete collection of serum samples by June 1966 and submission for serological work up.

Publications: None

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Title: Embryonic tooth development in the rhesus monkey.

Previous Serial Number: None

Principal Investigator: Dr. Bertram S. Kraus

Other Investigators: Dr. W.H. Niemann

Cooperating Units: The University of Pittsburg, Cleft Palate Research Center

Man Years:

Total:	.2
Professional:	.1
Other:	.1

Project Description:

Objectives: 1. Odontogeny of M. Mulatta fetus. 2. Survey of mouth structures for malformation and anomalies.

Methods Employed: The heads of fetuses of various gestational ages are submitted for histological and gross examination, along with pertinent clinical data on each individual.

Major Findings: None. The submitted tissue is being processed.

Significance: Normative data on the developing tooth and its surrounding structures. The incidence of malformation of the mouth parts in the monkey fetus.

Proposed Course of the Project: Collection and examination of additional material.

Publications: None

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Reproduction and behavior in the ecology of the Rhesus monkey.

Previous Serial Number: None

Principal Investigator: Dr. John A. Morrison

Other Investigators: Dr. Hans Kummer
Dr. Emil Menzel

Cooperating Units: Delta Primate Research Center

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: 1. To compare the group behavior of a social band of 80 monkeys before and after transferring the band from Cayo Santiago to Desecheo Island. 2. To correlate group movements and activities with the results of standard methods for censusing population samples. 3. To observe annually cyclic changes in the gonads and accessory sex organs of both sexes and compare the time between the sexes.

Methods Employed: The group to be moved to a new environment is closely followed for several hours each week to observe and record routine characteristics of inter- and intra-group activity. Photographs are made of representative behaviorisms, spatial relationships measured, and the temporal duration of certain events noted. Population sampling censuses will be performed at feeding stations, along the existing trails, and along randomly established transect lines. Commencing with the 1966 mating season, laparotomies, biopsies, and autopsies will be conducted on selected individuals of both sexes to describe the extent and characteristics of change in the reproductive organs.

Major Findings: The social band to be moved to Desecheo Island currently contains 64 monkeys one or more years of age and 18 newborn infants. Four adult animals, 3 males and 1 female, are castrates and will be retained at Cayo Santiago for other studies.

The observations made to date have not been tabulated and analyzed. Emphasis is being placed on the following items: a) structure of the group and

relative intra-group location of individuals during various kinds of group activity, general group cohesion; b) displays of dominance, aggression, leadership; c) grooming and individual associations and attachments between individuals, play among juveniles; d) flight distances to presence of an observer; e) aspects of the physical environment commonly attended, arboreal and terrestrial activity, travel routes; f) seasons of mating and birth; g) daily routine of activity, preference for particular areas.

Observations on Cayo Santiago will become intensified in mid-June when Drs. Kummer and Menzel will arrive to participate. The group will be trapped and moved to Desecheo in late June or early July then observed intensively for two weeks to describe its behavior while encountering an entirely new environment that is lacking in other, antagonistic social groups of Rhesus monkeys. Periodic observations will be performed to note the long-term aspects of adaptation, and ultimately more Rhesus groups will be introduced and inter-group reactions described.

Preliminary observations on general group activity and movement have been made throughout Cayo Santiago and the influence of the weekly feeding schedule noted. With the termination of the birth season at hand, a relatively stable population level will exist for two months during which time the weekly population samples will be censused. All samples will be made in areas of known size and an average density of monkeys per unit area computed. Knowing the actual total population will permit an evaluation of the accuracy with which the techniques estimate population size. Repeating the census after the large population removal during the next year will provide further opportunity to check the census accuracy.

During the 1965 mating season and in the months that followed it, mating activity and general sexual behavior were closely observed to become familiar with the characteristics of the Rhesus monkey at Cayo Santiago. No attempts were made then to investigate morphological changes associated with reproduction. This will commence in 1966 and continue into 1967. The reproductive organs will be studied grossly and histologically.

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Social behavior in confined Rhesus monkeys.

Previous Serial Number: None

Principal Investigator: Dr. Halsey M. Marsden

Other Investigators: Dr. John G. Vandenberg
Dr. Stephen Vessey

Cooperating Units: North Carolina Department of Mental Health

Man Years:

Total: 2.0
Professional: 1.0
Other: 1.0

Project Description:

Objectives: 1. To investigate the daily, seasonal, and lifelong social behavior of enclosed populations of primates, principally Rhesus monkeys (Macaca mulatta) with emphasis on inter- and intra-group relations, reproduction, and social behavior.

2. To determine the influences on social behavior of internal and external environmental factors.

Methods Employed: Primarily direct observation of marked monkeys confined in enclosures on Cueva island. Group structure is altered by removing or introducing animals. In some instances experimental procedures are employed on individual monkeys.

Major Findings: In Parguera, on Cueva island, studies were initiated with the two existing social groups confined in 1/4 acre enclosures. In an artificially formed group a preliminary study of manipulation of social structure emphasizing induced changes in the social rank of adult females has been completed. By replacing the dominant male with a strange adult male and removing and subsequently re-introducing the top-ranking female, the adult female dominance hierarchy was reversably altered. Patterns of aggression were observed in this study while family groupings and associations of individual animals were carefully followed. The changes in rank of offspring of the adult females almost exactly paralleled the changes in the rank of the mother, supporting the findings of Koford and Sade on the free ranging bands of Cayo Santiago. In addition, considering the dynamics of the rank changes, the

young not only reflected the mother's rank but participated in rank changes by suffering simultaneous aggression with the mother when her rank fell or directly contributing to the aggression against other members of the population when the mother's rank rose.

Proposed Course of Project: This study will be repeated using another social group and will be expanded to include aspects other than aggression, including studies of the relationship of various "dominant" males to the social groups under study. This latter will involve an effort to determine whether individual male dominants have important individual traits or, whether, in their role as dominants, their behavior is largely stereotyped. The ability to artificially manipulate social rank, if perfected to a somewhat greater degree, will present an excellent method to study physiological correlates of aggression and dominance patterns.

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Group behavior, reproduction, and population dynamics in free-ranging rhesus monkeys

Previous Serial Number: None

Principal Investigator: Dr. Stephen H. Vessey

Other Investigators: Dr. Halsey M. Marsden
Dr. John G. Vandenberg

Cooperating Units: North Carolina Department of Mental Health

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: 1. To investigate the daily, seasonal, annual and lifelong behavior of free-ranging primates, principally rhesus monkeys (M. mulatta), with emphasis on reproduction, group relations, and population dynamics.

2. To determine the influences on behavior of internal and external environmental factors.

Methods Employed: Primarily field observation of marked free-ranging rhesus monkeys on three islands. Selected animals are captured, examined, and released. Some are modified by castration, hysterectomy, implants, caging or other procedures. Two groups are permanently held in compounds for intensive observation.

Major Findings: Population Dynamics: As of February, 1966 the populations on La Cueva Island and Guayacan Island have increased to 179, 23 of which are maintained in enclosures. Forty births occurred in 1965 with a peak in May and June, the same as in 1964. Twenty-eight of 32 free-ranging infants survived, a mortality percentage of 12.5. Although the population density on La Cueva and Guayacan Islands is much lower than on Cayo Santiago, mortality rates were similar for all three islands. The total of 40 births was distributed among 54 adult females, giving a birth rate of 74%, lower than last year's 87%.

Reproduction: The peak in yearly rainfall occurs between the months of

August and November on La Cueva Island and Guayacan Island, whereas the yearly peak on Cayo Santiago is between May and August, about three months earlier on the average. The onset of mating activities occurs shortly after the peak in rainfall on all three islands, thus mating and births take place about three months earlier on Cayo Santiago than on La Cueva and Guayacan. Increased growth of vegetation resulting from these peaks in rainfall could be directly responsible for the initiation of breeding.

Social Dynamics: Three heterosexual bands occupy La Cueva Island, consisting of 20, 36 and 60 individuals each. The all-male band reported last year gradually fused with the largest band during the 1965 breeding season. Guayacan Island is occupied by a single band of 39 animals. Two young males are solitary, one on each island.

Proposed Course of Project: The relationship between ecological factors and the onset of the breeding season will be further studied. Various meteorological as well as phenological changes will be compared with the timing of births and breeding.

The effects of tubal ligation will be examined because one enclosed female with such an operation continued to have estrus cycles after normal females had ceased. The oviducts of four free-ranging females have been ligated to date, with plans to operate on two more.

The annual molt will be studied with respect to the overall timing in the population and individual patterns. Twenty-one animals of various ages, including two castrates, have been dyed black on one side to facilitate observation of new hair growth.

A study of interband relations has begun, with emphasis on the mechanisms of band dominance. Preliminary observations on group movements indicate that, although territoriality is not shown, bands have preferred locations which are determined to a large extent by the lack of higher ranking bands. Conditioning seems to be important in determining which group will dominate another. A few individuals of a high-ranking band can displace an entire low-ranking band even when greatly outnumbered. Removal of selected individuals and groups of individuals will show whether or not conditioning is involved and will also show which members of the group are responsible for the rank of the group. The first such removal will involve the dominant male of the middle ranking group. Subsequently, adult females will be removed one by one until an effect on the rank of the group is noted. Such manipulations will also yield information about relationships among animals of the same group, supplementing work being done in enclosures.

Serial No. NDB(CF) 61-LPP 820

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Effects of controlled early experience on development of the Siamese kitten.

Previous Serial Number: Same

Principal Investigator: Dr. Gilbert W. Meier

Other Investigators: None

Project Terminated: Investigator no longer associated with the laboratory.



Serial No. NDB(CF) 61-LPP 821

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Early development of emotional learning in monkeys

Previous Serial Number: Same

Principal Investigator: Dr. Gilbert W. Meier

Other Investigators: None

Project Terminated: Investigator no longer associated with the laboratory.

Serial No. NDB(CF) 61-LPP 822

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The effects of infantile trauma and social isolation during rearing upon adult reproductive behavior in the rhesus monkey Macaca mulatta.

Previous Serial Number: Same

Principal Investigator: Dr. Gilbert W. Meier

Other Investigators: None

Project Terminated: Investigator no longer associated with the laboratory.

Serial No. NDB(CF) 63-LPP 1075

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The effects of neonatal damage to the inferior colliculus upon the physiological and behavioral development of the rhesus monkey (*Macaca mulatta*).

Previous Serial Number: Same

Principal Investigators: Dr. Gilbert W. Meier
Dr. Donald P. Foshee

Other Investigators: Dr. Orlando Andy

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 63-LFP 1076

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Infantile Trauma: The role of genetic and environmental factors.

Previous Serial Number: Same

Principal Investigator: Dr. Gilbert W. Meier

Other Investigators: None

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 63-LPP 1077

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Studies on the development of sleep and wakefulness patterns in the Macaca mulatta.

Previous Serial Number: Same

Principal Investigators: Dr. Gilbert W. Meier and Dr. Ralph J. Berger

Other Investigators: None

Project Terminated: Investigators no longer associated with the laboratory.

Serial No. NDB(CF) 63-LPP 1080

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Experimental ketosis in the pregnant rhesus monkey.

Previous Serial Number: Same

Principal Investigator: Dr. John J. Schrufer

Other Investigators: Dr. Wendell H. Niemann

Project Terminated: Investigator no longer associated with the laboratory.



Serial No. NDB(CF) 63-LPP-1081

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Electrophoretic analysis of the hemoglobins of a large colony of *Macaca mulatta*.

Previous Serial Number: Same

Principal Investigator: Dr. John J. Schrufer

Other Investigators: Dr. M. A. Naughton
Dr. F. C. Battaglia
Dr. A. E. Hellegers

Project Terminated: Investigator no longer associated with the laboratory.

Serial No. NDB(CF) 63-LPP 1082

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: In vitro metabolism of ketone bodies by gestational and fetal tissues of the *Macaca mulatta*.

Previous Serial Number: Same

Principal Investigator: Dr. John J. Schrufer

Other Investigators: None

Project Terminated: Investigator no longer associated with the laboratory.

Serial No. NDB(CF) 63-LPP 1083

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Electrophoretic analysis of cord blood hemoglobins and infant hemoglobins in the *Macaca mulatta*.

Previous Serial Number: Same

Principal Investigator: Dr. John J. Schruefer

Other Investigators: Dr. André Hellegers
Dr. Frederick C. Battaglia
Dr. M. A. Naughton

Project Terminated: Investigator no longer associated with the laboratory.

Serial No. NDB(CF) 63-LPP 1088

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Mechanisms of vasomotor response observed after i.v. administration of a muscle relaxant in the cat.

Previous Serial Number: Same

Principal Investigator: Dr. Walter L. Stiehl

Other Investigators: Dr. C. C. González
Dr. Rafael Berríos

Project Discontinued

Serial No. NDB(CF) 63-LPP 1089

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Quantitative study of the growth of the skull and brain in the cat from birth to seventeen weeks of age.

Previous Serial Number: Same

Principal Investigator: Dr. Walter L. Stiehl

Other Investigators: Dr. Carmen Eva Cabrera

Project Discontinued

THE UNIVERSITY OF CHICAGO
DEPARTMENT OF CHEMISTRY

RESEARCH REPORT
NO. 1234
BY
J. D. SMITH
AND
M. E. JONES
1955

Serial No. NDB(I) 65-LPP 1265

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Responsiveness of denervated diaphragmatic muscle to immunological stimulation.

Previous Serial Number: Same

Principal Investigators: Dr. F. Alonso de Florida
Dr. José del Castillo

Other Investigators: None

Project Discontinued

Serial No. NDE(1) 65-LPP 1266

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The circulation of the lungs.

Previous Serial Number: Same

Principal Investigator: Dr. Walter L. Stiehl

Other Investigators: None

Project Discontinued



Serial No. NDB(I) 65-LPP 1267

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The effect of temperature change on muscle activity in the extremities after upper motor neuron lesions.

Previous Serial Number: Same

Principal Investigators: Dr. Walter L. Stiehl
Dr. Rafael Berríos

Other Investigators: Dr. C. C. González

Project Discontinued

ANNUAL REPORT
July 1, 1965 through June 30, 1966
Associate Director for Collaborative and Field Research
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

This year has been a significant one in the development of new collaborative programs and the strengthening of existing programs. Despite fiscal and space limitations, important progress has been made on all fronts of the Collaborative and Field Research Program. A brief summary of these specific accomplishments follows:

(1) Special Chronic Diseases Studies:-

Strides have been made in the slow and latent virus studies of chronic diseases of the central nervous system. Primates innoculated with cultures obtained from victims of "Kuru", a progressive disease of the nervous system prevalent in the Southwest Pacific area, have shown a positive reaction. Autopsies of animals who have died or been sacrificed have shown viral infections with clinical manifestations in part similiar to human victims. As a result of these preliminary findings, efforts are being expanded, within fiscal limitations, so that more data on this virus theory can be gathered and analyzed. This means additional animal experimentation coupled with broadened field activity in the Southwest Pacific area.

(2) Biometrics Branch:-

The Model Reporting area for Blindness Statistics Program was expanded and received significant new direction during the past year. Two new states were admitted to the Model Reporting area, bringing the total membership to 14. In addition, contracts are in effect in three states and the District of Columbia to support the completion and upgrading of their registers to qualify them for membership in the Model Reporting area. These 17 states, plus the District of Columbia represent nearly one third of the total U.S. population. This collaborative statistical project serves as a means for wide-scale data on prevalence, incidence, visual acuity, and cause of blindness is made available. They are the only data of their kind. With increased growth predictable, statistics on blindness in the U.S. will be significantly useful in planning programs of prevention and control. The MRA program has received wide support and endorsement throughout the country. Plans are underway to start evaluative studies, in line with recommendations of the Review Board which studied the MRA activities. An attempt will be made to determine the completeness of reporting of diagnosed blindness from a survey of all reporting sources in Vermont, with the cooperation of the Vermont Division for the Blind and with the endorsement of the pertinent professional associations and agencies in that State. There will also be an attempt to check the accuracy of recorded

medical and other data for blind persons on the New York State Blindness Commission register by comparing the register information with that abstracted from the medical records of blind persons known to a number of hospitals, clinics, homes for the aged, etc.

The Biometrics Branch is also supporting a study of blindness statistics in Egypt. This project, conducted by the University of Alexandria, Egypt, and supported by P.L.480 funds, is underway, and is proving to be quite productive even in its early phases.

(3) Epidemiology Branch:-

Significant program development advances have taken place under the leadership of Dr. Jacob A. Brody. Dr. Brody assumed duties as Chief of the Branch on July 1, 1965. He replaced Dr. Leonard T. Kurland who retired in September, 1964. Major areas of activity during Fiscal Year 1966 have been reevaluation of the Guam activity, development of laboratory facilities, development of new programs in prevalence of diseases of the eye, and genetic studies of neurologic diseases.

During this year, several long term studies at the NINDB Research Center, Guam, were completed. Considerable effort has subsequently gone into the planning of future studies on Guam in order to assure full utilization of the unusual opportunity to study neurologic diseases which is afforded to the program on this island. In order to redirect this planning and thinking, a team, including the Associate Director and Chief, Epidemiology Branch spent some time on Guam.

During Fiscal 1966, the Section on Ophthalmic Field and Developmental Research was established in the Branch. This section's activities are supervised by Dr. J. Theodore Schwartz. During the past year this new section has undertaken studies of chronic simple glaucoma in collaboration with the Division of Indian Health, EMS; studies of the influence of a synthetically derived aglycone of cycasin on ocular tissues in collaboration with the Laboratory of Experimental Pathology, NIAMD; and twin studies in Ophthalmology, utilizing a registry of 360 pairs of twins residing in the greater Washington area.

(4) Special Projects Branch:- Epilepsy Program:-

Departmental Committee on Epilepsy, composed of representatives from the Public Health Service, the Bureau of State Services, the Vocational Rehabilitation Administration, Office of Education, and the Welfare Administration, was established on June 14, 1965 for the primary purpose of coordinating the activities within HEW regarding the epilepsies. The first meeting of the Committee was held on July 9, 1965.

In December 1965, an Advisory Committee was appointed by the Surgeon General of the Public Health Service from the scientific community at large to assist in the development of programs concerning research and research training and service and service training in the field of

epilepsy. This body is jointly sponsored by NINDB and the Division of Chronic Diseases, Bureau of State Services. The Associate Director for Collaborative and Field Research, NINDB, was appointed Executive Secretary of the Committee.

The Section on Epilepsy:-

A protocol was developed for the pilot studies of a collaborative study by the Directors of four Clinical Centers and the Associate Director for Collaborative and Field Research. Of invaluable assistance in this was the statistical aid from the staff of the Perinatal Research Branch, C&FR. The proposals for the first phase of this study were approved by the National Advisory Neurological Diseases and Blindness Council at its March, 1966 meeting. Dr. James K. Penry will report for duty at NIH on July 1, 1966, as the Head of the Section on Epilepsy.

The Section on Head Injury:-

A Head Injury Planning Committee, sponsored by the NINDB Council, the American Neurological Association, the American Physiological Society, and the Society of Neurosurgeons, developed the plans for a conference on Head Injury that took place at the University of Chicago in February, 1966.

The proceedings of this conference will be published in June, 1966, and is intended to serve as a basis of a major NINDB program for the investigation of Head Injury.

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

PHS-NIH
Project Report
July 1, 1965 through June 30, 1966

Part A

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

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

Sub-Project II: Workshop-symposium on slow, latent, and temperate virus infections.

Sub-Project III: Fluorescent antibody technique in localizing neurotropic virus antigen in whole animals.

Sub-Project IV: Studies on the ecology, epidemiology and pathogenesis of arbovirus infections.

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

Other Investigators: Michael Alpers, M.D., Nancy Rogers, Mint Basnight, Pavel Albrecht, M.D.

Technical Assistants: Michael Sulima, Helene Gilbert, Edward van Steinberg, Alfred Bacote, Michael Nicholson, Philip van Steinberg

Student Assistants: Joel Kaplan, William Heilman, Valerie Gandler

Man Years (computed for 12 month period)

Total	8.25
Professional:	2.25
Other:	6.00

The studies reported in this section are being conducted in the NINDB laboratory of Slow, Latent, and Temperate Virus Infections at the Patuxent Research Center, Laurel, Maryland, in collaboration with the Bureau of Wildlife and Sports Fisheries, U.S. Department of Interior. Additional associated facilities are with the Laboratory of the Study of Child Growth and Development and Disease Patterns in Primitive Cultures, of which this project is a part and from which it originates (see Project Report Serial No. NDB (CF)-1282 (I-LX)).

Part A

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

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

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

Other Investigators: Michael Alpers, M.D., Pavel Albrecht, M.D. and Nancy Rogers

Cooperating Investigators: John C. Steele, M.D., University of Toronto, Canada; David Poskanzer, M.D., Massachusetts General Hospital, J. Craighead, M.D., Peter Bent Brigham Hospital, Boston; Richard T. Johnson, M.D., Cleveland Metropolitan Hospital; L.A. Zilber, M.D., Gamalaya Institute, Moscow, U.S.S.R.; Leonard T. Kurland, Mayo Clinic, Rochester, Minnesota; John T. Sever, M.D., NINDB; J.A. Morris, Ph.D. and H. Hopps, DBS; B. Dessel, M.D., Veterans Hospital, Wood, Wisconsin; A.J. Kenyon, D.V.M., University of Connecticut, Storrs; Mrs. E. Beck and Prof. P.M. Daniel, Department of Neuropathology, Maudsley Hospital, London; P.A. Palsson, D.V.M., M. Gudnadottir, M.D. and H. Thormar, Ph.D., Institute of Experimental Pathology, Keldur, Iceland; C.M. Eklund, M.D. and W. Hadlow, D.V.M., Rocky Mountain Laboratory, NIAID; J. Hourrigan, R. Reisinger and H. McDaniel, Animal Research Section, USDA; E. Dustman and C. Herman, Patuxent Research Center, Department of Interior; J. Dorman, M.D., NINDB; J. Brody, M.D., Epidemiology, NINDB; C. Colson, M.D., Columbia Presbyterian Hospital, New York; R. Katzman, M.D., Yeshiva University, New York; M. Schaeffer, M.D., City of New York Department of Health, and H. Fischman, D.V.M., Otisville, New York.

Project Description:

Objectives: The objectives of this long-term project remain as established: 1) to attempt to demonstrate infectious etiology for progressive degenerative diseases of the nervous system of man and animals by transmission to experimental hosts and suitable isolation techniques; 2) to determine the epidemiological significance of established prototype strains of viruses that cause slow, latent, temperate and chronic infections of the nervous system of man and animals; 3) to continue to elicit the biological, physical and chemical characteristics of the prototype strains and newly isolated strains of viruses known to induce slow infections, in order to define the basic bio-

logy of the new group of infectious and pathogenic microorganisms; 4) to develop new techniques for the successful isolation, identification, and characterization of the etiological agents of degenerative diseases of the nervous system of man and animals; 5) to develop and modify serological tests for detection, identification, and relationship of established and newly isolated agents; 6) to prepare, maintain, establish, and explore cell culture lines of autopsied and biopsied tissues from the central nervous system and other organs of man and animals affected with diseases under study, and to apply these techniques to the study of masking, latency, and temperateness of viruses that cause these slowly progressive degenerative diseases of the nervous system.

Methods Employed: In general, standard and classical techniques for transmission and isolation of viruses are employed with certain notable exceptions: a) the demonstration that the genetic mechanism of the host can determine the host resistance or susceptibility to expression of diseases of interest to our group has required inoculation of specimens into an extensive array of higher primates, i.e. apes and chimpanzees, and a number of species of lower primates, including both old and new world monkeys, as well as domestic animals, i.e. sheep, goats, pigs; and birds--such as ducks, geese, chickens and turkeys--in addition to the entire spectra of ordinary laboratory animals, rabbits, hamsters, guinea pigs, rats, and as many as 17 genetically defined lines of mice; b) a wide variety of tissue and cell cultures from animal and avian sources, as well as explants of tissue from naturally affected hogs; c) inoculated animals are held in isolation under carefully controlled observation for a minimum of five years in the case of large animals and up to three years in the case of mice and other small short-lived animals; d) pre-bleedings on all animals are tested to determine base-line antibody spectra, and serial bleedings collected during the course of observation are tested to determine sero-conversion to known agents; in addition, these sera are studied to determine newly formed antigenic properties; e) clinically ill animals are sacrificed with the approval and by recommended procedures of the Animal Care Panel, and serial transmission is attempted by employing the central nervous system and other organs to inoculate the discussed variety of animals; f) detailed neurohistological, as well as general, histological studies are made of each animal that is sacrificed or that has died from nonspecific causes during the study; g) well-established criteria for the selection of cases to be studied, acquisition of central nervous system tissue, preferably at surgical biopsy or at autopsy within a maximum of two hours of death of the patient, and optimal preservation in liquid nitrogen are followed. The following diseases are under study in this laboratory: kuru, Guamanian amyotrophic lateral sclerosis and related Parkinsonism dementia, amyotrophic lateral sclerosis occurring in patients resident in the continental U.S., subacute sclerosing leucoencephalitis, Schilder's disease, Parkinson's disease, multifocal leucoencephalopathy, metachromatic leucoencephalopathy, multiple sclerosis, progressive supranuclear palsy, necrotizing encephalitis. Additional methods being employed in these studies are concerned with that part of the program devoted to laboratory studies designed to more fully define the nature of scrapie virus as a model virus that causes a slow, but progressive, degeneration of the central nervous system of sheep, goats, and mice. Finally, all inocula are tested for the presence of other micro-

organisms such as bacteria, rickettsia, PPL0, fungi, parasites, etc.

Major Findings: Animal inoculation with human materials: As described in previous annual reports, inoculation of primates with kuru (7 cases), amyotrophic lateral sclerosis (6 cases), and the related Parkinsonism dementia (6 cases) was accomplished in January, August and September 1963. During 1964 and 1965 additional animals were inoculated with suspension of tissue from patients affected with diseases already described in this report. In all, until the beginning of the period covered by this report, some 12 chimpanzees and more than 300 other primates were inoculated and housed in the same facility with at least one uninoculated chimpanzee and approximately 25 uninoculated monkeys representative of the species under study, none of which developed a clinically recognizable neurological or other type of disease. During the period covered by this report a clinical syndrome, astonishingly akin to kuru in man, has developed in SEVEN of the EIGHT chimpanzees inoculated in mid-1963 and early 1964. The incubation period has ranged from 18 months to 30 months, average 24.5 months, after intracerebral inoculation with brain suspension from different kuru patients. The course of the clinical illness has ranged from 3 to 9 months, average 5.5 months. This fatal syndrome with progressive cerebellar ataxia of the trunk and all limbs and incoordination leading to increased disability, has not been seen as a spontaneous disease of apes and is the first convincing evidence of the transmissibility of one of the subacute or chronic human central nervous system diseases under investigation in our program. Further, of the 36 chimpanzees and more than 300 smaller monkeys in these transmission experiments from human tissue no animals have developed a chronic progressive neurological disorder, other than the SEVEN kuru-inoculated chimpanzees. At autopsy of these SEVEN animals there was no gross neuropathological lesions other than a fine needle scar at the site of the inoculations in the frontal lobe. Histological studies, as yet incomplete, have revealed the diffuse non-specific changes seen in the brain of kuru victims, such as generalized astrocytic hypertrophy, widespread status spongiosus and more system-bound degeneration of the cerebellum. The remarkable clinical correspondence of a disease developing successively in SEVEN of EIGHT chimpanzees inoculated, in all but one instance in which two of the subsequent affected chimpanzees were injected with the same material, with brain material from a different kuru patient, the onset in each after a similar long incubation period, the fact that there is no such syndrome of chimpanzees known to occur spontaneously or seen at present in our many control animals, and the remarkable similarity of the neuropathological findings, in the cases thus far examined, to those observed in kuru victims, lead us to conclude that kuru has been transmitted experimentally to these chimpanzees.

Primary cell cultures of human and animal origin: Employing standard tissue culture techniques we have successfully cultivated central nervous system tissues in vitro from human kuru specimens, as well as from freshly biopsied CNS tissue specimens from patients with multiple sclerosis and multifocal leucoencephalopathy, amyotrophic lateral sclerosis, subacute sclerosing leucoencephalitis; a thymus cell line has been established from a patient with myasthenia gravis, and successful explants are being cultivated from normal chimpanzee tissue as well as that from chimpanzees affected with the kuru-like

syndrome. These in vitro studies are being closely followed for determination of antigenic fractions that may react with known specific viral antisera as well as for electron microscopy for detection of viral-like intracellular particles. In order to establish potentially susceptible cell lines we have successfully cultivated CNS and thymus tissue from normal and scrapie infected mice and posterior dorsal root ganglia from newborn rhesus monkeys. Cell line susceptibility studies to known viruses are underway to determine interference by latent or masked viruses.

Scrapie: During the period covered by this report we have determined the scrapie virus to have an approximated density in saturated cesium chloride of 1.32 showing it not to be a naked nucleic acid nor totally lipid in nature; we have determined by serial gradacol membrane filtration that the size of the total virus particle is in the range of 17-26 μ in diameter which places the virus in the small size group of viruses and suggests the possibility of a "helper" virus for clinical infection; expanded immunological studies have consistently been negative in attempting to elicit antibodies to scrapie virus by neutralization, complement fixation, direct and indirect fluorescent staining as well as mouse passive protection-type neutralization in vivo tests. Current and continuing experiments to develop usable antigen antibody reactive tests include adaptation of phage neutralization test of primary incubation of undiluted serum and high concentration of virus followed by inoculation of animals with post-incubation serially diluted material, addition of accessory factors (fresh guinea pig serum and fresh monkey serum) to determine the effects of complement on successful neutralization of infectivity, and, hyperimmunization of animals with partially purified and concentrated suspension of scrapie infected animal tissue.

Significance to bio-medical research and the program of the Institute: The development of a kuru-like syndrome in SEVEN of EIGHT chimpanzees inoculated with human kuru brain material is the first transmission of a chronic progressive degenerative disease of the nervous system of humans to an experimental animal host in our program. This development amplifies the need and justification for the intensified search for an infectious etiology in chronic degenerative neurological diseases based on the concept of slow viruses dependent upon genetic factors of the host for development and expression of disease following a prolonged incubation period. These long term studies continue to represent the first major effort to experimentally induce certain neurological diseases of humans in experimental animals and to isolate their causative agents. They formed the major foundation for the convening during last year of a highly successful symposium on slow, latent and temperate virus infections at the National Institutes of Health with participants from several of the states, as well as from foreign countries. The meetings resulted in a monograph that is expected to serve as the only current available reference on diseases of this nature. Further, from the detailed physical, chemical and biological characterization of known slow viruses, such as scrapie, we are developing techniques for the study of degenerative diseases of man, not only in our own laboratory, but throughout the world.

Proposed course of the project: 1) Identification and characterization of the agent causing disease in chimpanzees; 2) continued long term observation of inoculated animals. Continued serial studies on the fractionation of serum specimens from these animals for the determination of shifts in the

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

Part B included: Yes

Part B: Publications

- Alpers, M. and Gajdusek, D.C.: Changing patterns of kuru: epidemiological changes in the period of increasing contact of the Fore people with Western civilization. *Amer. J. Trop. Med. Hyg.*, 14: 852-879, 1965.
- Beck, Elisabeth, Daniel, P.M. and Gajdusek, D.C.: A comparison between the neuropathological changes in kuru and in scrapie, a system degeneration. *Proceedings of the Vth Inter. Cong. Neuropathology*, Excerpta Medica Foundation, Amsterdam, in press.
- Morris, J.A., Gajdusek, D.C. and Gibbs, C.J., Jr.: Spread of scrapie from inoculated to uninoculated mice. *Proc. Soc. Exper. Biol. Med.*, 120:1 (Oct.), 108-110, 1965.
- Gibbs, C.J., Jr. and Gajdusek, D.C.: General considerations of slow virus infections. *Proceedings of the Inter. Symposium on Rabies (Geneva)*, in press.
- Gajdusek, D.C. and Gibbs, C.J., Jr.: Slow, latent and temperate virus infections of the central nervous system. Monograph 43 of: *Research in Nervous and Mental Disease*, Williams and Wilkins, Baltimore, in press.
- Gajdusek, D.C., Gibbs, C.J., Jr. and Alpers, M.: Experimental transmission of a kuru-like syndrome to chimpanzees. *Nature*, 209: (Feb.), 794-796, 1966.
- Kakulas, B.A., Lecours, A.-R. and Gajdusek, D.C.: Further observations on the pathology of kuru (a study of two cerebra in serial section). *Jour. Neuropath. Exper. Neurol.*, in press.
- Edgar, G.W.F. and Gajdusek, D.C.: Chemical assay of demyelination of kuru. *Nature*, in press.
- Beck, Elisabeth and Gajdusek, D.C.: A note on the variable size of the septal nuclei in man. *Nature*, in press.
- Gibbs, C.J., Jr.: Search for infectious etiology in chronic and subacute degenerative diseases of the central nervous system. *Bacteriological Reviews*, in press.

Part A

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

Sub-Project II: Workshop-Symposium on Slow, Latent and Temperate
Virus Infections.

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

Other Investigators: Michael P. Alpers, M.D. and Paul W. Brown,
M.D.

Cooperating Investigators: See: List of Participants and Table of
Contents in: Slow, Latent and Temperate Virus
Infections. Editors: D. Carleton Gajdusek, Clarence
J. Gibbs, Jr. and Michael P. Alpers, National
Institutes of Health, 1965. (Monograph of papers
presented at the Workshop-Symposium).

Part B included: Yes

Part B: Publications

Gajdusek, D.C., Gibbs, C.J., Jr. and Alpers, M.P., editors:
Slow, Latent and Temperate Virus Infections, NINDB, National
Institutes of Health, U.S. Government Printing Office, Washington,
D. C.

(Monograph on Workshop and Symposium held
at the National Institutes of Health, Bethesda,
Maryland, December 7-9, 1964; 44 papers, 7
definitive bibliographies, subject index and
authors index)

Contributions in above Monograph from this section:

Gajdusek, D.C.: Kuru in New Guinea and the origin of the NINDB
study of slow, latent and temperate virus infections of the
nervous system of man.

Gibbs, C.J., Jr. and Gajdusek, D.C.: Attempt to demonstrate a
transmissible agent in kuru, amyotrophic lateral sclerosis,
and other subacute and chronic progressive nervous system
degenerations of man.

Alpers, M.: Epidemiological changes in kuru, 1957 to 1963.

Gibbs, C.J., Jr., Gajdusek, D.C. and Morris, J.A.: Viral
characteristics of the scrapie agent in mice.

Morris, J.A., Gajdusek, D.C. and Gibbs, C.J., Jr.: Spread of
scrapie from inoculated to uninoculated mice.

Definitive Bibliographies:

Kuru in New Guinea

Scrapie with References on Rida

Visna

Maedi

Aleutian Mink Disease with References on Chediak-Higashi
Syndrome

Chediak-Higashi Syndrome

Hemorrhagic Fevers in South America

Part A

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

Sub-Project III: Fluorescent antibody technique in localizing neuro-
tropic virus antigen in whole animals

Principal Investigators: Pavel Albrecht, M.D. and D. Carleton
Gajdusek, M.D.

Other Investigators: Paul W. Brown, M.D., Michael P. Alpers, M.D.,
Nancy Rogers, Clarence J. Gibbs, Jr., Ph.D. and
Mint Basnight

Cooperating Investigators: J. Hotchin, M.D., Division of Laboratories,
New York Department of Health, Albany

Project Description:

Objectives: Use has been made of lymphocytic choreomeningitis virus as a model in establishing the methodology to be used in studies of other central nervous system diseases wherein a suspicion of possible virus etiology is entertained and to define the pathogenesis of LCM. To prepare fluorescent animal anti-sera to antigens in brain tissue of kuru, amyotrophic lateral sclerosis and other chronic central nervous system degenerative disorders; to purify and concentrate scrapie virus and prepare FA reagents in a search for specific antigens in infected tissues.

Methods Employed: Lymphocytic choreomeningitis in mice is used as a developmental tool in establishing fluorescent antibody techniques in the localization of virus antigen during a slow virus infection involving the central nervous system. Scrapie virus is subject to fluorocarbon deproteinization purification and ultracentrifugation concentration. A variety of experimental hosts are being immunized with vaccines and serum fractionation procedures are being employed to elicit an immune response.

Significance and Course: Study of LCM as an example of the slow or latent virus infection and, particularly, as an example of the phenomenon of acquired immune tolerance to infection in the mouse. This work on the pathogenesis of lymphocytic choreomeningitis is used both as a valuable addition to our knowledge in its own right and to establish proceedings which may aid in localizing virus antigen if any is present in other chronic

Serial No. NDB (CF)-62 OAD 969 (III)
Continued Project 1963 Serial No.
NDB (CF)-63 OAD 1140

central nervous system diseases wherein virus etiology is suspected. Diseases in which we will use the techniques are kuru, ALS, scrapie and Aleutian mink disease. Satisfactory preparation and staining of cross-sections of whole mice has been developed. The course and distribution of LCM has been defined in neonatally infected primary tolerant immune mice sacrificed at intervals after infection. The distribution of virus has also been defined in primary tolerant immune mice afflicted with 'late disease' occurring eight to 12 months after infection. Hyperimmune sera are being prepared to human brain material from cases of chronic and subacute central nervous system disease. In order to more firmly establish scrapie as a virus, to determine the onset and course of disease and to define the possible relationship of scrapie to kuru and other disease the development of a workable Ag-Ab system is paramount.

Part B included: Yes

Part B: Publications

Publications other than abstracts from this project:

Brown, Paul: Immunofluorescent Studies of Lymphocytic Choriomeningitis Infection in Neonatal Mice. J. Exper. Med. (in press)

Hotchin, J., and Brown, P.: The pathogenesis of late onset disease of mice with persistent tolerated infection by lymphocytic choriomeningitis virus. Manuscript, 1966.

Part A

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

Sub-Project IV: Studies on the ecology, epidemiology and pathogenesis of arbovirus infections.

1. Epidemic hemorrhagic fever in Calcutta.
2. Seroepidemiology of arbovirus infections in ecologically isolated primitive indigenous populations:
 - a) Seroepidemiology of Alaskan populations.
 - b) Seroepidemiology of the populations of the Caribbean and Central and South American countries with particular reference to Puerto Rico, Bolivia and Paraguay
 - c) Seroepidemiology of Australasian populations

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

Other Investigators: M. Alpers, M.D., Paul Brown, M.D., Nancy Rogers, Mint Basnight and P. Albrecht, M.D.

Cooperating Investigators: K. Shah, M.D. and F. Bang, M.D., Johns Hopkins University School of Public Health, Baltimore; B.K. Aikert, M.D., Institute of Post-Graduate Medical Research, Calcutta; J. Casals, M.D., Yale University, J. Brody, M.D., Epidemiology, NINDB; R. Randall, D.V.M., WRAIR, WRAMC; J. Sever, M.D., NINDB; C. Wisseman, E.B. Helwig, M.D. and Rosenzweig, E., Ph.D., University of Maryland Department of Microbiology; T. Work. M.D., C.D.C., Atlanta; R. Hornabrook, Kuru Research Center, New Guinea; F. Schofield, M.D., Public Health College, Gandar, Ethiopia; P. Allen, Ph.D., V and R division, Ft. Detrick; R. Taylor, M.D., Univ. California School of Public Health

Work has continued into the epidemiology, ecology and antigenic relationships that exist among the arboviruses in an effort to determine their public health importance and prevalence in selected populations. During the period covered by this report new studies were initiated in collaboration with the Epidemiology Branch of NINDB designed to determine the neurological sequellae in humans that may have suffered inapparent infections during the perinatal period with Jap B encephalitis on the island of Guam. Future plans call for completion of the serological survey and detailed neurological examination by resident neurologists.

Serial No. NDB (CF)-65 QAD 1282 (I-XI)

1. Collaborative-Field Research
 2. Office of Associate Director
 3. Bethesda, Maryland
- Projects NDB (CF)-62 QAD 970,
NDB (CF)-63 QAD 1138 and 1139 have
now been integrated into this project.

PHS-NIH

Project Report

July 1, 1965 through June 30, 1966

Part A

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

- Sub-Project I: Study of the developmental patterning of the human nervous system (A cybernetics of human development)
1. A research archive for ethnopediatric film investigation of styles in the patterning of the nervous system.
 2. Analysis of child care and behavior patterns in primitive cultures from photographic recording (development of techniques and methods).
 3. Investigation of non-recurrent phenomena (objectives and selectivity to be used in documentation of aperiodic phenomena to preserve maximum information).
 4. Analysis of culturally determined methods of approach to symbolic representation from drawings and art forms of children and adults in primitive societies.

- Sub-Project II: Human evolutionary study in isolated primitive groups
1. Kuru.
 2. Motor neuron disease and other degenerative diseases in New Guinea and in other inbred Pacific Island populations.
 3. Blood group genetic studies in Australasian (Melanesian and Micronesian) and South American indigenous groups.
 4. Studies on polymorphism of hemoglobins, red cell enzymes, and serum proteins in Australasian (Melanesian and Micronesian) and South American indigenous groups.

- Sub-Project III: Studies of isolated Micronesian populations
1. Child development and behavior on Ulithi, Ifaluk and Lamotrek atolls and Fais Island.
 2. Response to live measles virus vaccine in immunologically virgin populations without circulating measles virus (with special attention to response in susceptible adults and pregnant women and their offspring).
 3. Influenza A2: virgin soil epidemics (epidemiological, clinical and immunological response and discovery of populations without previous experience with Type A or

Type B influenza).

4. Study of infectious disease patterns in remote individual island populations.

5. Genetic characterization of the population of the Caroline Islands.

Sub-Project IV: Studies of isolated New Guinea populations

1. Child development and behavior in the Western Dani, Asmat, Tjitjak, Auyu, Kayagar groups of West New Guinea, the Kukukuku and Eastern Highland people of the Territory of Papua and New Guinea, the Mangsing, Mamusi and West Nakanai of New Britain.

2. Study of infectious disease in the diverse ecologically isolated New Guinea populations.

3. Genetic characterization of New Guinea populations.

4. Hereditary and genetic disease patterns in New Guinea.

5. The Kukukuku peoples: an intensive longitudinal study of child growth, development and behavior and disease patterns, human genetics and communication in an archaic Highland population of New Guinea.

6. Research cinema films on behavioral patterns of children in New Guinea cultures.

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

1. Child development and behavior on Tongariki and the Banks and Torres Islands and with the Ipayato of Espiritu Santo.

2. The Tongariki project: an intensive study of human evolution in the Shepherd Islands.

3. Human genetics and disease patterns survey of the Banks and Torres Islands.

4. Infectious disease seroepidemiology of the New Hebrides.

Sub-Project VI: Studies on Australian aborigines

1. Arbovirus seroepidemiological studies on Australian aboriginal communities of Cape York.

2. Infectious disease patterns survey of aborigines of Bentnik-Mornington Islands, the Kimberly, the Haast's Bluff and Cape York regions of Australia.

Sub-Project VII: Studies on Central and South American Indians

1. Human genetics and disease pattern studies of the Guayaki and Chaco Indian tribes and of the Mennonite colonists in the Chaco of Paraguay.

2. Children of the Guayaki Indians of Southeastern Paraguay.

3. Children of the Ayore (Moro) Indians of Bolivia and Paraguay.

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

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

1) Investigation of the social parameters producing "jumpiness" in mice.

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

1) Notational systems for human movement.

2) Ciphers and notation for human form, including ciphering for physiognomy, physique, palm printing, and ear form and hair.

3) Theoretical study of notational problem in mathematics, linguistics, music and dance.

4) Alphabet: a theoretical investigation of their relation to linguistics and their application to non-linguistic information.

5) Form recognition: a) neuroanatomic and genetic determination of preferential recognition; b) inter-relationship of the problem in computer programming and the arts.

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

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

Other Investigators: Clarence J. Gibbs, Jr., Michael Alpers, M.D., Pavel Albrecht, M.D., Nancy Rogers, E. Richard Sorenson, Mint Basnight.

Student Assistants: Max King, Allen Havens, Polly Sackett, Joanne Samuelson, Steven Oslon and Kathy Proper.

The section on Child Growth, Development and Behavior, and Disease Patterns in Primitive Cultures has continued all sub-projects listed in the Annual Report of 1964, with considerable expansion of collaborating investigators and institutions which are reflected in the authorship of all publications listed in Part B. The titles of the sub-projects and their subdivisions are sufficiently explicit to constitute in themselves the project description.

Part B: Yes

Part B: Publications

- Gajdusek, D.C.: Factors governing the genetics of primitive human populations. Cold Spring Harbor Symposia on Quantitative Biology, 29: 121-135, 1964.
- Simmons, R.T., Graydon, J.J., Gajdusek, D.C., Schofield, F.D. and Parkinson, A.D.: Blood group genetic data from the Maprik area of the Sepik District, New Guinea. Oceania, 35: (March), 218-232, 1965.
- Curtain, C.C., Gajdusek, D.C., Kidson, C., Gorman, J., Champness, L. and Rodrigue, R.: Serum pseudocholinesterase levels and variants in peoples of Papua and New Guinea. Amer. Jour. Trop. Med. Hyg., 14: (July), 671-677, 1965.
- Curtain, C.C., Gajdusek, D.C., Kidson, C., Gorman, J., Champness, L. and Rodrigue, R.: A study of the serum proteins of the peoples of the Territory of Papua and New Guinea. Amer. Jour. Trop. Med. Hyg., 14: (July), 678-690, 1965.
- Curtain, C.C., Baumgarten, A., Gorman, J., Kidson, C., Champness, L., Rodrigue, R. and Gajdusek, D.C.: Cold haemagglutinins: unusual incidence in Melanesian populations. Brit. Jour. Haematol., 11: (July), 471-479, 1965.
- Alpers, M. and Gajdusek, D.C.: Changing patterns of kuru: epidemiological changes in the period of increasing contact of the Fore people with Western civilization. Amer. Jour. Trop. Med. Hyg., 14: (Sept.), 852-879, 1965.
- Brown, P., Basnight, M. and Gajdusek, D.C.: Response to live attenuated measles vaccine in susceptible island populations in Micronesia. Amer. Jour. Epid., 82: (Sept.), 115-122, 1965.
- Simmons, R.T., Graydon, J.J., Gajdusek, D.C. and Brown, P.: Blood group genetic variations in natives of the Caroline Islands and in other parts of Micronesia. With a supplement by S.H. Riesenbergl: Tables of voyages affecting Micronesian islands. Oceania, 36: (Dec.), 132-170, 1965.
- Curtain, C.C., Gajdusek, D.C., Kidson, C., Gorman, J., Champness, L. and Rodrigue, R.: Haptoglobins and transferrins in Melanesia: relation to hemoglobin, serum haptoglobin and serum iron levels in population groups in the Territory of Papua and New Guinea. Amer. Jour. Phys. Anthropol., 23: (Dec.), 363-379, 1965.
- Sorenson, E.R. and Gajdusek, D.C.: The study of child behavior and

Part B: Publications (continued)

- development in primitive cultures. A research archive for ethnopediatric film investigations of styles in the patterning of the nervous system. *Pediatrics*, 37: (Part 2) (Jan.), 149-243, 1966.
- Brown, P., Gajdusek, D.C. and Morris, J.A.: Epidemic A2 influenza in isolated Pacific Island populations without pre-epidemic antibody to influenza virus types A or B, and the discovery of other still unexposed populations. *Amer. Jour. Epid.*, 83: (Jan.), 176-188, 1966.
- Simmons, R.T. and Gajdusek, D.C.: A blood group genetic survey of children of Bellona and Rennell Islands (B.S.I.P.) and certain northern New Hebridean Islands. *Archaeol. and Phys. Anthropol. in Oceania*, 1:1, 1966.
- Gajdusek, D.C., Gibbs, C.J., Jr. and Alpers, M.: Experimental transmission of a kuru-like syndrome to chimpanzees. *Nature*, 209: 794-796, 1966.
- Simmons, R.T. and Gajdusek, D.C.: Blood group genetic variations in natives of West New Guinea (West Irian). *Amer. Jour. Phys. Anthropol.*, in press.
- Beck, E., Daniel, P.M. and Gajdusek, D.C.: A comparison between the neuropathological changes in kuru and scrapie, a system degeneration. *Proc. Vth Inter. Cong. Neuropath., Excerpta Medica Foundation*, in press.
- Gajdusek, D.C. and Gibbs, C.J., Jr.: Slow, latent and temperate virus infections of the central nervous system. *Research in Nervous and Mental Disease*, Monograph 43, Williams and Wilkens, Baltimore, 1965, in press.
- Beck, E. and Gajdusek, D.C.: A note on the variable size of the septal nuclei in man. *Nature*, in press.
- Kakulas, B.A., Lecours, A.-R. and Gajdusek, D.C.: Further observations on the pathology of kuru (a study of two cerebra in serial section). *Jour. Neuropath. Exper. Neurol.*, in press.
- Edgar, G.W.F. and Gajdusek, D.C.: Chemical assay of demyelination of kuru. *Nature*, in press.
- Gibbs, C.J., Jr. and Gajdusek, D.C.: General considerations of slow virus infections. *Progress in Immunobiological Standardization*,

Part B: Publications (continued)

- S. Karger, Basel, in press.
- Gajdusek, D.C., Gibbs, C.J., Jr. and Alpers, M., editors: Slow, Latent and Temperate Virus Infections. NINDB Monograph No. 2, National Institutes of Health, U.S. Government Printing Office, Washington, D.C., in press.
- Gajdusek, D.C. and Alpers, M.: Kuru in childhood: disappearance of the disease in the younger age group. Pediatrics, in press.
- Nicholson, M.K. and Gajdusek, D.C.: "Jumpiness" of adult C57BL/6 mice as a marker for sibling deprivation in infancy. Use of the infant mouse as a rapidly maturing and reproducing and genetically stable mammal for the study of early physical and social factors influencing adult behavior. Pediatrics, in press.
- Morris, J.A., Gajdusek, D.C., Shaw, C.W. and Brown, P.: Epidemic A2 influenza in isolated Pacific Island populations. J. Microbiol., in press.
- Brown, P.: Immunofluorescent studies of lymphocytic choriomeningitis infection in neonatal mice. J. Exper. Med., in press.
- Gajdusek, D.C.: Slow virus infections. New Eng. J. Med., in press.
- Morris, J.A., Jr., Elizan, Teresita S. and Gajdusek, D.C.: Delayed neurological disease produced by ingestion of karwinskia humboldtiana. In preparation.
- Simmons, R.T., Gajdusek, D.C., Gorman, J.G. and Kidson, C. and Hornabrook, R.W.: The presence of the Duffy blood group gene Fy^b demonstrated by Melanesians. Nature, in press.
- Lehman, H., Gajdusek, D.C., Guiart, J. and Kirk, R.L.: Note on a new alpha chain mutation in hemoglobins in New Hebridean populations. Nature, in press.
- Alpers, M.: Kuru. In: A Manual of Tropical Medicine, IVth Edition. Hunter, G.W., Frye, W.W. and Swartzwelder, J.C., editors. W.B. Saunders, 1965.
- Beck, E., Gajdusek, D.C. and Kariks, T.: Neuroanatomical variation in the structure of the human brain in diverse peoples. In preparation.
- Gajdusek, D.C., Alpers, M., Shwachman, H. and Kopito, L.: Lead levels in the hair, nails, and serum of isolated primitive populations in contrast to the mounting lead levels in Americans. In preparation.

Gajdusek, D.C., Alpers, M. and Steinberg, A.G.: Distribution of Gc factor polymorphisms in South Pacific populations. In preparation.

Gajdusek, D.C., Alpers, M., Kitchin, D. and Bearn, A.G.: Distribution of Gm factor polymorphisms in South Pacific populations. In preparation.

Gajdusek, D.C., Alpers, M., Kitchin, D. and Bearn, A.G.: Aboriginal Gm factor in kuru and in natives of the kuru region. In preparation.

ANNUAL REPORT
July 1, 1965--June 30, 1966
Biometrics Branch - Collaborative and Field Research
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

A. SCOPE OF PROGRAM

The activities of the Biometrics Branch during the period July 1, 1965--June 30, 1966 may be divided into the following general programs:

1. Model Reporting Area for Blindness Statistics (MRA).
2. Research Projects with Other Investigators.
3. Statistical Consultation and/or Service Given to Investigators Outside of NINDB on Other Projects.
4. Statistical Consultation and/or Service to Clinical, Basic and Collaborative Research Investigators at NINDB in the Areas of Neurology and Blindness.

The status of each of the above programs is given below.

1. The Model Reporting Area for Blindness Statistics.

(a) Primary Objectives

The purposes of the Model Reporting Area are to make better statistics available on blind persons and to stimulate research in the field of blindness. To accomplish these objectives, the Model Reporting Area encourages complete reporting of blind persons to the registers maintained by member State agencies; improves the records concerning the reported blind; uses a common definition of blindness; standardizes the recording of essential information; and disseminates statistics derived from the registers to all who are interested in the problems of blindness.

(b) Significant Accomplishments, July 1, 1965--June 30, 1966

Each of the States comprising the Model Reporting Area, which was organized in 1962, maintains a State-wide register of blind persons. On July 1, 1965 the Area was composed of twelve such States, the States of New Mexico and Virginia having been added to the membership earlier in the year. However, as indicated below, a number of other States were actively engaged in improving their registers so as to meet the requirements of the Model Reporting Area. This resulted

in the addition of two such States to the Model Reporting Area, raising the total membership to 14 States by June 30, 1966.

All but one of the member States now have their registers on a tabulating type punch card system, but even that State has also expressed an interest in converting its present register to such a system with the assistance of the Biometrics Branch. The use of punch cards throughout the Model Reporting Area provides great flexibility in the production of tabulations of blindness statistics derived from the program.

Acting upon request from various States, the Biometrics Branch provided technical and consultative assistance to a number of state agencies interested in membership in the Model Reporting Area. Five States were under contract during the year to establish or improve their existing registers so as to meet the requirements of the Model Reporting Area. Upon satisfactory completion of their contracts, two of the States (South Dakota and Utah) were admitted to the program. The other three States should achieve membership during the next fiscal year. The 17 States which are members or actively working toward membership represent almost one-third of the total United States population.

Tabulations on the incidence and prevalence of blindness and severe vision impairment were produced for the Model Reporting Area member States covering the calendar year 1964. These data were published as the 1964 Statistical Report of the Model Reporting Area, the third in a series of annual Statistical reports. Included in the Report were tabulations on additions to the registers during the year (i.e. incidence), number on the register at the end of the year (i.e. prevalence), and removals from the register. The tables were prepared according to such characteristics as sex, age, race, degree of vision, and cause of blindness.

The Model Reporting Area program is providing a means for wide-scale adoption of a Standard Classification of Causes of Severe Vision Impairment and Blindness, which has been developed and sponsored by the National Society for Prevention of Blindness. The Biometrics Branch has played an integral part in revising the Standard Classification and the Index to the Classification during the past year. Through a quality control program, the Biometrics Branch is attempting to achieve a high degree of uniformity in the coding of causes of blindness throughout the entire Model Reporting Area. In the quality control program the Biometrics Branch staff reviews the coding of causes of blindness of all or a random sample of all ophthalmological eye reports processed by the member States. The Model Reporting Area program has also aided in the adoption

by a number of Model Reporting Area States of a standard eye report form prepared by the National Society for the Prevention of Blindness.

A review of the Model Reporting Area program by an Ad Hoc Review Panel was made during the fiscal year. Following are the Panel's recommendations on matters within the limits of its charge: (1) The MRA should be continued since the data being collected are of definite value in carrying forward an important mission of NINDB; (2) If and when more efficient and productive methods in collecting such data are developed, such methods should replace those now used by MRA; (3) In the event that MRA develops into an operation of wide coverage of prospective permanence, it is recommended that the function of MRA within NINDB should then be as a collection and integration point for the data from the several State registers, and a coordinating center for the separate operations; (4) It is most important, for the time being, that validation of the methods being used by MRA, and any others being developed, be expedited and that such validation should not only precede any further expansion, but also be built into any further expansion of MRA. A start has been made on the implementation of these recommendations.

(c) Major Plans for Fiscal Year 1967

Plans are underway to start evaluative studies, in line with recommendations of the Review Board, mentioned above. An attempt will be made to determine the completeness of reporting of diagnosed blindness from a survey of all reporting sources in Vermont, with the cooperation of the Vermont Division for the Blind and with the endorsement of the pertinent professional associations and agencies in that State. There will also be an attempt to check the accuracy of recorded medical and other data for blind persons on the New York State Blindness Commission register by comparing the register information with that abstracted from the medical records of blind persons known to a number of hospitals, clinics, homes for the aged, etc.

It is planned to continue providing technical and financial assistance and support to States interested in membership in the Model Reporting Area, until the program has achieved a significant representation of the United States population. An annual conference will be held if sufficient funds are included in the budget for this purpose.

The Model Reporting Area program continues to receive wide support throughout the country. In addition to previous endorsements received from the American Optometric Association and the Council of State Directors of Vocational Rehabilitation, the Model Reporting Area was endorsed by the American Academy

of Ophthalmology and Otolaryngology and a similar endorsement was made by the Section on Ophthalmology of the American Medical Association, at its Annual Convention in June 1965.

2. Research Projects with Other Investigators.

(a) Primary Objectives

The objective of this program is to conduct significant research in the area of neurological and sensory disorders of interest to the NINDB.

(b) Significant Accomplishments, July 1, 1965--June 30, 1966

(1) A study of association between perinatal factors and blindness in children: the purposes of this retrospective study are to determine (a) whether the birth weight distribution of children classified as blind due to prenatal factors is different from that of an appropriate control population of live births, and (b) whether the pregnancies of mothers of such blind children are characterized by an excess of selected perinatal disorders. The study was undertaken with the cooperation of the New York State Commission for the Blind and the New York City and the New York State Departments of Health. Included in the study are approximately 600 blind children and a control group composed of a stratified sample of approximately 8,000 recorded live births during the study period in New York State. Data pertaining to conditions of pregnancy, labor and delivery for both cases and controls, were obtained from the confidential medical supplements of the respective birth certificates. All of the necessary information has been abstracted, tabulations have been prepared, the final analysis is in progress, and a report of the study is in preparation.

(2) American Public Health Association Monograph on Neurological and Sense Organ Disorders: this is one of the series of monographs, sponsored by the American Public Health Association, which includes members of the Biometrics Branch among the authors. This monograph will contain information on the mortality, morbidity, and epidemiology of neurological and sense organ disorders, including a review of pertinent literature already published. Detailed tabulations were prepared on mortality from various neurological disorders to be included in the monograph, and these will be incorporated, where pertinent, in the various chapters of the monograph. A meeting of the authors was held and plans for the completion of the monograph were detailed.

- (3) A study of association between perinatal factors and strabismus in Negro children: the objectives were to determine (a) whether the birth weight distribution of children classified with strabismus due to prenatal factors is different from that of an appropriate control population of live births, and (b) whether the pregnancies of such strabismic children are characterized by an excess of selected perinatal disorders. The records of various eye clinics in Baltimore were used for selection of study cases. Birth certificates in the Baltimore City and the Maryland State Health Departments were used for selection of controls. Information on prenatal and obstetric complications was obtained from hospital records. The case group consisted of some 400 children meeting specified criteria with regard to diagnosis of strabismus, age, place of birth, and other factors. An appropriately matched control group was used for comparison. Data have been collected, and analyzed. The major findings were: Lower birth weight of the strabismic subjects was associated with shorter pregnancies, possibly indicating that the strabismic babies were immature rather than undersized. These children were not found to be undersized relative to control infants born after gestational periods of the same length. Strabismic infants weighed less even when gravidity was controlled, a result which suggests that strabismus is directly associated with birth weight. Thus, a reduction in prematurity should result in lowered incidence of strabismus.

The mothers of strabismic children had no more complications of pregnancy and delivery than the control mothers. They were the women delivering premature infants without recognizable cause. They had suffered more previous pregnancy loss (abortion or stillbirth) than control mothers of similar gravidity. And women with a previous history of pregnancy loss are at an increased risk of having premature babies, particularly without a specific clinical cause for the prematurity.

A significantly higher proportion of strabismic infants than controls evidenced major abnormalities at birth or shortly thereafter, especially respiratory conditions causing anoxia. A majority of the abnormalities among subjects and controls were those usually associated with prematurity, a finding re-emphasizing the importance of the relationship between prematurity and childhood morbidity.

This study was presented at the October 1965 annual meeting of the American Public Health Association, Chicago, Illinois, and is expected to be published in the December 1966 issue of the Journal of the Public Health Association.

- (4) Study of awareness, attitudes, and performance in reporting persons to blindness registers: this study is being done under contract with the American Foundation for the Blind. It is concerned with the actual reporting behavior of ophthalmologists, EENT specialists, and optometrists, as well as their awareness of, and attitudes regarding, the reporting of blind persons to registers. Also under study are the operations of State registration agencies, including the communications which have evolved between these agencies and the reporting sources with whom they deal. This study will supplement the work of the Model Reporting Area for Blindness Statistics (MRA) by focusing on the key problem of obtaining complete reporting of blind persons. After a number of pre-tests of drafts of questionnaires, final versions of questionnaires for directors of blindness registration agencies, their consulting ophthalmologists, and ophthalmologists and optometrists in the field were designed and approved for study. Field interviewing began June 1, 1965 and was completed by July 15, 1965. The next phase of the study was concerned with the preparation of tabulations and analysis of the data. Twenty-nine States were included for study. These included States in the MRA and all States with mandatory blindness registers so that comparisons of reporting behavior and attitudes might be made for ophthalmologists and optometrists separately in States having mandatory reporting against those who do not; in MRA member States versus non-member States, by region of country; and by the amount and type of information provided (regarding registers) by the registry agencies. In addition to the 29 register agency directors and the 29 consulting ophthalmologists to these agencies, 439 ophthalmologists located in the 195 cities of the 29 States with 50,000 population or more (as of April 1, 1960) and 192 optometrists (located in the same cities). All interviews were conducted by trained interviewers of a commercial research firm under sub-contract and were face-to-face interviews. Endorsements for the study were obtained from the American Academy of Ophthalmology and Otolaryngology and the American Optometric Association.
- (5) Evaluations of the levels of ocular tension in an Italian-American population and comparison of tensions obtained by three methods: the purpose of the project, conducted jointly with the Epidemiology Branch, was to describe tension levels in an Italian-American community, Nesquehoning, Pennsylvania. The project as described in the Annual Report of the Epidemiology Branch. Since a similar population in Roseto, Pennsylvania, has shown an unusually low frequency of deaths due to myocardial infarction, it was believed that a survey in the Nesquehoning area would offer valuable information

pertaining to the possible association between chronic vascular disease and chronic simple glaucoma. Three methods of tonometry were employed in examining each individual: applanation sitting, applanation supine and Schiötz. As the descriptive facet of the survey was deemed most important, bias due to confounding change of position and the resultant time interval in the two applanation series could not be resolved. Preliminary investigation has disclosed no striking peculiarities in the population but significant differences among tonometric methods. See Epidemiology Branch's Individual Project Report Serial No. NDB (CF) - 64 E 1186.

- (6) A study of association between perinatal factors and deafness in children: the purposes of this retrospective study are to determine (a) whether the birth weight distribution of children classified as deaf due to prenatal factors is different from that of an appropriate control population of live births, and (b) whether the pregnancies of mothers of such deaf children are characterized by an excess of selected perinatal disorders. The study was undertaken with the cooperation of the New York State Education Department, the New York City Board of Education, and the New York State and New York City Departments of Health. Included in the study were approximately 900 deaf children and a control group composed of a stratified sample of approximately 7,000 recorded live births during the study period in New York State. Data pertaining to conditions of pregnancy, labor and delivery, for both cases and controls, were obtained from the confidential medical supplements of the respective birth certificates. All of the necessary information has been abstracted.
- (7) A study of blindness statistics in Egypt: the objectives of this study are (a) to ascertain the feasibility of developing a blindness register on a pilot study basis in an Egyptian community as a mechanism for producing statistics on blindness (including severe vision impairment) needed for prevention and control activities and (b) to determine to what extent modifications may be made in procedures developed by the Model Reporting Area for Blindness Statistics (MRA) in the United States that, when applied in Egypt, will permit production of statistics comparable to those of MRA. By adapting the standards of the MRA to the collection of data on blindness in urban areas of Alexandria and rural areas outside the city, it is hoped to determine prevalence and incidence rates of blindness that will be compared with those derived from MRA data. The project has three phases. Phase I has been completed. In Phase I a random sample of 2,000 households (consisting of about 10,000 persons) was selected. This sample consisted of

persons from urban and rural areas, roughly in proportion to their numbers in the study population. A listing of all households in the urban and rural areas provided an up-to-date complete frame of households from which to sample. When possible, all members of households falling into the sample were tested for central visual acuity, and for peripheral field where such visual acuity was better than 6/60 in the better eye with best correction. Visual acuity was obtained with the use of portable vision testers which ensured standard lighting and distance, as well as privacy, during such examinations. These vision testers were modified so that they could be used equally well in homes with or without electricity and in homes using either 110-volts or 220-volts. The survey also included refraction (without drops) and measurement of peripheral field vision by means of portable perimetry. Trained examiners were used for all testing. All persons determined by the survey to be blind, according to definition, were examined later by ophthalmologists. These physicians not only confirmed the visual acuity, refractive and perimetric data determined by the survey, but also attempted to reach a diagnosis and, possible, the underlying cause of the condition. Attempts were made periodically throughout the duration of this Phase to ensure uniformity in vision testing by examiners and in diagnosis by ophthalmologists. The purpose of Phase II will be to build up a blindness register by attempting to have all the residents of the study areas (approximately 250,000 persons) visit the health centers or the University Hospital voluntarily for a vision examination identical to that given in Phase I. Again, all persons picked up as blind will be referred for ophthalmological examination. In addition, ophthalmologists and all agencies, hospitals and clinics serving the blind will be canvassed to get information on their known blind and will be encouraged to report blind cases to the register on a continuing basis. The results of the involuntary screening of Phase I will be compared with those of the voluntary screening in Phase II in order to determine which demographic groups are under-represented among those reporting for examination. This will permit better focusing of educational efforts to bring about completeness of voluntary screening. Phase III will be a repeat of Phase I with re-examination of persons in the original sample of 10,000 persons still resident in the study areas in order to determine new cases of blindness that have developed in the interim and all cases that have had their vision restored. Emphasis will be placed on standardization of procedures in the uniform collection of data and, thus, assure as far as possible the comparability of data between the MRA and the Egyptian pilot study. It is expected that

interest to NINDB. It is hoped that such efforts would produce research of a sound nature, avoid duplication of efforts by various agencies, help to coordinate existing or conflicting programs and help to yield a uniform and meaningful approach to the problem areas concerned.

(b) Significant Accomplishments, July 1, 1965--June 30, 1966

The Chief of the Biometrics Branch served on the following committees:

Biostatistics Special Review Panel, Division of Research Grants, NIH
Advisory Committee on Operational Research, National Society for the Prevention of Blindness
Steering Committee, Blinded War Veterans Project, American Foundation for the Blind
Ad Hoc Committee on Mental Retardation Program Statistics, DHEW
Scientific Advisory Council, Myopia Research Foundation
Scientific Advisory Committee, Massachusetts Institute of Technology Sensory Aid Evaluation and Development Center

Consultation was given to the Executive Director and professional staff of the New York Association for the Blind on various statistical aspects and record keeping of the Model Reporting Area for Blindness Statistics.

A Biometrics Branch staff member represented the NINDB at a work conference sponsored by the American Association on Mental Deficiency (AAMD), on "Improving the AAMD Classification Through Research."

Consultation was rendered to the Project Director of the National Study of Maternity Care, American College of Obstetrics and Gynecology on research areas of possible mutual interest.

Consultation on, programming of, and instruction in the use of the programs and the use of the Mathatron was given to members of the NINDB staff.

A Biometrics Branch staff member was invited to attend the Third Psychiatric Case Register Meeting sponsored by the Office of Biometry, NIMH, and be a member of a panel concerned with "Cooperative Register Experience Regarding Other Disorders and Conditions."

(c) Major Plans for Fiscal Year 1967

Continuation of these activities.

this study will provide precise information to indicate the extent to which data collection procedures of a highly developed country may and should be modified so that, for purposes of statistical evaluation, they are usable in a less sophisticated environment.

- (8) Evaluation of the levels of ocular tension in an American Indian population on the Parker Indian Reservation and comparison of three methods of tonometry and tonography: The purpose of the project is described in the Annual Report of the Epidemiology Branch, NINDB. The Biometrics Branch is concerned solely with work-ups and analysis of data. Comparison of the tension levels of the Indian population with others similarly examined, evaluation of the performance of different tonometric techniques and comparison of tonometry and tonography findings are the primary aims of the project. See Epidemiology Branch's Individual Project Report Serial No. NDB (CF) - 64 E 1323.
- (9) Evaluation of the levels of ocular tension in an American Indian population and comparison of tension obtained by four methods and blood pressure: The purpose of the project, conducted jointly with the Epidemiology Branch, was to describe tension levels in an Indian population on the Salt River Reservation, Phoenix, Arizona. The project is described in the Annual Report of the Epidemiology Branch. The comparison of four methods of tonometry, applanation sitting, applanation supine, Schiotz and the modified Maklakov applanometer method will afford an opportunity to assess the performance of each technique relative to the others. Population distribution of ocular tension may be directly compared to those obtained under similar circumstances but with different ethnic groups. [See (5) and (8)] See Epidemiology Branch's Individual Project Report Serial No. NDB (14) - 66 B 1324.

(c) Major Plans for Fiscal Year 1967

Work will be continued on the projects described above and papers will be prepared on those projects which will have been completed during the year. Where it is possible to undertake new projects, within the limits of personnel and budget, this will be done.

(d) Special Activities

None.

3. Statistical Consultation and/or Service Given to Investigators Outside of NINDB on Other Projects.

(a) Primary Objectives

The purpose of this program is to provide guidance or service upon request, to various agencies concerned with programs of

(d) Special Activities

None.

4. Consultation and/or Service Furnished to Clinical, Basic and Collaborative Research Investigators at NINDB in the Areas of Neurology and Blindness.

(a) Primary Objectives

The purpose of this program is to provide consultative and computational services in the design, analysis, and evaluation of research and experiments conducted by the clinical, basic and collaborative research investigators at the Institute.

(b) Significant Accomplishments, July 1, 1965--June 30, 1966

Various units of NINDB were provided with consultative and computational services in the design, analysis, and evaluation of research and experiments conducted by the clinical, basic and collaborative research investigators at the Institute.

Below are indicated the units in NINDB that received aid from the Biometrics Branch from July 1, 1965 through June 30, 1966

(1) Medical Neurology Branch

Statistical assistance and consulting was rendered as follows:

- a. Consultation and analysis of muscle tension data by use of regression analyses and analyses of variance.
- b. Standard errors, t-tests, and a determination of sample sizes in analyzing data on conductive velocity.

(2) Surgical Neurology Branch

Cooperation in the preparation of a manuscript to be published as follows:

Ommaya, A.K., and Sadowsky, D.: A system of coding medical data for punched-card machine retrieval. Part II - As applied to head injury. To be published in J. Trauma. Sept. 1966.

Consultation, assistance, analyses and programming were given for the following projects:

- a. Large scale research on factor analyses. Set up, explained, taught, and programmed mathematical model for obtaining weights and regression weights for estimating the factor scores on the Wechsler-Bellevue I.Q. scores on Forms I and II.

- b. Research and preparation of a mathematical model for an analysis of variance for a mixed model with unequal n's.
- c. Research on, instruction in, and setting up of mathematical model for varimax analysis of factor loading. Also helped to program method of solution on H-800.
- d. Programmed the Mathatron for obtaining Chi-square, analyses of variance, etc.
- e. Review of papers on psychological investigations, on the application of computer techniques to clinical research, on artificial intelligence, and on Apgar indices.
- f. Consultation and statistical assistance in the set up of a punched-card file for electrode implantation including organizing the code list, constructing the code sheet, processing the cards, and analyzing a sampling of the data.
- g. Research on and setting up of model for a problem on probabilities in brain mapping.
- h. Consultation on, setting up of mathematical model for, analyses of, and interpretation of data on induced head injuries. This includes probit analyses, t-tests, chi-square tests, regression analyses, etc.
- i. Preparation of code list, code sheets, and tabulation of medical records on stimulation in epilepsy.

(3) Laboratory of Neuroanatomical Sciences

- a. Instruction in and programming of the Mathatron for analyses of data on animal fiber counts, area of bundles, and area of spinal cord in fish.
- b. Spike heights vs. mm. from crush for from 1 to 27 days after operation on dogs analyzed by correlation, regression, graphing, analyses of variance, etc. These analyses programmed for and computed by CDP under our direct supervision.
- c. Cell measurements analyzed by t-tests. Verification of philosophy of experiments and statistics used in a paper.

(4) Ophthalmology Branch

Consulting and statistical assistance in analyses of data was given on the following projects:

- a. Correlation, regression analyses, analyses of covariance, t-tests, etc., on the I.O.P. turnover

vs. K out, K out and volume vs. controls, and I.O.P. drug analyses.

- b. Correlation, regression, and regression analyses for data on I.O.P., K out, and inflow of normal and abnormal rabbits (done with Dr. Philip R. McMaster I-IGAR).
- c. Comparison of I.O.P. and flow with the data from a paper by Dr. Marvin L. Sears. Obtained measures of skewness and normalized Sears' data and then compared by t-tests to available data.
- d. Analyzed by means of standard deviations, t-tests, etc., relative ophthalmological thresholds at various degrees from fovea and normal eyes vs. eyes under drug dosages.
- e. Parametric statistics (means, standard deviations, t-tests, analyses of variance, etc.) and non-parametric statistics (non-parametric correlations and Cochran Q tests) on data on intraocular pressures on outflow for normal, glaucoma, and glaucoma suspect patients for various drugs for pre-drug and various periods post-drug.
- f. Instruction and illustrations of the use of pre-wired programs and programs written especially for the Mathatron.
- g. Set up and instructions in the use of analysis of variance for fixed effects model, Model I, and mixed and random effects model, Model II.

(5) Laboratory of Neuropathology

- a. Consultation on a long-term brain weight study including advice on the methods of analysis of the data, the number of animals necessary for the experiments, and preliminary analyses obtaining means, standard deviations, t-tests, etc.

(6) Collaborative and Field Research

- a. Writing program for computation of maximum likelihood estimates of gene frequencies for ABO, MNS, Rh, etc., blood groups.
- b. Review methods of collection and analyses of data for an epilepsy study.

(7) Epidemiology Branch

- a. Consultation dealing with sample size of a proposed study of chromosomes in abortuses occurring within the first trimester. Study would involve analysis of blood samples to be obtained from women in their pregravid and gravid states in order to detect rubella or other findings and relate such results to the chromosome findings in the abortuses.
- b. Consultation on design and data collection involved in a study of malignant neoplasms of the eye, including the obtaining of data through the National Vital Statistics Division, and from death certificates in almost all States of persons dying from malignant neoplasms of the eye.

(c) Major Plans for Fiscal Year 1967

Continuation of the provision of consultative and computational services to clinical, basic and collaborative research investigators at the Institute.

(d) Special Activities

None.

B. TIME DISTRIBUTION OF BRANCH ACTIVITY

Below are indicated data representing Biometrics Branch man-years of work for the 12-month period, July 1, 1965-June 30, 1966 for all activities and for each major project.

Biometrics Branch, NINDB
Man-Years of Work

All Activities

Total:	17	1/10
Professional	7	9/10
Other	9	1/5

Model Reporting Area for Blindness Statistics

Total:	4	19/20
Professional	3	9/10
Other	1	1/20

Man-Years of Work

Perinatal Study of Blindness in Children

Total:	1/2
Professional	9/20
Other	1/20

Strabismus Study

Total:	1/2
Professional	7/20
Other	3/20

Italian-American Tonometry Survey

Total:	1/5
Professional	1/10
Other	1/10

Parker Indian Reservation Tonometry Survey

Total:	3/10
Professional	1/10
Other	1/5

Salt River Indian Reservation Tonometry Survey

Total:	1/2
Professional	3/10
Other	1/5

Study of Awareness, Attitudes and Performance
in Reporting Persons to Blindness Registers

Total:	3/5
Professional	2/5
Other	1/5

United States Mortality from Neurological and
Sense Organ Diseases: A.P.H.A. Monograph Series

Total:	1	1/20
Professional		3/10
Other		3/4

Perinatal Study of Deafness in Children

Total:	2/5
Professional	3/20
Other	5/20

Egyptian Blindness Study

Total:	1/5
Professional	3/20
Other	1/20

Mathematical Statistical Consultation

Total:	2 1/5
Professional	4/5
Other	1 2/5

Miscellaneous

Total:	1 2/5
Professional	2/5
Other	1

Administration

Total:	4 3/10
Professional	1/2
Other	3 4/5

C. PROBLEMS ENCOUNTERED

As a result of a Congressional increase for investigative studies of blindness, there was a restoration to the Biometrics Branch of four of the eight positions lost in 1963. The ceiling on promotions within the Branch continues to make difficult the retention or recruitment of senior personnel necessary to maintain the optimum conduct of the Model Reporting Area for Blindness Statistics and other program activities within the Branch.

D. PROPOSED OBJECTIVES

To date, the energies of the Biometrics Branch have been directed, within the limits of staff and budget, to the aspects of the programs mentioned above. The Branch has and will continue to work for improved statistics on the blind as well as to make the statistics from State to State more comparable by continuing its present program of furnishing help to States in developing and improving their blindness registers in order to meet the standards for admission to the Model Reporting Area. Where necessary and possible, contracts with State register agencies for the blind will be undertaken in order to assist such agencies that could not otherwise meet the Model Reporting Area standards. The Biometrics Branch will stimulate States to utilize their blindness registers with greater effectiveness for administrative and research uses. Finally, it will attempt to initiate new research studies in the area of neurologic and

sense organ disorders, in addition to carrying out those on which it is presently engaged.

The Branch will continue to furnish statistical consultation to serve clinical and basic research scientists at NINDB as well as outside investigators.

1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Model Reporting Area for Blindness Statistics

Previous Serial Number: SAME

Principal Investigators: Hyman Goldstein and Irving D. Goldberg

Other Investigators: Geoffrey G. Dell'Osso, Candler M. Hawkins,
George J. Inada, Ronald L. Jacobson,
Allen R. Lewis and Helen B. Moorhead

Cooperating Units: Connecticut Board of Education of the Blind
Kansas Department of Social Welfare
Louisiana Department of Public Welfare
Massachusetts Department of Education
New Hampshire Department of Health and Welfare
New Jersey Commission for the Blind
New Mexico Department of Public Welfare
North Carolina Commission for the Blind
Oregon Commission for the Blind
Rhode Island Department of Social Welfare
South Dakota Service to the Blind and
Visually Handicapped
Utah Department of Public Instruction
Vermont Department of Social Welfare
Virginia Commission for the Visually Handicapped

Man Years

Total:	4 19/20
Professional:	3 9/10
Others:	1 1/20

Project Description:

Objectives: The Model Reporting Area for Blindness Statistics is a voluntary association of States which maintain registers of persons with serious visual impairment, and is organized under the sponsorship of the National Institute of Neurological Diseases and Blindness. (For convenience, the MRA defines the term blindness to include severe visual impairment as described below.) Each member State has agreed to uphold the standards for membership, and may continue in membership as long as

it upholds them. Periodic evaluation of the performance of each member State will be made by the NINDB in order to insure maintenance of these standards.

The two basic objectives, to which each member State subscribes and agrees to uphold, are to make better statistics available on blind persons and to stimulate research in the field of blindness. In order to accomplish this, the MRA will:

1. Encourage complete reporting of the blind, so that the number registered may more nearly reflect the true prevalence of blindness, and so that additions to the registers may more nearly reflect the true incidence;
2. Seek to improve the records concerning the reported blind, so that the causes of blindness and the characteristics of the blind may more easily be studied;
3. Use a common definition of blindness and standardize recording of essential information, so that data from different States can be more meaningfully compared or pooled in an effort to arrive at national statistics on blindness.

Methods employed: Each member State accepts for statistical purposes the MRA definition of blindness, which includes severe visual impairment as follows: "Visual acuity of 20/200 or less in the better eye, with best correction; or visual acuity of more than 20/200 if the widest diameter of the field of vision subtends an angle no greater than 20 degrees." Individuals with a progressive eye condition which does not yet meet the MRA definition are excluded. As understood here, the term best correction does not include devices which cannot be used continuously or which improve visual acuity to more than 20/200 only by restricting the field of vision to 20 degrees or less.

Each member State will attempt to include on its register all residents who fall within the MRA definition of blindness. Such registrants will constitute its MRA Register. The State may also register other persons under a wider definition of blindness, but only if these persons can be easily distinguished for statistical purposes. The MRA Register will not be confined to persons who have applied for services; nor will there be any arbitrary exclusion of persons on the basis of age, race, or any other factor outside the criteria of the MRA definition of blindness.

Each member State will attempt to collect all of the items of information listed below for each registrant. It will routinely make efforts to clarify ambiguous reports, and to obtain the data missing from incomplete reports. The required items must appear on the register card (or its equivalent) for ready access. These items are the essential minimum; however, States are encouraged to collect additional pertinent information.

- (a) Date of addition to the MRA register
- (b) Type of addition to the MRA register (i.e. whether first addition or readdition)
- (c) County of residence (or its equivalent)
- (d) Date of birth
- (e) Sex
- (f) Race (except where recording such information is contrary to law)
- (g) Age at onset
- (h) Date of eye examination
- (i) Discipline of examiner
- (j) Degree of vision
- (k) Standard classification of causes of severe vision impairment and blindness
- (l) Date of removal from MRA register
- (m) Reason for removal from MRA register

Each member State will continually correct the essential items on its register cards in accordance with any reports received of reinterviews or reexaminations of registrants. It will immediately remove from the MRA Register any person known to have died, moved out of State, or recovered vision beyond the MRA definition of blindness. Furthermore, it will redetermine the residence and blindness status of every person on its MRA Register during an annual clearance. A registrant may be considered cleared if he has received any service for the blind or has otherwise been contacted since the beginning of the calendar year, provided that there is no evidence that his status has changed since then. However, intensive efforts must be made to contact all other registrants. Contact may be made by mail inquiry, telephone call, or personal visit to the registrant himself, his relatives or neighbors, his physician, or an institution in which he resides; a personal visit will be made, if feasible when other methods fail. These intensive efforts will be completed before the end of the calendar year, and registrants who have not been located will be removed from the MRA Register by that time. It may then be assumed that those persons who remain on the MRA Register at the end of the calendar year are still resident in the State and still fall within the MRA definition of blindness.

A quality control program was initiated in the Model Reporting Area in 1965 with the primary objective of obtaining uniform classification of causes of blindness by a variety of coders. The necessity of this control is recognized by all member States, and it is planned that this will be a continuing program. Copies of the coded physicians reports are sent monthly by the member States to the Biometrics Branch where coding is verified by or under the supervision of the Medical Record Librarian. Problem cases are referred to the consulting ophthalmologist. After review, the reports are returned to the States with an explanation of the reasons for any corrections which are made. All new cases will be reviewed for States with less than 400 additions to the register annually; for States with more than 400 additions annually, a sampling will be submitted.

Each member State will prepare certain tabulations of data from its MRA Register or will prepare a duplicate deck of IBM cards for each calendar year, in accordance with the specifications of the NINDB, and will submit them to the NINDB by February 15 of the following year.

Major findings: Data based on the 1964 annual tabulations have been compiled and have been published in the third report of MRA tabulations from 12 States for prevalence (and 11 States for incidence) that comprised the MRA in 1964. The major findings were as follows:

- (a) There were 5,414 first additions and 107 readditions to the MRA registers in 1964. The rate per 100,000 population for all additions to the MRA registers was 16.0, ranging by State from a low of 8.7 per 100,000 in New Jersey to a high of 25.6 per 100,000 in North Carolina.
- (b) The total number of persons on the MRA registers at the end of 1964 was 51,910, or a rate of 150.2 per 100,000 population.
- (c) The rates for additions generally remained low under 45 years of age and increased only slightly with age up to age 45. Thereafter the rates increased markedly with age in each sex. Although the rates among the aged were somewhat higher for females, there did not appear to be any major sex differences in the rates for additions, except for the oldest age group, namely 85 years and over, where females showed a markedly higher rate.
- (d) Persons reported with vision of exactly 20/200 represented 26.5 percent of the MRA total. This high proportion probably indicates a lack of precise visual acuity measurement or lack of careful recording by the eye examiner, particularly because this level of acuity is sufficient to distinguish the "legally" blind. Some six percent of all additions were totally blind, and about six percent had vision greater than 20/200 but with visual field restricted to 20 degrees or less. An additional 11 percent of all additions had vision limited to either light perception or light projection.
- (e) For all additions the three most frequent causes of blindness were principally cataract due to senile degeneration, other retinal affections due to diabetes, and glaucoma with etiology "unknown to science."

Significance to Bio-medical Research and the Program of the Institute: Data on prevalence, incidence, visual acuity, and cause of blindness available through the MRA represent the only data of its kind, with uniform definition of blindness, for a population of this magnitude (i.e., about 18 percent of the United States population in 1963). As the MRA grows and becomes more representative of the country geographically and demographically, it may become possible to project estimates of incidence and prevalence for the country as a whole with respect to age, sex, race, visual acuity, and cause of blindness. Such estimates are urgently needed for planning programs of prevention and control.

Proposed Course of Project: In view of the fact that a State commits itself to maintenance of its blindness register along the standards of the MRA, once it is admitted as a member, the project will continue for an indefinite period of time with little cost to NINDB. Additional qualified States will be added to the MRA until adequate representation of the United States is achieved, within the resources of the Branch.

Honors and Awards: None

Publications:

Author: Biometrics Branch, NINDB: Annual Tabulations of the Model Reporting Area for Blindness Statistics: 1964 Statistical Report. U.S. Dept. of Health, Education, and Welfare, Public Health Service Publ. No. 1419. Washington, D. C., U. S. Government Printing Office, 1966.

Rogot, E.: Note on mortality among the diabetic blind. Publ. Health Reports 80: 1025-1026, 1965.

Rogot, E.: Survivorship among the aged blind. New Outlook for the Blind 59: 333-338, 1965.

Rogot, E., Goldberg, I.D., and Goldstein, H.: Survivorship and causes of death among the blind. J. Chron. Dis. 19: 179-197, 1966.

Rogot, E., and Goldberg, I.D.: A proposal Index for measuring agreement in test - retest studies. J. Chron. Dis. (accepted for publication)

1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Study of Association between Factors of Pregnancy, Labor, and Delivery and the Occurrence of Blindness in Children

Previous Serial Number: SAME

Principal Investigator: Irving D. Goldberg

Other Investigators: Hyman Goldstein and Eugene Rogot; Dana Quade,
University of North Carolina

Cooperating Units: New York State Commission for the Blind
New York State Health Department
New York City Health Department

Man Years

Total:	1/2
Professional:	9/20
Others:	1/20

Project Description:

Objectives: To determine whether mothers of blind children are characterized by an excess of selected prenatal and perinatal disorders as compared to mothers of the total population of live births surviving the neonatal period; and to determine whether the distribution of birth weights among the blind children was different from that of the total population of live births surviving the neonatal period.

Methods employed: The study population comprises all children (about 600 cases) who meet the following five criteria:

1. Born during the period 1948-1959;
2. Classified blind by the New York State Commission for the Blind during the period 1948- 1960;
3. Having blindness presumed to be due to unspecified prenatal or genetic factors or to otherwise unknown factors as indicated in the records of the Commission;
4. With birth certificate on file at the New York City or the New York State Department of Health;
5. Single-born.

The control group consists of a stratified sample of 2 in every 1,000 recorded live births in New York during the years 1948-1959 (some 8,000 controls in all).

Information collected include:

- (a) Cases and controls: items appearing on birth certificates, viz. date and county of birth, sex of child, weight at birth, race of mother, age of mother, number of previous children, complications of pregnancy, complications of labor, operative procedure, Rh factor, presence or absence of congenital abnormality or birth injury.
- (b) Cases only: cause of blindness (site/type and etiology), year classified blind.

Analysis consists of a comparison between cases and controls on factors under (a) above. Insofar as possible, different categories (type/site) of blindness will be studied separately.

Major findings: Data still in process of analysis.

Significance to Bio-medical Research and the Program of the Institute: This is, to the knowledge of investigators, the first attempt to relate a wide range of prenatal and perinatal disorders and the occurrence of blindness in children. If such association is found this may furnish information that would lead to improvement in prevention of certain types of blindness in children.

Proposed Course of Project: To finish analysis and interpretation of data and publish report of findings.

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 62 B 968

1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Monograph on Neurological and Sense Organ Diseases:
A.P.H.A. Vital and Health Statistics Monographs Series

Previous Serial Number: SAME

Principal Investigators: Irving D. Goldberg and Hyman Goldstein

Other Investigators: Leonard T. Kurland, Mayo Clinic
Gail R. Williams, University of Michigan
Jerome D. Schein, Gallaudet College
Rick Heber, University of Wisconsin

Cooperating Units: American Public Health Association

Man Years

Total:	1 1/20
Professional:	3/10
Others:	3/4

Project Description:

Objectives: To produce a monograph on the subject of neurological and sense organ diseases as part of the American Public Health Association's Vital and Health Statistics Monographs Project. The purpose of the latter project is to produce a series of monographs based on specific and general topics, analyzing United States mortality data during 1959-1961 as well as data from local surveys or clinical experience, in this country or elsewhere.

Methods employed: This is one of a series of monographs, sponsored by the American Public Health Association, which includes members of the Biometrics Branch among the authors. This monograph will contain information on the mortality, morbidity, and epidemiology of neurological and sense organ disorders, including a review of pertinent literature already published. Detailed tabulations were prepared on mortality from various neurological disorders to be included in the monograph, and these will be incorporated, where pertinent, in the various chapters of the monograph. An integral part of this monograph are data derived from tabulations on mortality in the United States in 1959-1960 prepared by the National Center for Health Statistics specifically for this monograph series. The monograph will also contain mortality data from other countries.

Major findings: Monograph is still in process of preparation.

Significance to Bio-medical Research and the Program of the Institute: This monograph on neurological and sensory organ diseases will be one of 17 volumes in the series (16 specific topics and a review volume) providing current and historical information on vital and health statistics, to serve as a major reference source for a review of the nation's health.

Proposed Course of Project: Work on the monograph will continue and completion is expected in 1967.

Honors and Awards: None

Publications:

Williams, G., Kurland, L.T., and Goldberg, I.D.: Morbidity and mortality with Parkinsonism. J. Neurosurgery 24, No. 1, Part 2, 138-143, 158, 1966.

Serial No. NDB (CF) - 63 B 1134

1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Study of Perinatal Factors Associated with Strabismus in Children

Previous Serial Number: SAME

Principal Investigator: Hyman Goldstein

Other Investigators: Candler M. Hawkins and Irving D. Goldberg;
Maureen Henderson, George Entwisle,
University of Maryland School of Medicine

Cooperating Units: Wilmer Eye Clinic of Johns Hopkins Hospital, Baltimore, Maryland
Presbyterian Hospital, Baltimore, Maryland
Baltimore City Eye-Ear-Nose-and-Throat Hospital, Baltimore, Maryland
Baltimore City Health Department, Baltimore, Maryland
Maryland State Health Department, Baltimore, Maryland

Man Years

Total:	1/2
Professional:	7/20
Others:	3/20

Project Description:

Objectives: To determine whether mothers of Negro children with strabismus were different from mothers of Negro control children in reported prenatal and obstetric complications.

To determine whether the distribution of birth weights of children with strabismus was different from that of children selected as controls.

Methods employed: The case study group (400 children) met the following criteria:

1. They must have had a diagnosis of strabismus made at either the Wilmer Eye Clinic of Johns Hopkins Hospital, Presbyterian Hospital Eye Clinic, or Baltimore City Eye-Ear-Nose-and-Throat Hospital in Baltimore. Strabismus is defined as a constant extra ocular muscle imbalance characterized by esotropia or exotropia (cases of

- esophoria and exophoria were excluded). The cases need not be now alive nor current clinic cases.
2. The child must have been born in a Maryland hospital on or after January 1, 1950 and diagnosed with strabismus before January 1, 1963.
 3. Single-born (confirmed from hospital records).
 4. Selection was by date of diagnosis beginning with most recently diagnosed cases.

The control group was selected from birth certificates and matched with the cases in the following variables: (1) date of birth (next registered birth matching on all other relevant variables); (2) place of birth (same institution); (3) sex; (4) maternal age (matched within following categories: (a) under 20 years; (b) 20-34 years; (c) 35 years and over). All originally matched controls who die in the neonatal period were replaced by appropriately matched neonatal survivors.

Information to be collected is as follows:

(a) Cases and controls

In addition to the data necessary for matching from the birth certificate, detailed information about the study pregnancy, the study delivery, and the outcome of pregnancy--including infant weight--have been extracted from hospital records in a manner such that the abstracter did not know whether the individual involved was a case or control in order to insure against potential bias in recording of information. The following variables were compared for the two groups: (1) birth weight distribution; (2) prenatal care; (3) prenatal complications; (4) past obstetrical history; (5) fetal presentation and position; (6) type of delivery; (7) length of labor; (8) toxemic, hemorrhagic, mechanical, and medical complications of delivery; (9) reported abnormalities of infant; (10) reported length of gestation.

(b) Cases only

Type of strabismus and muscle imbalance.

The analysis will include comparison between cases and controls on variables under (a) above. Insofar as possible, different categories of strabismus and muscle imbalance were studied separately.

Major findings: Lower birth weight of the strabismic subjects was associated with shorter pregnancies, possibly indicating that the strabismic babies were immature rather than undersize. These children were not found to be undersized relative to control infants born after gestational periods of the same length. Strabismic infants weighed less even when gravidity was controlled, a result which suggests that strabismus is directly associated with birth weight. Thus, a reduction in prematurity should result in lowered incidence of strabismus.

The mothers of strabismic children had no more complications of pregnancy and delivery than the control mothers. They were the women delivering premature infants without recognizable cause. They had suffered more previous pregnancy loss (abortion or stillbirth) than control mothers of similar gravidity. And women with a previous history of pregnancy loss are at an increased risk of having premature babies, particularly without a specific clinical cause for the prematurity.

A significantly higher proportion of strabismic infants than controls evidenced major abnormalities at birth or shortly thereafter, especially respiratory conditions causing anoxia. A majority of the abnormalities among subjects and control were those usually associated with prematurity, a finding re-emphasizing the importance of the relationship between prematurity and childhood morbidity.

Significance to Bio-medical Research and the Program of the Institute: To the knowledge of investigators this represents the first attempt to relate a wide range of prenatal and perinatal disorders to the occurrence of strabismus in children. In view of the fact that strabismus is often associated with the occurrence of amblyopia, the finding of a relationship between prenatal and perinatal disorders and strabismus might furnish clues how to prevent amblyopia.

Proposed Course of Project: The study was reported at the annual meeting of the A.P.H.A. in October 1965 and is scheduled for publication in the December 1966 issue of the Journal of the American Public Health Association. Abstracts of the paper have appeared in the January 1966 issue of Pediatric Herald and the March 1966 issue of Public Health Reports.

Honors and Awards: None

Publications:

Goldstein, H., Henderson, M., Goldberg, I.D., Benitez, E., and Hawkins, C.M.: Perinatal factors associated with strabismus in Negro children. Amer. J. Publ. Health (accepted for publication).

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Significance to Bio-medical Research and the Program of the Institute: To the knowledge of investigators this represents the first attempt to relate a wide range of prenatal and perinatal disorders to the occurrence of strabismus in children. In view of the fact that strabismus is often associated with the occurrence of amblyopia, the finding of a relationship between prenatal and perinatal disorders and strabismus might furnish clues how to prevent amblyopia.

Proposed Course of Project: The study was reported at the annual meeting of the A.P.H.A. in October 1965 and is scheduled for publication in the December 1966 issue of the Journal of the American Public Health Association. Abstracts of the paper have appeared in the January 1966 issue of Pediatric Herald and the March 1966 issue of Public Health Reports.

Honors and Awards: None

Publications:

Goldstein, H., Henderson, M., Goldberg, I.D., Benitez, E., and Hawkins, C.M.: Perinatal factors associated with strabismus in Negro children. Amer. J. Publ. Health (accepted for publication).

Serial No. NDB (CF) - 63 B 1137

1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A Study of Awareness, Attitudes and Performance
in Reporting Persons to Blindness Registers

Previous Serial Number: SAME

Principal Investigator: Hyman Goldstein

Other Investigators: Irving D. Goldberg and Ronald L. Jacobson
Richard E. Onken, American Foundation
for the Blind

Cooperating Units: Blindness register agencies of twenty-nine
States with blindness registers

Man Years

Total:	3/5
Professional:	2/5
Others:	1/5

Project Description:

Objectives: Investigate the awareness of, attitudes toward, and reporting performance regarding State registration of the blind on the part of ophthalmologists, eye-ear-nose-and-throat (EENT) specialists and optometrists. This has been accomplished (a) through interview of such reporting sources, (b) through interview of register agency staff, and (c) study of (1) written materials and other communications directed to ophthalmologists, EENT specialists and optometrists by State register agencies; (2) the forms and communications these agencies receive from ophthalmologists, EENT specialists and optometrists.

Methods employed: The study populations consisted of:

1. State register agency directors and appropriate staff members including consulting or supervisory eye specialists in 29 States.
2. Ophthalmologists, EENT specialists and optometrists in the 29 States selected.

Information has been gathered from directors of register agencies and their staff members directly responsible for maintaining the register regarding:

1. The uses they make of the register.
2. The register law.
3. The operation of the register.
4. Services available to blind persons.
5. Written materials and other communications directed to ophthalmologists, EENT specialists and optometrists, particularly with respect to the type and extent of educational campaigns undertaken to inform potential reporting sources and professional associations of the desirability and need to report cases to the register.
6. The forms and communications received from such potential reporting sources.

Respondents were selected from directories of ophthalmologists, EENT specialists and optometrists in the selected States. These were located in urban areas with population of 50,000 or more (as of 4/1/60) in order to reduce interviewing costs. Letters were sent to each professional describing the purpose of the study and including documents of support from the respondent's professional society. Then a phone contact was made to arrange an appointment at the respondent's convenience.

Interviews took from one-half hour to an hour. The questionnaire included closed and open-ended questions for probing attitudes toward registers and the reasons behind the attitudes. Due to differing roles regarding the register, there was four separate questionnaires: one for directors of agency personnel and the staffs; a second for ophthalmologists who are advisors to the register agency; a third for other ophthalmologists and EENT specialists, and a fourth for optometrists. Interviewing was conducted by trained interviewers under direction of a commercial research firm under sub-contract. Interviewing started on or about June 1, 1965.

The basic hypothesis is that reporting of blind persons to register agencies by ophthalmologists and optometrists is based on their awareness of and a positive attitude toward registration of blind persons. Conversely, it is assumed that nonreporting is based on lack of information and/or on a negative attitude toward registration. In order to test this hypothesis, a typology of ophthalmologists, EENT specialists and optometrists will be constructed as follows:

1. Those who report all blind persons to the register agency.
2. Those who do not report all blind persons to the register agency since they are:
 - (a) Not aware of the register and the need to report all cases of blindness.
 - (b) Aware of the register but are unwilling to cooperate.

A comparison will be made between the selected States, based on the frequency and extent of their efforts to inform ophthalmologists, EENT specialists and optometrists of the need for the reporting of all cases of blindness.

A comparison will be made between the proportion of eye specialists and optometrists in each of these States who are aware of the register and information given by the register agency. The proportion who are aware of the register will then be related to the degree of effort in their State to inform them of the register.

Efforts will be made to determine: 1) what effect, if any, mandatory reporting had on completeness of reporting, and 2) the attitude of potential reporting sources toward mandatory reporting in States with or without such reporting laws.

It will also be determined whether ophthalmologists as compared to optometrists are more or less likely to report and why.

In all cases, an evaluation of the effort of State blindness agencies to orient potential reporting sources to the benefits of registration will be made so that results obtained may be viewed in relation to such orientation.

Since the basic hypothesis states that attitudes as well as awareness regarding the register influence the completeness of reporting, information collected on attitudes will be analyzed to determine those which lead to reporting and those which lead to nonreporting.

Since both closed and open-ended questions will be asked, codes and content analysis will be developed prior to data collection. The collected data will be tabulated by machine. The analysis of attitudes will include the construction of indices of attitudes toward the register agency, toward welfare agencies in general, toward blind persons, etc.

Major findings: Data have been collected but not analyzed as yet.

Significance to Bio-medical Research and the Program of the Institute: It is clear that this study will provide information of value in improving the completeness of reporting. It will reveal the extent to which attitudes hinder or encourage the achievement of complete reporting. Therefore, after the research findings have been analyzed, they will be reviewed and, it is hoped, will be translated into recommendations for implementation.

The study report and its evaluation may also serve as a basis for the implementation of the findings through the consultative services offered by the staffs of the American Foundation for the Blind and the Biometrics Branch, NINDB.

Through the information gathered in this study a measure for evaluating the efforts of the register agency to encourage complete reporting may be provided by relating the communications directed to eye specialists and optometrists with the degree of awareness of these communications on the part of the eye specialists and optometrists. If it is found that these professionals are not aware of the communications, it is hoped that recommendations may be made to register agencies for improving communication practices based on the practices of more successful States and practices adopted by the Model Reporting Area for Blindness Statistics. If, on the other hand, it is found that reporting sources are aware of the register and the need to report all cases of blindness but do not do so, the reasons for nonreporting will be determined. If it is found that the reasons for nonreporting lie in objection to the procedures involved in the registration then it may be recommended that consideration be given by the register agency to changing such procedures, or that they inform reporting sources of the need for these procedures.

Proposed Course of Project: To prepare tabulations and analyze the data with eventual publication of findings.

Honors and Awards: None

Publications: None

1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Study of Association between Factors of Pregnancy, Labor and Delivery and the Occurrence of Deafness in Children

Previous Serial Number: SAME

Principal Investigators: Hyman Goldstein and Irving D. Goldberg

Other Investigator: Geoffrey G. Dell'Osso

Cooperating Units: New York State Education Department
New York City Board of Education
New York State Department of Health
New York City Department of Health

Man Years

Total:	2/5
Professional:	3/20
Others:	5/20

Project Description:

Objectives: To determine whether mothers of deaf children are characterized by an excess of selected prenatal and perinatal disorders as compared to mothers of the total population of live births surviving the neonatal period; and to determine whether the distribution of birth weights among deaf children was different from that of the total population of live births surviving the neonatal period.

Methods employed: The study population consisted of all children (about 900 cases) who meet the following four criteria:

1. Born during the period 1950-1959 and appointed prior to September 30, 1964 to a State school for the deaf by the Division for Handicapped Children, New York State Education Department.
2. Having deafness presumably prenatal or genetic in origin, or not otherwise known to be adventitious. For purposes of this study, a deaf person is defined as one who is totally deaf or has impaired hearing such that his auditory loss is 70 db. or greater in the better ear.

3. Single-born.
4. With birth certificates on file at the New York City Department of Health or the New York State Department of Health.

The control group consisted of a stratified sample of 2 in every 1,000 recorded live births in New York during the years 1950-1959, inclusive.

Information collected included:

- (a) Cases and controls: items appearing in birth certificates, viz. date and county of birth, sex of child, weight at birth, race of mother, age of mother, number of previous children, complications of pregnancy, complications of labor, operative procedure, Rh factor, presence or absence of congenital abnormality or birth injury.
- (b) Cases only: diagnosis for each ear, date of earliest diagnosis, hearing loss in narrow speech range (500-2000 cps.) in db. for each ear.

Comparison will be made between cases and controls on factors under (a). Separate analyses will be made for those cases of deafness who have other complications such as blindness, brain injury, etc. Insofar as possible, different categories of deafness as well as different degrees of hearing loss will be studied separately.

Major findings: Data have been collected and analysis is in progress.

Significance to Bio-medical Research and the Program of the Institute: This is, to the knowledge of investigators, the first attempt to relate a wide range of prenatal and perinatal disorders and the occurrence of deafness in children. If such association is found, this may furnish information that would lead to improvement and prevention of certain types of deafness in children.

Proposed Course of Project: To analyze and interpret data and to publish report of findings.

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 64 B 1188

1. Collaborative & Field Research
2. Biometrics Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Study of Blindness Statistics in Egypt

Previous Serial Number: SAME

Principal Investigator: Hyman Goldstein

Other Investigator: Dr. Mohyi-Eldin Said,
University of Alexandria, Egypt

Cooperating Units: University of Alexandria, Alexandria, Egypt

Man Years

Total:	1/5
Professional:	3/20
Others:	1/20

Project Description:

Objectives: To ascertain the feasibility of developing a blindness register on a pilot study basis in an Egyptian community as a mechanism for producing statistics in blindness (including severe vision impairment) needed for prevention and control activities; determine to what extent modifications may be made in procedures developed by the Model Reporting Area for Blindness Statistics (MRA) in the United States that, when applied in Egypt, will still permit production of statistics comparable to those of MRA; document problems and their solutions in conducting such studies in developing countries.

Methods employed: The study is being conducted in two urban areas (approximate population 125,000 persons) and in 23 rural villages (approximate population 125,000 persons). The study consists of three phases. Phase I has been completed. In Phase I a random sample of approximately 1,000 households (approximately 5,000 persons) was drawn from the urban study areas for house-to-house vision testing. The same size sample was drawn from the rural study areas. With an estimated blindness prevalence rate of 15 per 1,000 population (using a definition of blindness comparable to that of the MRA), the survey was expected to yield about 75 blind persons in the urban areas and the same number in the rural ones in this Phase. Actually, using the MRA definition of blindness, the prevalence rate in the urban area was 14 per 1,000 and in the rural area, 45 per 1,000. This may

be compared with an estimated prevalence rate in this country of about 2 per 1,000. In Phase III there will be a re-examination, about one and one-half years later of persons in the original sample of 10,000 persons still resident in the study areas to determine new cases of blindness. In both phases, the examined population found not to be blind, is the control group. In Phase II, an attempt will be made to examine every resident in the study areas by persuading such residents to come to health centers voluntarily for a vision examination. This will establish a blindness register. Assuming that its prevalence rate of 31 per 1,000 population found in Phase I, obtains in Phase II, approximately 7750 persons should be described as blind in this Phase.

As mentioned above, in Phase I, persons in the control group were those found not to be blind by definition, or 10,675 of the 11,023 actually examined. The same will be true of Phase III. In Phase II which attempts to establish a blindness register, approximately 7750 persons will be cases and 242,250 persons will be controls.

Information collected included:

- (a) Cases and controls: date and place of birth, sex, nationality, race, marital status, education, occupation, distance vision both uncorrected (or with present corrections) and with best correction for each eye, refractive power of lenses, peripheral field of vision where better eye with best correction has visual acuity greater than 6/60.
- (b) Cases only: probable age of onset of severe vision impairment, history of severe ocular infections, operations, etc., with age at occurrence, blood relationship, if any, between parents, history of similar ocular conditions in blood relatives, near vision both uncorrected (or with present correction) and with best correction for each eye, intraocular pressure, cause of severe vision impairment (site and etiology), prognosis and recommendations for treatment, if any.

Comparison will be made of total and cause-specific incidence and prevalence rates for the various age, sex, urban and rural, and other groups. Comparison of distributions of certain demographic variables in the cases and control groups.

Major findings: Data are still being collected. The study will continue until March 1968.

Significance to Bio-medical Research and the Program of the Institute: This is considered to be the first attempt to apply standards of highly sophisticated data collection procedures (MRA) to those of a developing country in order to determine what modifications may be made in such standards when they are applied in the developing country and still permit the production

of comparable statistics. The utilization of such a "tool," in cooperation with scientific sampling methodology in underdeveloped countries has tremendous potential in the derivation of comparable international statistics of blindness. Since the prevalence rate of blindness in Egypt is one of the highest in the world, the development of comparable statistics for that country may provide valuable clues to the role of environment and other factors in the etiology of blindness. Specific information on age, sex and other demographic variables in the blind population will help pinpoint high risk groups who need to be screened for early signs of eye disorder.

Proposed Course of Project: To collect, process, analyze and interpret data and to publish report.

Honors and Awards: None

Publications: None

ANNUAL REPORT
JULY 1, 1965 THROUGH JUNE 30, 1966
EPIDEMIOLOGY BRANCH
NATIONAL INSTITUTE OF NEUROLOGICAL
DISEASES AND BLINDNESS

Introduction

The major areas of activity of the Branch during the period of this report have been: (1) the programs relating to the NINDB Research Center on Guam; (2) ophthalmic field and developmental research; (3) liaison and coordination of further studies on the toxicity of cycad; (4) development of laboratory facilities within the Epidemiology Branch; (5) epidemiologic and genetic studies of neurologic diseases; and (6) international collaborative studies - U.S.S.R., Japan, and Nigeria.

A reorganization of personnel and structure of the Branch has occurred during this year. As a result a major effort during the year has been organizational. On July 1, 1965, Dr. Jacob A. Brody became Chief of the Epidemiology Branch. On that date Dr. Richard Need left the service and Dr. Kwang-ming Chen became Officer-in-charge of the Guam Research Center. At the beginning of this fiscal year Dr. Asao Hirano, neuropathologist at Montefiore Hospital, left the Branch to accept a faculty appointment at Montefiore. Also Dr. Antonio Stazio, neurologist, finished his multiple sclerosis field work and left the Branch to join the staff at the Veterans Hospital in Washington, D.C., and Dr. Gail Williams left the Branch to continue his training in genetics at the University of Michigan. In September, 1965, Mrs. Nancy Wetmore, Public Health Nurse, joined the staff on Guam. Also in September, Mr. Chris Plato was sent by the Branch to the University of Wisconsin to complete course work toward his Ph.D. in genetics. He will return to the Branch in September, 1966. In January, 1966, Dr. David L. VerLee, ophthalmologist, joined the Branch.

As of July 1st, 1966, a major influx of personnel will occur which will enable the further implementation of the programs described below. The final recruitment is not complete in either the laboratory or field programs. Those who will definitely join the Branch are Dr. Elliot Wilner, neurologist; Dr. Thomas Henson, neurologist, Dr. Roswell Eldridge, internist and geneticist; Dr. David Plank who is finishing his internship; and Dr. Richard E. Goldberg, ophthalmologist.

Major administrative changes occurred during the year. Among them were the creating of a new Section on Ophthalmic Field and Developmental Research which is headed by Dr. J. Theodore Schwartz; the establishment of a laboratory within the Epidemiology Branch; and, as of June 30, 1966, the discontinuance of our participation in the liaison and coordination of studies on the toxicity of cycads.

I. Projects related to the NINDB Research Center, Guam

Studies of amyotrophic lateral sclerosis and Parkinsonism-dementia have been conducted on Guam since 1953. Since 1956, NINDB has had a permanent research staff on Guam. Until September 1964, Dr. Leonard Kurland was in charge of these studies. In the final three years of his direct participation a large staff of investigators were on Guam doing intensive studies of the neurologic diseases on that island and on neighboring islands in the Marianas. During the present year final analysis and publication of the data accumulated in these studies has taken place. Four major papers have already been published and one more is in the final stages of preparation. These papers are the climax of Dr. Kurland's studies on Guam and will undoubtedly receive considerable attention in the neurological world. The papers already published are:

1. Elizan, T. S., Chen, K. M., Mathai, K. V., Dunn, D., and Kurland, L. T.: ALS and PD Complex -- A Study in Non-Chamorro of the Mariana and Caroline Islands. Archives of Neurology 14:347-355, April 1966.
2. Elizan, T. S., Hirano, A., Abrams, B. M., Need, R. L., Van Nuis, C., and Kurland, L. T.: The ALS/PD Complex of Guam -- Neurological Re-evaluation. Archives of Neurology 14:356-368, April 1966.
3. Reed, D., Plato, C., Elizan, T. S., and Kurland, L. T.: The ALS/PD Complex -- A Ten-year Follow-up on Guam. Part I. Epidemiologic Studies. American Journal of Epidemiology 83:54-73, January 1966.
4. Hirano, A., Malamud, N., Elizan, T. S., and Kurland, L. T.: ALS and PD Complex of Guam -- Further Pathologic Studies. Archives of Neurology (in press).

In the next few months the manuscript on genetic studies on Guam by Chris Plato will be submitted to the American Journal of Human Genetics.

During the present year considerable effort has gone into the planning of future studies on Guam in order to assure ourselves that we are taking full advantage of the unusual opportunity to study neurologic diseases which is afforded us on this island. In order to redirect the thinking and planning of studies, the Chief of the Epidemiology Branch spent two months on Guam.

I-A. EPIDEMIOLOGY. Considerable effort is going into establishing a central record system for all known Guam cases. This will involve re-evaluation and updating of family data, clinical information and places of residence of cases. A preliminary review of available information reveals that since 1945, there have been 265 cases of ALS of which 41 are still alive. Since 1948, the number of deaths has been fairly constant. The first death ascribed to Parkinsonism-dementia was in 1954. To date there have been 135

cases with PD, some of whom also had symptoms of ALS. There are currently 27 living PD cases. The most important hints from the preliminary analysis of available information were that the rate of ALS and PD may be declining and is definitely shifting from areas which previously had very high incidence. In four villages, almost all cases of either ALS or PD were among males. There was some suggestion that small geographic foci of cases exist within villages. These findings tend to support the theory that there are exogenous rather than genetic causes of ALS.

The epidemiological studies will be continued with expanded effort at updating the records and exploring the unusual sex concentrations and possible geographic foci. By 1 January 1967, a duplicate of the case summary will be available at NIH for coding and evaluation with the Biometrics Branch.

I-B. CLINICAL. The clinical course of ALS on Guam has been adequately described. More work is probably needed on the PD complex and in relating the course of this disease to other presenile dementias. We will attempt to recruit someone whose main interest will be in the description of this unusual degenerative disease.

I-C. PATHOLOGY. At present we are continuing to collect and hold pathological material from cases of ALS and PD on Guam. When we are able to perform an autopsy within three hours of death, we routinely freeze various sections of the brain and cord in liquid nitrogen for histochemical, immunological, and virological studies. We are collecting between 15 and 20 brains as controls from people who die of diseases other than ALS or PD. We are concentrating on individuals who die of other chronic degenerative diseases such as cancer and tuberculosis. There is evidence that the degenerative processes seen in PD and to a lesser extent in ALS occur also among Guamanians who die of other chronic wasting diseases. This would suggest that the Guamanians have a tendency toward Alzheimer's neurofibrillary degeneration in a characteristic distribution in the CNS. A definitive study of this is essential. Every attempt will be made to work collaboratively with a single group of pathologists who will work-up both case and control materials doing general and neuropathology according to a rigid protocol and schedule.

At present we are collaborating with Dr. Augustus Rose's group at UCLA in a study of enzyme patterns in single anterior horn cells of ALS patients and controls. Dr. John Andrews, a resident in neurology at UCLA, spent two weeks with us on Guam in connection with these studies.

I-D. NON-CHAMORRO CASES. After completing the updating of cases on Guam, we will pursue studies among non-Chamorros with greater intensity. The focus of five Carolinian cases of spinal ALS among fewer than 2,000 Carolinians on Saipan is intriguing. On Guam we are following three

Filipinos, one with ALS and one with questioned ALS or progressive spinal atrophy, and the third with possible PD. In addition we are following Mr. Throne who has stationary Parkinsonism without dementia and slowly progressive ALS.

The new Medical Officer of the Trust Territories has indicated a desire to continue our training course for Medical Practitioners. This program should provide us with information concerning neurologic diseases on remote islands.

Since three and possibly five cases of ALS occurred in recent years among Caucasians who lived on Guam, we are attempting to evaluate the nonmilitary Caucasian population in terms of duration of stay on Guam and incidence of ALS.

I-E. METABOLIC DISEASES. An abstract by Dr. Thomas Burch (Burch, T. A., O'Brien, W. M., Kurland, L. T., Need, R., and Bunim, J. J.: Hyperuricemia and Gout in the Marianas Islands. Arthritis and Rheumatism 7:296-297, 1964) confirmed our impression that there is a high incidence of disturbances of glucose metabolism and elevated uric acid levels on Guam. An analysis of glucose tolerance among Guamanians by Dr. Richard Need which will be published shortly in Diabetes provides background data on this observation and suggests a possible relationship with PD and abnormal glucose tolerance. Dr. Kurland has commented in previous reports that there is a possible relationship between the high incidence of hepatoma, diaphysical aclasia, hyperuricemia and ALS. Observation of these metabolic abnormalities is of interest in their own right and also from the point of view that they are related to the neurologic diseases we see. It is possible that the end product of these metabolic errors is CNS degeneration. Laboratory and field investigation to test this hypothesis are being planned. It is important to note that collagen changes in skin described by Dr. Harold Fullmer (Fullmer, H. M., Siedler, H. D., Krooth, R. S., and Kurland, L. T.: A cutaneous disorder of connective tissue in amyotrophic lateral sclerosis. Neurology 10:8;717-724, 1960) were observed in healthy controls as well as patients with ALS and PD.

I-F. STATESIDE GUAMANIANs. It is estimated that between 5,000 and 10,000 Guamanians are currently living on the Mainland. Within the next two months we will have a fairly complete list of these people and their addresses. A means will be worked out for contacting these people in order to determine their rate of ALS/PD. This study may require several years of follow-up, although certain information might be learned through a single visit. If there is a difference in the age-specific attack rate among Stateside dwelling Guamanians, it would tend to incriminate environmental factors on Guam as the cause of illness. If no major differences are apparent, it could mean that the disease is genetic or that some early exposure on Guam caused the pathology.

I-G. EEG STUDIES. The EEG seems to be our most sensitive, objective tool for measuring PD. These studies will be broadened by testing the members of the families of PD cases. Although the ALS cases have shown no specific changes, it might be worthwhile to check some older people with other chronic degenerative diseases.

I-H. VIRUS STUDIES. Material for isolation and antigen studies are being collected on Guam. To date brain suspensions from three patients have been inoculated into chimpanzees by Drs. Gibbs and Gajdusek, and a larger number are in other primates and laboratory animals. We have no positive results at this time.

I-I. OTHER NEUROLOGIC DISEASES. The myotonia dystrophica cases and the cases of Charcot-Marie-Tooth disease are being followed and manuscripts are in preparation describing their occurrence. We are not currently studying the cases of diaphysial aclasia but will do so when time permits.

I-J. OTHER FIELD AND LABORATORY STUDIES ON GUAM. All specimens of serum and tissue are now being formally logged in according to definite protocols. At present we have several thousand bloods in the serum bank from humans and are collecting bloods from swine, chickens, and dogs. We also have autopsy specimens from 16 patients and controls on Guam.

We are collaborating with Dr. Gibbs in studies of Japanese B encephalitis and from preliminary testing, there is a good possibility that a small or smoldering focus of JBE or other Group B ARBO virus is present on Guam. This is of great importance at present because of the military situation and the recent observation that a very efficient mosquito vector has become abundant on Guam. We are also doing screening for toxoplasmosis with the possibility of expanding this into a major study of the ecology of toxoplasmosis on Guam.

We are engaged in a study of hearing among the school children in three villages and also of the incidence of acute and chronic otitis media among children born since 1 January 1961, in three villages. This study will run for one year and the data will be referable to the National Health Survey and similar studies conducted in Alaska.

II. Projects related to Collaborative and Field Research in Ophthalmology

II-A. TWIN STUDIES IN OPHTHALMOLOGY. During the past year the "Twin Registry for Eye Examinations" has been expanded to include 360 pairs of twins residing in the Greater Washington Area. With the addition of necessary staff, the Section is now implementing its investigations of the relative role of heredity and environment in the development of certain ocular characteristics.

A general ocular evaluation and zygosity determination is being scheduled for the entire twin population. Subsequently, more definitive and detailed examinations will be provided for designated subgroups of the registry. The earliest definitive examinations will include components of refraction as determined by optical and ultrasonometric techniques, careful motility evaluation, and investigation of known parameters of chronic simple glaucoma.

II-B. FIELD INVESTIGATIONS IN CHRONIC SIMPLE GLAUCOMA. Much of our field work continues to be directed toward understanding the importance of some of the variables affecting reliability of tonometric procedures. Analysis of our data on the Nesquehoning, Pennsylvania survey was completed during the year and revealed a marked influence of certain variations in technique on the results of tonometric measurements. Differences in the patient's posture and differences in types of tonometers were shown to have a marked influence on the measurement of ocular pressure. Results of tonometric testing performed during our field investigation among American Indians at the Colorado River Reservation in Arizona were consistent with these findings.

Our studies of techniques of measuring intraocular pressure serve to emphasize the need for carefully standardized field techniques in order to collect comparable epidemiologic data on the frequency of ocular hypertension. This point of view was discussed in our presentation "Problems Relating to the Epidemiology of Chronic Simple Glaucoma" at a recent Symposium held at the Royal College of Surgeons in London. During the past year, this Section has continued to explore the possibility of reaching agreement among investigators with regard to a practical tonometric field technique for epidemiologic studies. This matter will be pursued informally at the forthcoming XX International Congress of Ophthalmology.

A new field study was undertaken among the American Indian population at Salt River, Arizona. Ocular tensions were measured with the Schiotz tonometer, the Maklakov tonometer as modified by Posner and Inglis, and the Goldmann applanation tonometer in both the sitting and the supine positions. Beyond obtaining further descriptive data on ocular hypertension among American Indians, this study will provide a comparison of tonometric results using those instruments commonly employed at the present time by the ophthalmologists of other nations. In order to investigate the relationship between systemic blood pressure and ocular pressure, and the influence of postural change on each measurement, standard blood pressure measurements were also obtained on the patients examined at Salt River. These measurements were obtained through collaboration with the Hypertension Section, Heart Disease Control Program, B.S.S.

II-C. INFLUENCE OF A SYNTHETICALLY DERIVED AGLYCONE OF CYCASIN ON OCULAR TISSUES. This investigation is being undertaken in collaboration with the Laboratory of Experimental Pathology, NIAMD. The aglycone of

cycasin has been shown to be capable of producing tumors in certain experimental animals. Mice have been noted to develop hepatomas, adenomas of both kidneys, multi-locular hepatic cysts and hemangio-endotheliomus of the liver, following administration of aglycone or painting of artificial skin ulcers with aqueous emulsions of the cycad nut. In the mouse, rat, and guinea pig, tumors have variously developed in the liver, stomach, lung, kidney and bile ducts following administration of cycasin by other routes.

This is a pilot investigation of the effect of intracameral application of the aglycone of cycasin on the ocular tissues of young experimental animals.

II-D. MALIGNANT NEOPLASMS OF THE EYE. This is a study of United States mortality experience with malignant neoplasms of the eye undertaken in collaboration with Biometrics Branch, NINDB. During the past year we have obtained pertinent data from 1,024 or 1,108 death certificates in which the primary cause of death was assigned to Rubric 192 (malignant neoplasm of the eye) during the years 1959 through 1961. These data have been classified according to histologic type of tumor as reported on the death certificate. The occurrence of death due to various types of intraocular tumor is also being classified according to epidemiologic features such as age, race, sex, and geographic distribution. It is anticipated that additional information will be solicited on certain cases, and where necessary, attempts will be made to recover surgical or autopsy specimens.

II-E. OPHTHALMIC SURVEY AMONG POPULATIONS OF THE SOLOMON ISLANDS. The Section of Ophthalmic Field and Developmental Research has been invited to join a survey team of medical specialists and anthropologists to participate in field investigations among defined populations on Bouganville Island and Malaita Island, both in the Solomon Chain. Members of the Department of Anthropology at Harvard University have recently completed approximately two years of preliminary anthropologic field work among the study populations, and the Department is now preparing to follow this preparatory activity with epidemiologic investigations. The later will include the general medical evaluation, internal medicine, cardiology, pediatrics, dentistry, ophthalmology, general metabolic investigations and anthropometry. Additional populations of the Solomon Islands will be studied by the Harvard group each summer for the next seven years.

This is a unique opportunity to obtain descriptive ophthalmic data among well defined, relatively nonacculturated populations which have been thoroughly and recently studied by anthropologists, and which will be evaluated simultaneously by other medical disciplines. Our participation with this field team will provide an excellent opportunity to undertake and gain field experience among "primitive" populations along with the opportunity to estimate the frequency of normal ocular characteristics and common ocular diseases among such populations.

II-F. PROGRAM PLANS AND PROPOSED FUTURE OBJECTIVES OF THE SECTION ON OPHTHALMIC FIELD AND DEVELOPMENTAL RESEARCH.

1. Television Ophthalmoscopy. The interests of this Section in the development of Television Ophthalmoscopy and the problems and potential value of developing such an instrumentation system were discussed and evaluated at a workshop described in the last annual report (see Introduction, Section I-D). The refinement of such instrumentation from the stage of development to practical clinical usage requires the collaboration of science, industry and medicine as well as the collaboration of many different disciplines within. It was recommended by participants in the workshop that the Section on Ophthalmic Field and Developmental Research should provide appropriate coordination and should participate in the development of such instrument systems. In line with this opinion, after evaluation of organizational methods of approach, this Section strongly recommends the establishment of an Instrumentation Development Unit within the Section on Ophthalmic Field and Developmental Research. Budget estimates have been prepared and submitted and exploratory recruiting has been undertaken. Proceedings of the Workshop on Television Ophthalmoscopy have been prepared for publication. Further action must await the availability of required resources.

2. Development of an eye study community. It is becoming increasingly evident that a large, well-defined population must be studied in order to answer questions encountered by our developing program. Presently, for example, we need to evaluate the relative efficiency of glaucoma screening when tonometric examinations are performed by different techniques. Descriptive data on the prevalence of ocular characteristics must ultimately be drawn from defined populations. Prospective or follow-up examinations on appropriate cohorts within such populations could provide highly important additions to our knowledge of the natural history and incidence of certain eye diseases.

In recognition of the need for accurate descriptive information by service programs as well as epidemiologic interests, we are exploring the possibility of collaborating with the Bureau of State Services in the careful organization of pilot activity within a potential "eye study community." In view of the potential value of such an investigation, extensive evaluation of this possibility is warranted. It must be recognized that such an undertaking would require careful liaison with the community and wide-spread interest and participation. Serious consideration of on-going examinations must await successful pilot activity.

3. Trachoma Studies. The Division of Indian Health and the University of California are preparing to undertake a five year trachoma investigation and control program in the Southwestern United States.

This section has been invited to collaborate in the development and execution of epidemiologic investigations in conjunction with the five year program. Exploratory planning sessions will be undertaken during the current fiscal year.

II-G. CONSULTATION OR SERVICES FURNISHED TO OTHER ACTIVITIES.

1. Collaboration with the National Health Examination Survey in expanding and improving the ophthalmology protocol for phase III examinations.
2. Continuing ophthalmic consultation for the Model Reporting Area for Blindness Statistics sponsored by the Biometrics Branch, NINDB.
3. Continuing assistance to Peace Corps, Washington, D. C. and Food and Drug Administration, Washington, D. C., in capacity of consulting ophthalmologist.

III. Projects related to liaison and coordination of further studies on the toxicity of cycads

III-A. FOOD, MEDICINAL AND TOXIC PLANTS AND THEIR RELATION TO NEUROLOGICAL DISEASE. Two compilations of the plants used locally for food and medicine in Guam and in Mitogawa, Japan, geographic areas with high incidence of amyotrophic lateral sclerosis, are completed. It has been planned that plants reputed to have neurotoxic effects on either animal or man will be selected from the lists for further study.

To date, attempts to coordinate a cooperative project between the Government of Guam and pharmacognosicists for multiple screening and a follow-up study of the medicinal and toxic plants from Guam have failed. A proposal from Dr. Norman Farnsworth, School of Pharmacy, University of Pittsburgh, is under consideration by the Governor of Guam. As soon as a program with Guam is implemented, it is anticipated that a similar arrangement can be made for investigation of the plants of the Mitogawa area of Japan.

Several supplemental reports have been received from Dr. Joseph Forgacs (Contract PH43-64-606) and are ready for distribution and publication. The reports describe toxic fungi identified in a wide range of foods and chews from Guam and Japan. Samples, collected in 1963-64 by Dr. Whiting, include betel and cycads from Guam, and winter-stored rice, katsuobushi (dried bonito), miso soup (manufactured with cycad starch), ahu (a small fish common in the Mitogawa streams), _____ (a fermented bean relish), and zen mai (Osmunda japonica Thunb.) from Japan.

III-B. DIETARY METHODOLOGY. From a backlog of work on Dietary Survey Methodology with which Dr. Whiting has been involved for several years, a major report has been completed and accepted for publication by the Journal of Clinical Nutrition - Dietary Appraisal: Problems in Processing Dietary Data by Juanita A. Eagles, Marjorie Grant Whiting and Robert E. Olson.

Computer processing of food intake from a dietary study of a large sample of Navajo at Many Farms, Arizona by M. Whiting and E. Green has recently been completed. This report will be published as part of the Cornell Medical School study of Many Farms (1958-59).

Translation has been completed and an index to nutrition research in the U.S.S.R. for the period 1950-59 is now available as a mimeo for limited distribution.

III-C. CYCAD RESEARCH. Various characteristics of the cycad continue to hold the interest of workers from many different disciplines. Annual expenditures on cycad research is estimated as more than \$200,000 for Institutes at NIH other than NINDB and at least \$100,000 for institutions other than NIH.

Dr. Whiting has continued to maintain close relationships with researchers on cycads throughout the world. Visitors from abroad during the year included Dr. Peter Magee, Medical Research Council Laboratory, Surrey, England; Dr. Clifford Gallagher, McMaster Animal Health Laboratory, Sydney, Australia; and Dr. Noel Riggs, University of New England, Armidale, Australia. Dr. Riggs is spending his sabbatical leave with Dr. Frank Strong at the Natural Products Laboratory at the University of Wisconsin where his research centers around inositol, pinotol, and sequoyitol and related compounds and the azoxycompounds. All of these compounds are obtained from cycads.

1. Cycad starch and its derivatives. Dr. Gert Laqueur, Chief of the Laboratory of Experimental Pathology, NIAMD and his group continue as the chief investigators of the hepatotoxic and tumorigenic properties of cycads. One aspect of their work pertinent to the interests of neurologists is the research of Dr. Maria Spatz (NAMM) reported at the Federation meetings in April 1966. Dr. Spatz has demonstrated that with feeding of crude cycad meal to rats during the first five days of pregnancy, transplacental tumors have been produced in the offspring. Dr. David Smith has published in Science (in press) on the mutagenic effects of MAM (methylazoxymethanol) on bacterial systems. A paper by Matsumoto and Laqueur on the production of duodenal tumors in rats by interperitoneal injection of MAM has been accepted for publication. Dr. Asao Hirano has several lines of cycasin-induced tumors successfully transplanted in both inbred and non-inbred strains of rats. He is also investigating the protection of rats against the acute toxic and

the lethal effects of cycasin. Hirano has confirmed Magee's report of induction of tumors in one-day old rats with a single subcutaneous injection of cycasin and is now studying the mechanism of cycasin breakdown in the newborn rat.

Work has started on collaborative mono-contamination experiments (D. Smith, M. Spatz, E. McDaniel, and G. Laqueur) designed to determine which bacteria are capable of splitting the glycoside cycasin in germ-free animals. It has been confirmed that germ-free animals develop tumors following ingestion of MAM-acetate.

Cycad projects in the NCI include feeding experiments with monkeys (Drs. Margaret Kelly and R. O'Gara) and with fish (Dr. M. Stanton).

Additional evidence has been reported to support the work of both Riggs and Matsumoto (April 1965) regarding the methylating action of MAM, the aglycone of cycasin.

Although radioactively labelled derivatives of MAM-acetate, with a label on both carbons, have been produced experimentally, preparation of a compound labelled on only one of the carbons has not yet been accomplished. Contract bids for development of this product have been announced by NCI.

Mickelsen and Yang (Michigan State University) have found that the dried husks of cycad seeds from Cycas circinalis in Guam produce hepatomas in rats. Recently Dr. Hoch-Ligeti (Veterans Administration, Martinsburg, West Virginia) has shown that the fresh green husks, when fed to rats, produce severe liver damage within a week. Both fresh and dried husks of cycad seeds are used as a chew in Guam.

Several hundred copies of the report of the Fourth Cycad Conference (April 1965) have been distributed.

2. Cycad Fronds. Progress has been made in locating and identifying lesions in cattle affected by the feeding of fronds of various species of cycads. Field observations and feeding experiments continue in several geographic areas using fronds from local cycad plants: Queensland and Northern Territory in Australia, Papua in New Guinea, Dominican Republic, and Puerto Rico. Opportunities in Puerto Rico appear to be excellent for a cooperative project to include planned feeding experiments supported by biochemical and pathological studies (Medical School, School of Pharmacy, and USDA). Ample supplies of cycad fronds and new sprouts of species of Zamia reputed to be toxic to cattle are being shipped from Puerto Rico to Dr. Matsumoto (University of Hawaii) for identification and isolation of the neurotoxic constituent.

Reports were presented and discussed at a meeting on Neurotoxins in Cycads held at NIH in December 1965.

Success in producing a paralysis with the feeding of cycad fronds has been reported in limited experiments with sheep and goats. Long-term feeding of rats with dried fronds from *Zamia debilis* obtained from the Dominican Republic produces liver damage and malignancies. Neither the cycad leaves nor husks contain more than traces of cycasin which was previously identified as the hepatotoxin and carcinogen in the starchy portion of cycads. Chemists (Matsumoto, Riggs, et al.) are now attempting to identify the carcinogenic agent in both leaves and husks.

IV. Laboratory Facilities

During the present year laboratory activities have been limited to the planning and setting up of our new laboratory and to collaborative studies with Dr. Sever's group in the Section on Infectious Diseases, Perinatal Research Branch, NINDB and with Dr. Gibbs' group in the Laboratory of Slow, Latent and Temperate Virus Infections, C&FR, NINDB. Attempts have been made to standardize transformation reactions of lymphocytes as a mechanism for determining hypersensitivity. Also serological testing for Japanese B encephalitis antibodies on Guam is underway.

The laboratory is being designed and set up for immunological studies and pathogenesis studies using tissue culture and small laboratory animals. The immunological studies will be designed to screen patients with chronic neurological diseases for evidence of hypersensitivity. Initially we will confine our work to transformation of lymphocytes in these patients. Our studies in pathogenesis will include trying to get a model of muscle degeneration in mice using myotropic viruses and attempting to work out a model for study of western encephalitis and St. Louis encephalitis based on the epidemiological observation that these viruses produce different symptoms in different age groups of the same populations. There is a suggestion that western encephalitis is neurotropic for dividing cells, while St. Louis encephalitis is primarily a vasculitis.

V. Patterns of Neurologic Disease

With an increased staff we are planning studies of several diseases which can best be performed by a national agency with highly trained personnel. These studies will include reviews of the epidemiology and genetics of Parkinsonism, stroke, senility and presenility, amyotrophic lateral sclerosis in the United States, and of the remote effects of cancer on the central nervous system. The long-term plan would be to create a section within the Branch concerned with these problems. At present the direction will have to come entirely from the Branch Chief, but we are actively recruiting for an epidemiologist with background in neurology who would be interested in this type of work.

A contract has been given by the Branch to Dr. Abraham Lilienfeld, Professor of Chronic Diseases, Johns Hopkins University School of Public

Health, to conduct studies in nine areas on the reliability of mortality reporting of stroke.

VI. International Studies

VI-A. U.S.S.R.

1. Vilyuisk encephalomyelitis. An official request through the International Division of the U.S.P.H.S. has been in the hands of Soviet officials for two years to permit Dr. Brody and Dr. Gajdusek of NINDB and Dr. Richard Johnson, Assistant Professor of Neurology, Division of Neurology, Cleveland Metropolitan General Hospital, and possibly Dr. Richardson, Pathologist at the Massachusetts General Hospital to go to Vilyuisk where a progressive debilitating disease exists among the Yakut people. This disease bears some resemblance to PD found on Guam. At one time the Russians claimed to have isolated a virus from these patients but apparently they now believe this virus was a contaminant in the mouse colony.

2. The 1964 Medical Delegation to the U.S.S.R. on Latent Infections, Chronic Intoxications and Genetic Disorders of the Nervous Systems of Man and Animals recommended several studies be conducted in the U.S.S.R. through assignment of American personnel. These included investigation of Soviet reports of Von Economo's disease in Siberia, further documentation of Vilyuisk encephalomyelitis, and a review of cases with the chronic sequelae following tick-borne encephalitis. We are trying to recruit personnel for this type of an assignment but of course are limited by the present uncertainty of negotiations with the Soviets.

3. It is important that long-term collaborative epidemiological studies be conducted between American and Soviet epidemiologists in fields such as multiple sclerosis, stroke and Parkinsonism. The U.S.S.R. is the only country in the world with a unified reporting system and a large enough population with sufficiently varied geographical settings to permit definitive studies of the epidemiology of these diseases. Negotiations for such collaboration are underway but are meeting with the difficulties mentioned above.

VI-B. JAPAN

During the year two members of the Branch (Drs. Brody and Chen) visited the Wakayama Medical College in Japan and the Kii Peninsula. An unusually high incidence of ALS and other motor neuron diseases occurs in this area. We intend to continue collaboration with the group at Wakayama Medical College in studies on Guam and in the Kii Peninsula.

VI-C. NIGERIA

Dr. G. L. Monekosso, Department of Medicine, University of Lagos, has invited our collaboration in the investigation of neurologic diseases occurring in certain villages in southwestern Nigeria. In some respects these diseases resemble syndromes associated with nutritional deficiencies. Dr. Maurice Victor, Chief, Neurology Service, Cleveland Metropolitan General Hospital, has expressed interest in participation as a consultant and will accompany members of the Branch to Nigeria to investigate the clinical and epidemiological situation and determine if a meaningful and productive study could be conducted. Should the funds be available during the coming fiscal year, we will implement this plan.

Serial No. NDB (CF) - 54 E 102
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Population survey and case-finding study of ALS-PD complex
in the Mariana Islands other than Guam, and the Caroline
Islands.

Previous Serial Number: NDB (CF) - 60 E 705.

Principal Investigators: T. S. Elizan

Other Investigators: K. M. Chen
K. V. Mathai
D. Dunn
L. T. Kurland

Cooperating Units: None

Man Years:

Total: 1/8
Professional: 1/16
Other: 1/16

Project Description:

Saipan, Tinian, Rota, and the Northern Marianas group of islands have been totally surveyed for the presence of ALS-PD complex. The high prevalence of this disease entity was previously considered to be confined to the Chamorro people of Guam, and the leading hypothesis as to etiology involved an hereditary factor operating in the Chamorro population. A non-Chamorro (Carolinian) population sharing a common environment with a vulnerable or afflicted Chamorro population exists in Saipan and in the Northern Marianas. Saipan has a total population of approximately 7,200 people, 1,100 of whom are Carolinians, and the rest Chamorros. These two ethnic groups reside in adjacent, and often in the same, communities. The Northern Marianas have five islands inhabited by both Carolinians and Chamorros, the former outnumbering the latter.

There is evidence at this time that Carolinians in Saipan may have a high prevalence of ALS (100/100,000) - which may be as high as the prevalence rate among Chamorros in Guam (127/100,000, as of June 1963).

OBJECTIVES & METHODS

There is also evidence that Chamorros in the rest of the Marianas, other than Guam, (Saipan, Rota, Northern Marianas) have likewise a high prevalence (50/100,000), though not as high as that of the Chamorros in Guam. These findings reflect a probable significant role of environmental factors on the

development of the disease in genetically vulnerable racial groups. Continuing clinical studies, including hospitalization and diagnostic work-up of suspicious cases, and postmortem follow-up of definite cases, are being made. Blood studies of these populations, including blood grouping, haptoglobins, hemoglobins, and carbonic anhydrase determinations, are being done by Mr. Chris Plato, geneticist, and constitute several separate projects.

Honors and Awards: None

Publications:

1. Elizan, T. S., Chen, K. M., Mathai, K. V., Dunn, D., and Kurland, L. T.: Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex in Non-Chamorro of the Marianas and Caroline Islands -- A Preliminary Report. Archives of Neurology 14:347-355, April 1966.

Serial No. NDB (CF) - 54 E 103
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Clinical studies on ALS-PD complex of Guam

Previous Serial Numbers: NDB(CF) - 59 E 604, NDB(CF) - 63 E 1094, NDB(CF) -
63 E 1095, NDB(CF) - 63 E 1096, NDB(CF) - 60 E 715.

Principal Investigator: T. S. Elizan

Other Investigators: A. Hirano
B. M. Abrams
R. L. Need
C. Van Nuis
L. T. Kurland

Man Years

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

Project Description:

OBJECTIVES

I. General medical aspects. Heretofore, the clinical evaluation of ALS-PD patients in Guam had been solely concentrated on the nervous system. This study was started to evaluate (a) the general medical condition of these patients, and (b) the occurrence of other associated diseases and/or abnormalities. All living ALS-PD patients and matched controls in the registry panels are given a thorough general physical examination, and the following laboratory work-up:

METHODS EMPLOYED

1. total serum proteins, A/G ratio, bilirubin, cephalin flocculation test, to evaluate liver function;
2. cholesterol and serum lipids are being done because of the apparent low incidence of arteriosclerotic changes in the heart and in the brain;
3. uric acid, Bentonite flocculation test for rheumatoid factor and glucose levels are being determined in a joint project with the staff of NIAMD;
4. blood urea nitrogen, for gross evaluation of renal function;
5. sodium and chloride;

6. serology; and
7. EKG to determine any abnormalities. Russian workers have reported nonspecific EKG changes in ALS.

The general pathology of organs outside of the central nervous system has not been adequately studied in the Guam ALS-PD cases. There are 32 autopsied cases of ALS-PD (1961-1963) whose nonnervous tissues and organs were available for gross and microscopic pathologic examination. Special attention was focused on the heart, liver, lungs, and kidneys.

OBJECTIVES

II. Neurological aspects. Detailed clinical analysis of motor neuron disease and Parkinsonian syndrome with associated organic mental changes, as found in Guam, was done on the case material of the NINDB from 1957 to 1964. A total of 104 ALS cases and 72 PD cases were available for study.

METHODS EMPLOYED

The following items were evaluated:

1. onset and sequence of development of symptoms and signs; patterns of involvement of the CNS;
2. interrelationships between the PD picture and motor neuron features; peculiarities of the "mixed" types of cases in contrast with the "pure" types;
3. differences and similarities between the "Guam disease" and the classical motor neuron disease and Parkinsonian syndrome as observed in the West;
4. detailed characteristics of the "dementia" in the PD cases;
5. clinico-pathological correlations whenever possible;
6. analysis of age and sex characteristics and duration of illness with clinical progression of the disease; and
7. associated metabolic and other medical diseases with the PD and/or ALS picture.

An additional group of living patients consisting of (a) those with "pure" Parkinsonian features resembling, in some respects, that seen classically in the West, with no dementia, and (b) those who exhibit, at this time, only

suspicious or indefinite Parkinsonian or motor neuron features, will be further studied and followed up, to clarify a suspected spectrum of this disease complex, with all its "intermediate" forms, and to further analyze the actual evolution of the disease process as it becomes more definitive.

Honors and Awards: None

Publications:

1. Elizan, T. S., Hirano, A., Abrams, B. M., Need, R., Van Nuis, C., and Kurland, L. T. : The Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex of Guam -- Neurological Re-Evaluation. Archives of Neurology 14:356-368, April 1966.

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Epidemiological aspects of ALS/PD complex on Guam

Previous Serial Number: Same

Principal Investigator: D. M. Reed

Other Investigators: C. C. Plato
T. S. Elizan
L. T. Kurland

Cooperating Units: None

Man Years

Total: 1/16
Professional: 1/32
Other: 1/32

Project Description:

METHODS EMPLOYED

A. ALS/PD Case Control. Every ALS or PD patient included in the registry from 1958 to 1963 was matched with a "normal" control for age, sex, and village of residence. Patients and controls who are still alive at this time were studied for differences in diet, microenvironment, socio-economic status, occupation, and disease history. No significant differences were found between cases and controls.

B. Other Epidemiological Studies of ALS/PD. Using the ALS/PD cases ascertained during the past five years, village of residence, prevalence and incidence rates were calculated. These data are strikingly similar to the data collected by Kurland et al. in 1953. The incidence rate for ALS was found to be 53 per 100,000 population. The rate for PD was approximately 19 per 100,000.

Tabulation of rates by village of birth using the 1930 population census, and detailed checking of the villages of birth of all cases was done. There was an indication of a north to south increase in incidence of ALS but not PD.

Death certificates were checked for the past ten years for deaths due to ALS, PD, or suspiciously related neurological diseases. The known ALS cases who were alive in 1953 are also being traced. ALS accounted for 10 percent of all adult deaths, PD for five percent, as compared with one out of 1000 in the United States.

Honors and Awards: None

Publications:

1. Reed, D. M., Plato, C. C., Elizan, T. S., and Kurland, L. T.: The Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex: A Ten-Year Follow-Up on Guam. I. Epidemiologic Studies. American Journal of Epidemiology 83:1;54-73, January 1966.

Serial No. NDB (CF) - 61 E 832

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Studies on the natural history of multiple sclerosis.

Previous Serial Number: Same.

Principal Investigator: L. T. Kurland

Other Investigators: G. W. Beebe
J. F. Kurtzke
B. N. Nagler

Cooperating Units: National Research Council, National Academy of Sciences
Veterans Administration Hospital
Lynchburg Training School and Hospital

Man Years

Total: 1/16

Professional: 1/32

Other: 1/32

Project Description:

The study has proceeded in three phases: (1) progression from acute, idiopathic optic neuropathy to multiple sclerosis; (2) clinical patterns in the course of multiple sclerosis and the prognostic significance of a wide variety of factors, present at onset, and of subsequent residence; and (3) epidemiology, with attention to prior medical history, sociologic variables, and many factors in the natural environment, especially climatologic and other correlates of geography.

Phase (1) has been completed and the report was published. The other phases are underway based on comparisons of approximately 400 patients and an equal number of matched "controls."

Another study was made of 183 patients with an acceptable diagnosis of unilateral papillitis and unilateral or bilateral retrobulbar neuritis. These patients were followed-up through a variety of sources: 21 were judged "definite multiple sclerosis," three "possible multiple sclerosis," and the remaining "not multiple sclerosis." Thus, 13.1 percent (24/183) of the patients developed multiple sclerosis during the follow-up period, an incidence considerably lower than that reported by other investigators.

Honors and Awards: None

Publications:

1. Nagler, B. N., Kurland, L. T., Auth, T. L., Beebe, G. W., Kurtzke, J. F., Lessell, S., and Nefzger, M. D.: Studies on the natural history of multiple sclerosis. I. Objectives, designs, and methods. Acta Neurologica Scandinavica, Supplement 19 42:141-156, 1966.
2. Kurland, L. T., Auth, T. L., Beebe, G. W., Kurtzke, J. F., Lessell, S., Nagler, B. N., and Nefzger, M. D.: Studies on the natural history of multiple sclerosis. II. Progression from optic neuropathy to multiple sclerosis. Acta Neurologica Scandinavica, Supplement 19 42:157-176, 1966.

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Amyotrophic Lateral Sclerosis and Parkinsonism-Dementia Complex: Further Pathologic Studies.

Previous Serial Number: Same.

Principal Investigator: A. Hirano

Other Investigators: N. Malamud
T. S. Elizan
L. T. Kurland

Cooperating Units: Montefiore Hospital and Medical Center
Langley-Porter Neuropsychiatric Institute

Man Years

Total: 1/2
Professional: 3/8
Other: 1/8

Project Description:

Neuropathological examination of 35 ALS, 47 PD, and 29 control (non-ALS or PD) patients from Guam revealed a close similarity in pathological changes between the ALS and PD patients. These changes include nigral changes among all the ALS patients and spinal cord changes among approximately one-half of the PD patients. The authors conclude that ALS and PD are related as members of a spectrum of a single disease entity: ALS-PD. (This conclusion is not accepted by other investigators on the Guam project.) In addition, the relationship of this syndrome to other diseases is discussed on the basis of neuropathological findings. The pathogenesis of neurofibrillary changes is considered as is the possible etiology of ALS-PD.

Honors and Awards: None

Publications:

1. Hirano, A.: Neuropathology of Amyotrophic Lateral Sclerosis and Parkinsonism-Dementia Complex on Guam. 2000 Word Report. Vth International Congress of Neuropathology, Zurich.
2. Hirano, A., Malamud, N., Elizan, T. S., and Kurland, L. T.: Amyotrophic Lateral Sclerosis and Parkinsonism-Dementia Complex on Guam: Further Pathologic Studies. Archives of Neurology (in press).

- Serial No. NDB (CF) - 62 E 958
1. Collaborative & Field Research
 2. Epidemiology Branch
 3. Agana, Guam & Bethesda, Maryland

FHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A serological and hematological genetic survey of Chamorros in Guam

Previous Serial Number: Erroneously reported as 62 E 953 in last annual report

Principal Investigator: C. C. Plato

Other Investigator: M. T. Cruz

Cooperating Units: None

Man Years

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

Project Description:

OBJECTIVES

The object of this study is to obtain data on the distribution of the ABO, MNS, Rh, Kell, Duffy, Diego and other blood groups among the Guamanians, and to compare this distribution with the distribution of neurologic diseases in this population.

METHODS EMPLOYED

Over three hundred specimens have been collected from five Guamanian villages. In addition to the blood grouping studies, a portion of the red cells were hemolyzed for hemoglobin investigations. Serum protein electrophoresis was also performed for the determination of the frequencies of the various haptoglobin types among the Chamorros.

Concurrently with the population survey the same serological and hematological tests were performed on a number of ALS/PD patients. The results of the patients and random population samples will then be compared in order to determine whether there are associations between ALS/PD and any of the blood groups or haptoglobin types.

Additional data have been collected and are presently being analyzed.

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 62 E 959

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Further studies on the CA₁C variant of carbonic anhydrase enzyme

Previous Serial Number: Same

Principal Investigator: R. Tashian

Other Investigators: C. C. Plato

Cooperating Units: University of Michigan

Man Years

Total:	1/64
Professional:	1/64
Other:	0

Project Description:

MAJOR FINDINGS

In our biochemical and enzymatic studies we were fortunate to discover a new variant (CA₁C) of carbonic anhydrase. This we showed to be inherited and to be controlled by a single autosomal gene present in the Chamorros of Guam and Saipan, but apparently not in the Carolinians.

OBJECTIVES

The purpose of this study is twofold: (1) to test a random number of Chamorros in order to determine the frequency of the CA₁C gene in this population; and (2) to obtain larger quantities of blood from carriers of this gene in our effort to isolate this substance and study its enzymatic and biochemical properties in greater detail.

METHODS EMPLOYED

Over three hundred Chamorro blood specimens have been collected and are being tested for the presence of the CA₁C variant. Also, biochemical studies are being carried out at the University of Michigan for chemical identification and isolation of this enzyme.

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 62 E 961

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Five-year registry study of ALS/PD cases on Guam

Previous Serial Number: Same

Principal Investigator: C. C. Plato

Other Investigators: T. S. Elizan
D. M. Reed

Cooperating Units: None

Man Years

Total:	3/8
Professional:	3/8
Other:	0

Project Description:

OBJECTIVES

The role of heredity in the etiology of ALS has been a debatable issue since the discovery of the disease. Guam, with its peculiarly high prevalence of ALS, presents an excellent testing ground for the genetic hypothesis. In 1958, a point survey of patients and controls was conducted by Dr. Robert Krooth, a geneticist and former member of this Branch. Even though the results of that survey strongly suggested that ALS occurred more often among the sibs of patients than one would expect by chance alone, the data failed to show the same trend among the offspring and parents of patients. A similar analysis of the PD cases failed to offer conclusive indications of familiarity mainly because of the limited amount of data available at that time. In view of the inadequacy of the point survey, a new prospective study was initiated in 1958 by Dr. Krooth, with the following objectives: (1) to determine whether a familial concentration of the disease exists in both ALS and PD; (2) to determine if there is a significant tendency for a parent-offspring aggregation in these diseases; and (3) to conduct general comparisons among the relatives of the patients and controls.

METHODS

The basic principle of the prospective study was the accumulative registering of each and every new patient and a suitable control during a five year period, and if necessary, during a ten year period, after the initiation of the study. The living parents, sibs, offspring, and spouses of both patients and controls were likewise registered. Complete family histories were also recorded.

Case finding among the relatives of patients and controls consists of a thorough neurological examination; any suspicious or doubtful "cases" are reexamined by the staff neurologists, and either confirmed or rejected as a case.

MAJOR FINDINGS

Because of the possibility that ALS and PD are two forms of the same disease spectrum, the data for ALS and PD were pooled and analyzed as if we were dealing with one disease, ALS/PD.

After five years of existence the patient-control panels were closed and over 80 percent of 2,000 relatives registered were located and given complete neurological examinations. These examinations have been completed. The main results of this study are:

(1) ALS/PD of Guam is familial. At least 23 percent of the registered patients showed definite history of the disease in one or more of their 0.5 relatives (parents, sibs or offspring). The familiarity value of 23 percent is considered a minimal one since only relatives affected during the years 1952-1964 were considered and then only if clinical and/or pathological records of diagnosis were available in our NINDB files.

(2) The brothers of the registered ALS/PD patients have a higher incidence of the disease than the general male population of Guam or the brothers of the registered controls (weighted by age and sex). The sisters of the patients also demonstrated a tendency for higher incidence; however, statistical comparisons gave nonsignificant differences.

(3) The degree of parent to offspring transmission (a main indicator of genetic transmission) could not be estimated at this stage. This was due to the fact that the ages of the parents and offspring were well above and below the ages of high risk for ALS (respectively) which resulted in a very low expected value making statistical comparisons unreliable at this time. It is hoped that an answer may be obtained within five to ten years when most of the offspring of both patients and controls will advance to the ages of high risk.

The manuscript of this report has been prepared and will be submitted for publication in the very near future.

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 63 E 1090
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland & Agana, Guam

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Pedigree studies in Umatac

Previous Serial Number: Same

Principal Investigator: C. C. Plato

Other Investigator: M. T. Cruz

Cooperating Units: None

Man Years

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

Project Description:

Umatac is a small village in southern Guam which has the highest rate of ALS/PD on the island.

Several pedigrees collected earlier from this village suggest the possibility that ALS/PD is a genetically controlled disease transmitted through a single dominant gene with incomplete penetrance. This has not been confirmed since in a village as small and isolated as this one, with a presumably high degree of inbreeding, chance alone might be responsible for the high prevalence of familial occurrences.

OBJECTIVES

The objectives of the present study are: (1) to trace and construct pedigrees for all the ALS/PD patients in the village; (2) to estimate the degree of consanguinity in this village; and (3) to evaluate the role of inheritance regarding the high prevalence of ALS/PD in this village.

METHODS EMPLOYED

The first phase of this project has been completed. Almost 97 percent of the village population was formed into a single pedigree which we were able to trace back for seven generations.

We are presently engaged in the estimation of the inbreeding coefficient and the examination of the role played by genetic inheritance in the etiology of ALS/PD.

Honors and Awards: None

Publications: None

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Serological and hematological investigations among the Trukese in Micronesia

Pervious Serial Number: Same

Principal Investigator: C. C. Plato

Other Investigator: M. T. Cruz

Cooperating Units: None

Man Years

Total: 1/16

Professional: 1/16

Other: 0

Project Description:

METHODS EMPLOYED

Concurrent with the clinical neurological examinations, blood specimens were collected from three hundred and sixty Carolinians from the Truk and Yap districts. These specimens were typed for ABO, Rh, MNS, Kell, P, JK^b, and K^a systems. The plasma was used for haptoglobin determinations. With the hemolysater we carried out electrophoretic assays for detection of abnormal hemoglobins.

OBJECTIVES

The objectives of this project were: (1) to detect possible associations between ALS/PD and the standard genetic markers (blood groups); (2) to estimate the genetic relationship between the various isolates under study; (3) to compare the blood group gene frequencies of the various areas of Truk and Yap in an effort to determine the origin of the Carolinians who have emigrated to Saipan; and (4) to detect, if possible, abnormal hemoglobins in this area. The laboratory procedures have been completed, the data analyzed, and the calculations completed. A report has been prepared.

This project was formerly titled "Blood group frequencies of the Carolinians of Truk and Eastern Yap," and incorporates a former project titled "Hemoglobin and haptoglobin studies on the various Micronesian populations," Serial No. NDB (CF) - 62 E 963.

Honors and Awards: None

Publications:

1. Plato, D. C. and Cruz, M. T.: Blood Group and Haptoglobin Frequencies of the Trukese of Micronesia. Acta Genetica et Statistica Medica 16:74-83, 1966.

Serial No. NDE (CF) - 63 E 1092

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Dermatoglyphic evaluation of the Chamorros and Carolinians

Previous Serial Number: Same

Principal Investigators: C. C. Plato
J. Niswander

Other Investigators: None

Cooperating Unit: National Institute of Dental Research

Man Years

Total: 1/16
Professional: 1/16
Other: 0

Project Description:

OBJECTIVES

This project was undertaken for two reasons: (1) to correlate fingerprint patterns with the neurological diseases found in these populations; and (2) to define the dermatoglyphics of these populations and compare them to those of other isolates.

Dermatoglyphics, like blood groups, are widely used as genetic markers as well as means of evaluating the relationship of genetic isolates. Since dermatoglyphics (finger as well as palm prints) are controlled by more than one pair of genes, their distributions are less vulnerable to genetic drift and isolation than the blood groups which are controlled by single genes.

The objectives of the project in its present form are: (1) to obtain additional genetic information regarding the Trukese and to use it, along with blood group data, to estimate the relationships between the various isolates in this geographic area; and (2) to determine whether isolates with common high values of blood sugar and uric acid also have similar dermatoglyphic patterns. From earlier studies it was found that the people of Micronesia have very high values of uric acid and blood sugar. These same high values were found in other populations including South American Indians. Since the dermatoglyphics of these isolates are available this comparison was deemed valuable.

METHODS EMPLOYED

Finger prints and palm prints were collected from seventy Trukese and have been evaluated. A manuscript is under preparation for publication.

Honors and Awards: None

Publications: None

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Blood group investigations on the Carolinians and Chamorros of Saipan

Previous Serial Number: Same

Principal Investigator: C. C. Plato

Other Investigators: D. L. Rucknagel
L. T. Kurland

Cooperating Units: University of Michigan

Man Years

Total: 1/8
Professional: 1/8
Other: 0

Project Description:

OBJECTIVES

Amyotrophic lateral sclerosis is found to be of high prevalence among both ethnic groups (Chamorro and Carolinian) residing in Saipan. This finding of high prevalence among two populations residing and working side by side gave more importance to the question of nature vs. nurture in regard to the etiology of ALS. It became of interest to investigate whether the two groups were genetically related; or whether the common high prevalence of ALS was due to environmental factors shared by both ethnic elements, or due to interaction of both heredity and environment.

METHODS EMPLOYED AND MAJOR FINDINGS

The blood group investigations undertaken in this project were intended to supply evidence for or against genetic relationship between these two groups of Saipan. In all 143 Chamorros and 146 Carolinians were included in this study. Each one was typed for the following systems: ABO (including A_1 , A_2 , and A_x), MNS, M^g , Rh (five antigens), P, V^w , W_r^a , Kell (including Kp^g and Kp^b), Fy^a and JK^b . All C positive individuals were further tested for C^w and all D positive were investigated for D^u . The results gave evidence that the Carolinians and Chamorros of Saipan are genetically different.

Honors and Awards: None

Publications:

1. Plato, C. C., Rucknagel, D. L., and Kurland, L. T.: Blood Group Investigations on the Carolinians and Chamorros of Saipan. American Journal of Physical Anthropology March 1966 (in press).

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Clinical aspects of diabetes mellitus in Guam

Previous Serial Number: Same

Principal Investigator: R. L. Need

Other Investigator: T. S. Elizan

Cooperating Units: None

Man Years

Total:	1/8
Professional:	1/8
Other:	0

Project Description:

OBJECTIVES AND METHODS EMPLOYED

The clinical aspects of diabetes mellitus in Guam were studied. Of particular interest was the previously reported apparent absence of juvenile diabetes and of ketoacidosis in Chamorros. The study encompassed known diabetics seen at the Guam Memorial Hospital and in the NINDB clinics during 1963-1964 and included glucose tolerance tests, past medical history, physical findings and family histories.

MAJOR FINDINGS

Only one case of juvenile diabetes was seen and no instance of diabetic acidosis was observed. Moreover, very few cases of diabetic neuropathy were seen in these Chamorro patients.

A paper has been prepared and is presently being submitted for publication.

Honors and Awards: None

Publications: None

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

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

Previous Serial Number: Same.

Principal Investigators: T. S. Elizan
K. M. Chen

Other Investigators: Y. Yase
R. L. Need

Man Years

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

Project Description:

The NINDB has occupied a unique position on Guam since 1956. It is the only neurological consultation service available to the Chamorro, Filipino, and Caucasian populations on Guam and sees most neurological patients at the two hospital facilities; Guam Memorial Hospital and Navy Hospital. Therefore, most cases of significant neurological disease are eventually brought to its staff's attention.

METHODS EMPLOYED

Dr. Hirano classified 200 neurological patients seen from September 1, 1959 to June, 1960 without regard to dividing Chamorros from non-Chamorros.

The present study reanalyzes all Chamorro non-ALS, PD or suspect cases, as to neurological entities. If the patient presents any question of diagnosis and is still alive, he will be reexamined and have relevant diagnostic studies. All cases of rare neurological disease still alive will be reexamined.

If, in the 300-odd cases available for study by case history, any disease entity appears to obtain in greater numbers than would be expected in previous statistical experience, appropriate steps will be taken to describe it clinically and gather statistical data about its appearance on Guam with a further view to elucidating its etiology and pathogenesis.

A paper on dystrophia myotonica in Chamorro families of Guam is presently being prepared.

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 63 E 1106

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Screening of edible and medicinal legumes for neurotoxic constituents

Previous Serial Number: SAME

Principal Investigator: M. G. Whiting (Coordinator)

Other Investigators: F. Strong
E. A. Bell
Q. Jones

Cooperating Units: University of Wisconsin, Madison, Wisconsin
King's College, London, England
U. S. Department of Agriculture, Beltsville, Md.

Man Years

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

Project Description:

OBJECTIVES AND MAJOR FINDINGS

The objective of this research is to assess the neurotoxic components of a variety of edible and medicinal legumes from focal areas of high prevalence of neurological disease. Bell has found that seeds of Cycas circinalis were of greater interest to him than the seeds of leguminous plants and in a letter dated 26 April 1966, he reported the isolation of millogram quantities of the hitherto unknown basic amino acid in the endosperm of seeds of C. circinalis from Guam. He suggests that this amino acid may contain an imidazole or similar heterocyclic ring. At his request a larger supply of seeds have been shipped. A supply of fronds from the Dominican Republic has also been mailed.

PROPOSED COURSE OF PROJECT

Plans have been discussed but no action taken to have Bell apply his screening techniques to a selection of the seeds now being collected through a world-wide search and screened for fat and protein content through the office of Q. Jones at USDA. Bell's progress with cycads will henceforth be reported directly by him.

Honors and Awards: None.

Publications: None.

Serial No. NDB (CF) - 63 E 1107

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Food, medicinal, and toxic plants of Mitogawa, Japan.

Previous Serial Number: SAME

Principal Investigator: M. G. Whiting

Other Investigators: A. Ko
H. Matsushita
F. R. Fosberg

Cooperating Unit: Smithsonian Institution, Washington, D. C.

Man Years

Total: 3/8
Professional: 1/8
Other: 1/4

Project Description:

OBJECTIVES

The objective of this research is to provide a working botanical reference to commonly ingested plants from the Mitogawa area of Japan.

A compilation is completed which includes information concerning more than 150 plants collected in the Mitogawa area of Japan during the winter and early spring of 1963-64. These plants are used either for food or medicine or are reputed by residents of the area to be toxic. Voucher specimens have been deposited with R. Fosberg and have been identified by him and his associates. The completed material includes 1) local Japanese name; 2) botanical identification; 3) common English name; and 4) text which describes use in the mountain area as obtained from local residents.

PROPOSED COURSE OF PROJECT

It is hoped that from this compilation the plants, which are reported from other sources and areas to produce neurotoxic effects on man or animals, will be selected for further investigation. No further research on this project is planned by NINDB.

Honors and Awards: None.

Publications: None.

Serial No. NDB (CF) - 63 E 1108

1. Collaborative and Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Study of the toxic constituents of zen mai (Osmunda japonica Thunb.)

Previous Serial Number: SAME

Principal Investigator: M. G. Whiting (Coordinator)

Other Investigator: H. Matsumoto

Cooperating Unit: Agricultural Biochemistry Department, University of Hawaii,
Honolulu, Hawaii

Man Years

Total:	1/8
Professional:	1/8
Other:	0

Project Description:

PROPOSED COURSE OF PROJECT

No further work is being done by Matsumoto on the toxicity of the Mitogawa samples of Osmunda japonica Thunb. (zen mai). However, because of recent reports of the production of malignant tumors in rats following feeding of bracken, plans are now under consideration by Matsumoto for more extensive studies of this fern. Henceforth, progress on this research will be reported by him directly.

Honors and Awards: None.

Publications: None.

Serial No. NDB (CF) - 63 E 1109

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Study of toxic fungi in cycad and other plant material used for human or animal food in various areas of the world.

Previous Serial Number: SAME

Principal Investigator: M. G. Whiting

Other Investigators: J. Forgacs
W. T. Carll

Cooperating Units: Good Samaritan Hospital, Suffern, New York
Bluff Run Farm, Hopkins, South Carolina

Man Years

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

Project Description:

PROPOSED COURSE OF PROJECT

The final revised report and several supplementary reports (Mycotoxicoeses I, II, III, and IV) from Forgacs have been duplicated and are ready for distribution.

No further research has been done (or is planned by NINDB) on this aspect of cycad research. Forgacs has been urged to publish his reports.

Honors and Awards: None.

Publications: None.

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Plants from the island of Guam: Food, medicinal and toxic.

Previous Serial Number: SAME

Principal Investigator: M. G. Whiting

Other Investigators: F. R. Fosberg
N. Farnsworth
A. Won Pat

Cooperating Units: Smithsonian Institution, Washington, D. C.
University of Pittsburgh, Pittsburgh, Pennsylvania
Government of Guam, Marianas

Man Years

Total: 1/2
Professional: 1/8
Other: 3/8

Project Description:

OBJECTIVES

The objective of this research is to provide a basic botanical reference for use by research workers interested in environmental factors in relation to disease patterns in Guam.

METHODS EMPLOYED

A compilation is completed which includes information concerning more than 200 plants collected by M. Whiting in Guam during 1954, 1957-58, and 1963. These plants are used either for food or medicine or are reputed by residents of Guam to be toxic. Voucher specimens have been deposited with R. Fosberg and have been identified by him and his associates. The completed material includes 1) local Chamorro (or other) name; 2) botanical identification; 3) plant family; 4) common English name; and 5) description of use and preparation as obtained from local informants. Plants from this compilation which have been reported from other sources and areas to produce neurotoxic effects on either man or animals should be selected for further investigation.

PROPOSED COURSE OF PROJECT

A proposal for collaborative investigation of plants selected from this list has been prepared by Farnsworth and, through Won Pat, presented to the Governor of Guam. Progress on this research will henceforth be reported by Won Pat or Farnsworth. No further research on this program is planned by the Epidemiology Branch.

Honors and Awards: None.

Publications: None.

Serial No. NDB (CF) - 63 E 1111

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Neurotoxin(s) in fronds of cycad plants.

Previous Serial Number: SAME

Principal Investigator: M. G. Whiting (Coordinator)

Other Investigators: J. L. Anderson
C. Gallagher
W. T. K. Hall
J. A. Henderson
J. Innes
M. Mason
C. Hoch-Ligetl
M. Stanton
P. Healy
M. Manzano
H. Matsumoto
R. Bullock
N. Riggs

Cooperating Units: Division of Animal Industry, Department of Agriculture,
Stock & Fisheries, Territory of Papua and New Guinea,
Australia
McMaster Animal Health Laboratory, University of Sydney,
Sydney, Australia
Animal Research Institute, Yeerongpilly, Brisbane,
Queensland, Australia
College of Veterinary Medicine, Washington State
University, Pullman, Washington
Agricultural Biochemistry Department
University of Hawaii, Honolulu, Hawaii
Laboratory of Pathology, NCI
Bionetics Research Lab., Inc., Falls Church, Virginia
Mason Research Institute, Worcester, Massachusetts
Veterans Administration, Martinsburg, West Virginia
University of Puerto Rico, Rio Piedras, Puerto Rico
South Puerto Rico Sugar Company Trading Corporation,
New York, New York (Dominican Republic)
University of New England, Armidale, Australia

Man Years

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

Project Description:

OBJECTIVES

In the draft of a manuscript received in midsummer, Hall reported that, using the Marchi stain, he had located lesions in the c.n.s. of cattle which have been fed cycad fronds. Similar lesions have since been identified by Innes and Mason in animals from the Dominican Republic and by Gallagher and Healy in animals from Papua and Northern Territory.

Methods employed and Major findings:

Mason describes the lesions as follows: "The histologic lesions found in the spinal cords of affected cattle in the Dominican Republic were those of degenerated myelinated fibers of the fasciculus gracilis, dorsal spinocerebellar and some tracts in the lateral and ventral funiculi. These were demonstrated using the Marchi stain. They could not be clearly delineated when negative staining for myelin was used...There is a correlation between severity of the symptoms and lesions." Mason's research was completed under Order No. D-135089-6.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

Dr. Brody (NINDB), during a visit to Puerto Rico in September 1965, had opportunity to observe local cattle afflicted with cycad paralysis and to make a film showing the condition. This film from Puerto Rico, combined with a film from the Dominican Republic and another received from Hall in Queensland clearly demonstrates the similarity of clinical signs in animals of three different breeds. Several different species of cycads are implicated.

Manzano, a chemist for the Agricultural Experimental Station at the University of Puerto Rico, attended the Federation meetings in Atlantic City in April where he had ample opportunity for conferences with others engaged in cycad research. He has been assigned to proceed with an active program of research on the local species of cycad. Matsumoto has suggested collection and storage of a large supply of young sprouts from the spring growth in Puerto Rico. Manzano has been urged to set up a production plant for the extraction of the glycoside (macrozamin) from tubers of the Puerto Rican cycads since there is considerable demand for this compound and no source of a supply is now available.

Hoch-Ligetzi reports liver damage in rats following feeding of dry fronds of Zamia debilis obtained from the Dominican Republic.

Daily feeding of the dry fronds to fish (Lebistes reticulatus) has been toxic and lethal. Histological studies are not yet completed.

Matsumoto is feeding rats and Japanese quail with fractions obtained from an artificial rumen to which fresh sprouts and mature fronds of Zamia spp. have been added. Results are not yet available. Gallagher and

coworkers and Hall are also actively engaged in attempts to reproduce neurotoxic effects with the feeding of cycad fronds in small laboratory animals.

At least three laboratories, Hawaii, Sydney, and New Armidale, are actively engaged in attempts to identify the toxin(s) responsible for the neurological effects in cattle which follow ingestion of the fronds and sprouts of cycad.

PROPOSED COURSE OF PROJECT

Future research on the neurological effects in animals which follow ingestion of cycad fronds and identification of the neurotoxin will be reported by individual investigators and by institutions other than NINDB.

Honors and Awards: None

Publications:

Whiting, M., Spatz, M., and Matsumoto, H.: Research progress on cycads. Economic Botany. 20, 1:98-102, Jan.-Mar. 1966

Whiting, M. G.: New Advances in Research on Toxic Plants: Cycad. In Nutrition Notes 1:2, Oct. 1965.

Whiting, M. G.: Cycad Research, Report on Fourth Conference on Cycad Toxicity. Toxicon. 3:171, 1965.

Editor: Whiting, M.G.: Conference on the Toxicity of Cycads (Fourth). NINDB, NIH, 1965, 201 pp.

- Serial No. NDB (CF) - 63 E 1112
1. Collaborative & Field Research
 2. Epidemiology Branch
 3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Chemistry of the cycad (related to carcinogenic and hepatotoxic components)

Previous Serial Number: SAME

Principal Investigator: M. G. Whiting (Coordinator)

Other Investigators: H. Matsumoto
N. V. Riggs
C. Gallagher
B. H. Korsch
E. A. Bell
R. C. Shank
D. Mattocks
A. Ash
G. Laqueur

Cooperating Units: Agricultural Biochemistry Department, University of
Hawaii, Honolulu, Hawaii
Department of Organic Chemistry, University of New
England, Armidale, N.S.W., Australia
King's College, London, England
Toxicology Research Unit, Carshalton, Surrey, England
McMaster Animal Health Laboratory, University of
Sydney, Sydney, Australia
Ash Stevens Inc.
Detroit, Michigan
Laboratory of Experimental Pathology, NIAMD

Man Years

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

Project Description:

OBJECTIVES AND MAJOR FINDINGS

Chemical research on cycads has been directed toward the production of labelled MAM-acetate. Although uniformly labelled MAM-acetate has been produced experimentally, it is not available in quantity sufficient for biochemical experimentation. Contract bids for the preparation of this product have been announced by NCI. There is also a need to prepare MAM with radio-carbon label in only one of the carbons.

Methylazoxymethanol-acetate (MAM-acetate) is now available commercially from Ash Stevens; quoted price is \$50.00 per gram.

Several laboratories are actively engaged in attempts to isolate toxic metabolites other than MAM from various portions of the cycad plant.

PROPOSED COURSE OF PROJECT
future progress on this problem will be followed by NIAMD (Dr. Laqueur).

Honors and Awards: None.

Publications: None.

Serial No. NDB (CF) - 63 E 1113
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Effects on animals following administration of cycad plant material and of toxic compounds isolated from cycads.

Previous Serial Number: SAME

Principal Investigator: M. G. Whiting (Coordinator)

Other Investigators: G. Laqueur
M. Spatz
A. Hirano
M. Kelly
R. O'Gara
M. Stanton
N. Hoch-Ligeti

Cooperating Units: Laboratory of Experimental Pathology, NIAMD
Laboratory of Chemical Pharmacology, NCI
Laboratory of Pathology, NCI
Veterans Administration, Martinsburg, West Virginia

Man Years

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

Project Description:

OBJECTIVES

Considerable progress has been made toward an understanding of the carcinogenic effects in animals which follow administration of cycad plant material and toxic compounds isolated from the plant.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

The research of greatest interest to NINDB is the demonstration by Dr. Maria Spatz (NIAMD) that the toxic effects from cycads can be transmitted in rats from the pregnant mother to the fetus. Crude cycad meal was fed to 61 pregnant animals and to date, thirteen of the animals that have died have shown a total of 17 tumors. Tumors are located in brain, small intestine, kidney, colon, and uterus.

In preliminary feeding experiments with the fresh green husk from seeds of Cycas circinalis, Hoch-Ligeti has produced severe liver damage within a week. This effect is of interest because the husk, green or dry, is used as a chew by Guamanians.

Other research on the toxic effects of cycads on different animal species will be found in the reports of NIAMD (G. Laqueur) and NCI (M. Kelly, R. O'Gara, and M. Stanton).

PROPOSED COURSE OF PROJECT

Future progress will be followed by NIAMD (Dr. Laqueur) and NCI.

Honors and Awards: None.

Publications: None.

Serial No. NDB (CF) - 63 E 1114

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Effects of fresh cycad kernel meal on open wounds

Previous Serial Number: SAME

Principal Investigator: R. O'Gara

Other Investigators: M. G. Whiting
J. M. Brown

Cooperating Unit: NCI

Man Years

Total: 0
Professional:
Other:

Project Description:

PROPOSED COURSE OF PROJECT

This experiment has been repeated in the laboratory of Dr. O'Gara and will be reported by him directly.

Honors and Awards: None.

Publications: None.

Serial No. NDB (CF) - 63 E 1117
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Epidemiological studies of multiple sclerosis in New Orleans, Louisiana.

Previous Serial Number: Same.

Principal Investigator: A. Stazio

Other Investigators: L. T. Kurland
R. Paddison
R. Paterson
J. Paterson
J. Moosey
T. Soniat

Cooperating Units: Louisiana State University School of Medicine
Tulane University
Ochsner Clinic

Man Years

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

Project Description:

OBJECTIVES

A follow-up and re-evaluation of a 1951 epidemiologic survey of multiple sclerosis in New Orleans was conducted during 1964 and 1965. The purpose of this study was to reappraise all cases reported in the previous survey, to provide information on survivorship patterns and the natural history of multiple sclerosis in New Orleans, and to establish a comparison with the corresponding aspects of the disease in Winnipeg, Canada, where a similar study was completed two years before.

METHODS EMPLOYED AND MAJOR FINDINGS

Twenty-four of the 29 patients examined in the previous survey and included in the resident probable patient category, were accepted as such in our follow-up study, and four of the possible and unlikely cases of the previous survey could also be accepted as probable cases on the basis of our re-evaluation. An additional 31 patients, who in retrospect should have been eligible for the original study in 1951 but had not been examined then, were also accepted as probable cases of multiple sclerosis in our study. Our revised prevalence rate of probable patients on January 1st, 1951 was 8.6 per 100,000 population, as compared to the original estimate

of 6.0. On the basis of our re-evaluation studies in Winnipeg and New Orleans, the ratio of prevalence rates in the two communities is now estimated as about 4 to 1.

The mean age at onset, the rate of progression of the disease, the average duration of the disease, and the female to male ratio were observed to be essentially similar in New Orleans and in Winnipeg.

The Winnipeg to New Orleans ratios of incidence and mortality rates are estimated as 4 to 1, which is the same as the ratio of prevalences in the two communities.

A paper on multiple sclerosis in New Orleans, Louisiana, is presently being prepared for publication.

Honors and Awards: None

Publications: None.

Serial No. NDB (CF) - 65 E 1248
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Fine Structure of Cerebral Fluid Accumulation. VII.
Reactions of Astrocytes to Cryptococcal Polysaccharide
Implantation.

Previous Serial Number: Same.

Principal Investigator: A. Hirano

Other Investigators: H. M. Zimmerman
S. Levine

Cooperating Units: Montefiore Hospital and Medical Center
New York Medical College Center for Chronic Disease

Man Years

Total: 1/2
Professional: 3/8
Other: 1/8

Project Description:

OBJECTIVES AND METHODS EMPLOYED

Implantation of cryptococcal polysaccharides in rat brain resulted in the spread of exogenous material with fluid into the distant white matter. Changes of fibrous astrocytes in the area of implantation and in distant white matter were studied with light and electron microscopy.

MAJOR FINDINGS

Clear appearing astrocytes were observed in the vicinity of the implant during the acute stage. A watery and voluminous expansion of cytoplasm was accompanied by many minute vesicular profiles and a considerable increase of glycogen-like granules. In the chronic stage, the increase of glial fibrils was the most conspicuous phenomena.

Direct invasion of polysaccharide-rich fluid into the astrocytes was observed through ruptured plasma membranes at the site of implant. Diffuse invasion by a fluid mass left a large lake of polysaccharide within the affected astrocyte. In addition to diffuse infiltration of exogenous material, small cystic focal collections of fluid were observed in the affected astrocytes. The morphological texture of ingested material in these cells remained remarkably unchanged. Persistence of large amounts of intracellular polysaccharide was observed even 100 days after implantation.

Honors and Awards: None

Publications:

1. Hirano, A., Zimmerman, H. M., and Levine S.: Fine Structure of Cerebral Fluid Accumulation. VII. Reaction of Astrocytes to Cryptococcal Polysaccharide Implantation. Journal of Neuropathology and Experimental Neurology 24:386-397, 1965.

Serial No. NDB (CF) - 64 E 1250

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Reaction of the Nervous System to Cryptococcal Infection.
An Experimental Study with Light and Electron Microscopy.

Previous Serial Number: Same.

Principal Investigator: S. Levine
A. Hirano

Other Investigator: H. M. Zimmerman

Cooperating Units: New York Medical College Center for Chronic Disease
Montefiore Hospital and Medical Center

Man Years

Total:	1/2
Professional:	3/8
Other:	1/8

Project Description:

MAJOR FINDINGS

The cells of *Cryptococcus neoformans*, whether living or dead, elicited only a weak and delayed inflammatory reaction in the brains of experimental animals and some human patients. This is due, at least partly, to an inherent quality of brain tissue, since the cryptococcal organisms often caused vigorous inflammation in other organs. The cryptococcal capsule and cell wall were extremely resistant to digestion, even when engulfed by phagocytes in the brain. The purified capsular polysaccharide implanted into the brain enjoyed the same relative freedom from inflammatory reaction and from digestive degradation. It is likely that both the peculiar qualities of the capsular polysaccharide and the inherent characteristics of brain as a tissue result in an inadequate inflammatory response to the cryptococci. This may be partly responsible for the predilection of cryptococcosis for the brain, and for the chronic progressive character of the infection. Other factors inherent in the organism and in the biochemistry of the nervous system are undoubtedly important, as well.

This research was presented at the 44th Annual Meeting of the Association for Research in Nervous and Mental Diseases, New York, New York, December 5, 1964.

Honors and Awards: None

Publications:

1. Levine, S., Hirano, A., and Zimmerman, H. M.: The Reaction of the Nervous System to Cryptococcal Infection: An Experimental Study with Light and Electron Microscopy. In Zimmerman, H. M. (Ed.): Proceedings of the Association for Research in Nervous and Mental Disease, Infections of the Nervous System. Baltimore, Maryland, The Williams and Wilkins Company (in press).

Serial No. NDB (CF) - 64 E 1251
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Amyotrophic Lateral Sclerosis, Histopathological Considerations

Previous Serial Number: Same.

Principal Investigator: A. Hirano

Other Investigator: L. T. Kurland

Cooperating Unit: Montefiore Hospital and Medical Center

Man Years

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

Project Description:

This study covers the following five points:

1. The histopathologic findings in classic, sporadic cases of ALS based on a review of material at Montefiore Hospital and Medical Center.
2. Familial ALS in which the classic findings are frequently noted, but in some cases of which posterior-column demyelination and other atypical changes may occur.
3. Histopathologic features observed in Guam ALS cases.
4. Neuropathological features of PD complex on Guam, with special reference to Lewy's bodies in substantia nigra and electron microscopic study of fibrillary structures in the pyramidal cell layer of the Sommer's sector.
5. On the eosinophilic inclusion bodies observed and described in hereditary ALS from the U.S.S.R. by Bunina. Studies of spinal cord sections from the experimental monkeys used in studies by Zilber and colleagues in Moscow.

Honors and Awards: None

Publications:

1. Proceedings of Workshop Symposium on Latent, Slow and Temperate Virus Infections, NINDB, NIH, Bethesda, Maryland, December 7-9, 1964. U. S. Government Printing Office (in press).

Serial No. NDB (CF) - 65 E 1252
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Reaction of Ependyma to Cryptococcal Polysaccharide
Implantation.

Previous Serial Number: Same.

Principal Investigator: A. Hirano

Other Investigators: H. M. Zimmerman
S. Levine

Cooperating Units: Montefiore Hospital and Medical Center
New York Medical College Center for Chronic Diseases

Man Years

Total: 1/2
Professional: 3/8
Other: 1/8

Project Description:

Following intracerebral implantation of cryptococcal polysaccharide, large amounts of polysaccharide-rich fluid spread into the distended extracellular spaces of the subependymal region. Alterations of ependyma and subependymal structures were studied with light and electron microscopy after the whole brain was fixed in situ by perfusion. Considerable amounts of fluid were engulfed by subependymal phagocytic cells, while smaller amounts entered the cytoplasm of ependymal cells usually as small cystic collections. The adjacent ependymal cells generally maintained close contact, without obvious separation. Phagocytes with engulfed fluid, nevertheless, were capable of separating adjacent ependymal cells and were able to traverse the ependymal barrier into the lumen of the ventricle.

This report was presented at the Annual Meeting of the American Association of Neuropathologists on June 13, 1965 in Atlantic City, New Jersey.

Honors and Awards: None

Publications:

1. Hirano, A., Zimmerman, H. M., and Levine, S.: The Fine Structure of Cerebral Fluid Accumulation. Reaction of Ependyma to Cryptococcal Polysaccharide Implantation. Journal of Pathology and Bacteriology (in press).

Serial No. NDB (CF) - 64 E 1253

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Influence of small systematic errors on the results of tonometric screening

Previous Serial Number: Same

Principal Investigator: J. T. Schwartz

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1/64
Professional:	1/64
Other:	0

Project Description:

OBJECTIVES

Although glaucoma surveys have been performed among many populations, relatively few reports are truly comparable. In this study a graphic method was used to demonstrate the important influence of small differences in tonometric techniques on the outcome of mass tonometry surveys.

MAJOR FINDINGS

The frequency distribution of ocular tensions among a population of 414 retired persons was used to demonstrate that a systematic error of only 2 mm. of mercury would alter the proportion of positive screenees at 25 mm. of mercury by about 60 percent. The amplitude of potential systematic error resulting from a number of differences in tonometric techniques was estimated from available literature. The influence of these error values on tonometric survey results was then determined by graphic methods.

It was found that differences in examination procedure which might ordinarily hold minimal significance in clinical practice can exert considerable influence on the outcome of population surveys. This study concluded that a single, specific, and carefully defined tonometry technique must find broad acceptance in order to acquire comparable geographic data on the frequency of ocular hypertension.

Honors and Awards: None

Publications:

1. Schwartz, J. Theodore: Influence of small systematic errors on the results of tonometric screening. American Journal of Ophthalmology 60:1003, September 1965.

Serial No. NDB (CF) - 64 E 1254

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Comparison of measurements of parameters of chronic simple glaucoma among monozygous and dizygous twins

Previous Serial Number: Same

Principal Investigator: J. T. Schwartz

Other Investigators: None

Cooperating Units: None

Man Years

Total: 1/8
Professional: 1/16
Other: 1/16

Project Description:

OBJECTIVES

Comparison of concordance among monozygous and dizygous twins with respect to specific physical characteristics provides an evaluation of the relative role of genetic or environmental influence underlying the development of these characteristics.

A registry of local twins who will be available for ophthalmic examinations on an outpatient basis has been developed by the Section on Ophthalmic Field and Developmental Research. Over 350 pairs of twins have been located in the greater Washington area.

PROPOSED COURSE OF PROJECT: An ophthalmic screening examination and zygosity determination will be provided for the twin population. Selected pairs of adult twins will be scheduled for glaucoma evaluation. Examinations will include such tests as tonometry, tonography, gonioscopy, visual field, water provocation and evaluation of response to the topical steroid preparation. Concordance figures for those characteristics will then be collected and analyzed.

The former title of this project was "Comparison of glaucoma response following steroid provocation among monozygous and dizygous twins."

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 64 E 1255

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Tonometry survey in an Italian-American population

Previous Serial Number: Same

Principal Investigator: J. T. Schwartz

Other Investigator: G. G. Dell'Osso

Cooperating Unit: Biometrics Branch, C&FR, NINDB

Man Years

Total:	3/16
Professional:	1/8
Other:	1/16

Project Description:

OBJECTIVES

A community-wide tonometry survey was performed among adult Italian-Americans living in Nesquehoning, Pennsylvania. Americans of Italian descent living in a nearby Pennsylvania county are reported to experience a low mortality due to coronary artery disease. Nesquehoning was selected for pilot investigation largely because of our ultimate interest in investigating the possible association between chronic simple glaucoma and chronic vascular disease. [See Project No. NDB (CF) - 63 E 1122, 1963-1964 Annual Report.]

This survey was undertaken with the following specific objectives: (1) to establish the frequency distribution of ocular tension in a defined Italian-American population, (2) to determine postural influence (sitting vs. supine) on applanation tonometry measurements in a defined "total population," and (3) to compare the results of applanation and Schiotz tonometry in a defined "total population."

METHODS EMPLOYED

The investigation was undertaken in the form of a mass survey program. Community participation was organized through the cooperation of a team of local volunteers. The target population was 450 persons age 25 and over, of whom 84 percent were examined. Positive screenees were subsequently referred to attending physicians for definitive care.

MAJOR FINDINGS

Analysis of the data revealed: (1) An estimate of the net error inherent in current calibrations of Schiotz (7.5 gram weight) and Goldmann tonometers is about 3 mm. Hg. (2) Estimates of intraocular pressure by the Goldmann method showed an average increase of approximately 2 mm. Hg following a change

from the sitting to the supine recumbent position. Postural influence would sometimes be expected to constitute a serious confounding variable when a coefficient of ocular rigidity is estimated from paired Schiøtz and sitting Goldmann applanation readings. (3) Within a population, variation is found among individuals in the amount of difference between paired readings obtained with the Goldmann and Schiøtz tonometers when the examinee remains in the same supine recumbent position. Part of this variation may result from an inconstant biologic response to the test instrument.

The amplitude of difference in measurements of ocular tension as obtained with different techniques of examination emphasizes the need for uniform field techniques in the collection of comparable epidemiologic data on ocular hypertension.

Honors and Awards: None

Publications:

1. Schwartz, J. T. and Dell'Osso, G. G.: Comparability of Goldmann and Schiøtz tonometers in a community. Archives of Ophthalmology (in press).
2. Schwartz, J. T.: "Some Problems Relating to the Epidemiology of Chronic Simple Glaucoma," in Glaucoma: Proceedings of a Symposium on Epidemiology, Early Diagnosis, and Some Aspects of Treatment. L. B. Hunt, Editor, Edinburgh, Scotland, E. & S. Livingston Ltd. 1966.

- Serial No. NDB (CF) - 64 E 1256
1. Collaborative & Field Research
 2. Epidemiology Branch
 3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Tonometry survey among an American Indian population at Colorado River Reservation, Arizona

Previous Serial Number: Same

Principal Investigator: J. T. Schwartz

Other Investigators: G. R. Williams
G. G. Dell'Osso
D. Powers
B. Becker
N. Ballin
M. Newman

Cooperating Units: Biometrics Branch, C&FR, NINDB
Division of Indian Health
Washington University School of Medicine

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

Project Description:

American Indians have been reported to have a low frequency of chronic simple glaucoma. This study was a reservation-wide tonometry survey at the Colorado River Indian Reservation in Arizona, whose Indian population is of mixed tribal origin. Ocular tensions were measured on each subject by three different techniques: Goldmann applanation in the sitting position, Goldmann applanation in the supine position, and Schiøtz indentation in the supine position.

OBJECTIVES

The primary objectives of this survey were as follows: (1) to obtain pilot information on the frequency distribution of ocular tensions among a defined population of American Indians, (2) to provide a second evaluation of the influence of postural differences (sitting and supine) and instrument differences (Goldmann applanation and Schiøtz indentation) on tonometric measurements, in order to provide a cross-comparison with similar data previously obtained in a separate population [see Project No. NDB (CF) - 64 E 1255], (3) to correlate the results of tonometric screening by each of three techniques with the results of a definitive clinical evaluation for glaucoma, and (4) to appraise the physical practicability of employing Goldmann applanation tonometry, sitting

or supine, in population surveys performed under rigorous field conditions requiring a moderate degree of mobility.

METHODS

The field investigation was undertaken as a two-and-a-half week mass screening program. During this period the survey teams operated from five examination sites on the reservation. Community participation was organized by a field team from the Division of Indian Health. Diagnostic evaluation, including ophthalmoscopy, tonography and gonioscopy, was performed by a field team from Washington University. Tonometry was performed on 439 patients. Approximately 87 percent of the target population of adults age 30 and over were examined.

MAJOR FINDINGS

The data are being analyzed for Objectives (1) and (3).

Differences in the measurements of intraocular pressure found in association with different instruments and posture were very similar to those found in our previous study among Italian-Americans [Objective (2)].

The survey provided an opportunity for thorough appraisal of the practicability of performing applanation tonometry in the field using the applanation tonometer with slit lamp (sitting) or operating microscope (supine). Use of either method was satisfactory even under fairly rugged field conditions [Objective (4)].

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 64 E 1257
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: U. S. mortality experience with malignant neoplasms
of the eye

Previous Serial Number: Same

Principal Investigator: G. R. Williams

Other Investigators: J. T. Schwartz
D. L. VerLee
I. D. Goldberg

Cooperating Unit: Biometrics Branch, C&FR, NINDB

Man Years

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

Project Description:

Under the present system of classifying causes of death, all fatalities due to malignant neoplasms of the eye are assigned without subclassification to Rubric 192. Retrospective characterization of eye tumor mortality by histologic type requires, therefore, an examination of individual death certificates.

METHODS EMPLOYED AND OBJECTIVES

In the United States during the period 1959 to 1961, there were 1,108 certificates in which the primary cause of death was assigned to Rubric 192. A full copy of the microfilm image of these death records has been provided by the Division of Vital Statistics, National Center for Health Statistics, on 1,024 certificates. The data from these records has been recorded in a standard format and is now being analyzed in collaboration with the Biometrics Branch. Certain epidemiologic features of eye tumor mortality in the United States such as age, race, sex, and state of residence will be obtained from this analysis.

PROPOSED COURSE OF PROJECT

It is anticipated that additional information will be solicited on certain cases, and where necessary an attempt will be made to recover surgical specimens or autopsy materials. Dr. Lorenz Zimmerman, Chief, Ophthalmic Pathology Branch, Armed Forces Institute of Pathology,

has agreed to examine the pathologic material which we recover. Attending physicians, referring physicians, hospitals, clinics, tumor registries, and other sources of professional information will be contacted in order to obtain data on cases which are of special importance.

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 66 E 1312
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Comparison of components of refraction and ocular motility among monozygous and dizygous twins

Previous Serial Number: None

Principal Investigator: J. T. Schwartz

Other Investigator: D. L. VerLee

Cooperating Units: None

Man Years

Total: 1/8
Professional: 1/16
Other: 1/16

Project Description:

OBJECTIVES

Comparison of concordance of physical characteristics in monozygous and dizygous twins is valuable as an indication of the relative roles of heredity and environment in the expression of these characteristics.

The Section on Ophthalmic Field and Developmental Research has compiled a registry of approximately 350 pairs of monozygous and dizygous twins who will be available for examination.

PROPOSED COURSE OF PROJECT

The first phase of the project will consist of obtaining a detailed medical history and performing screening ophthalmic examinations on each twin. It will also include blood typing and finger printing in an effort to determine zygoty with a high level of probability.

In subsequent examinations of selected pairs of twins, the components of refraction (including total refractive power, corneal curvature, anterior chamber depth, lens thickness and curvatures, and ultrasonic determinations of axial length) as well as parameters of ocular motility (including heterophoria, AC/A ratio, vergence amplitudes, and accommodation amplitudes) will be measured. Concordance figures for these characteristics will then be calculated and analyzed.

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 66 E 1313
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Ophthalmic survey of population groups of the Solomon Islands

Previous Serial Number: None

Principal Investigator: D. L. VerLee

Other Investigators: J. T. Schwartz
A. Damon
D. L. Oliver
W. W. Howell

Cooperating Unit: Department of Anthropology, Harvard University

Man Years

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

Project Description:

The Section on Ophthalmic Field and Developmental Research has been invited to join a survey team of medical specialists and anthropologists to participate in field investigations among defined populations on two islands in the Solomons; Bouganville and Malaita. Members of the Department of Anthropology at Harvard University have recently completed approximately two years of preliminary anthropologic field work among the study populations, and that Department is now preparing to follow this preparatory activity with medical and epidemiologic investigations. These investigations will include a general medical evaluation, evaluations in internal medicine, cardiology, pediatrics, dentistry, ophthalmology, general metabolic status, and anthropometry. Additional populations of the Solomon Islands will be studied by the Harvard program each summer for the next seven years.

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 66 E 1314
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Influence of a synthetically derived aglycone of cycasin on ocular tissues

Previous Serial Number: None

Principal Investigators: J. T. Schwartz
.. D. L. VerLee

Other Investigator: G. L. Laqueur

Cooperating Unit: Experimental Pathology Branch, NIAMD

Man Years

Total: 1/16
Professional: 1/16
Other: 0

Project Description:

The aglycone of cycasin has been shown to produce tumors in certain experimental animals by investigation in NIAMD. Hepatic, renal, gastric, pulmonary, and other tumors have been found in laboratory animals following administration of the aglycone and other derivatives of the cycad nut.

This study is a pilot investigation of the effects on ocular tissues of intraocular injections of the aglycone in rabbits, dogs, and cats.

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 66 E 1315
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Tonometry survey among American Indian population of Salt River Reservation, Arizona

Previous Serial Number: None

Principal Investigator: J. T. Schwartz

Other Investigators: G. G. Dell'Osso
D. Powers
R. Inglima
R. Banner
H. Hall

Cooperating Units: Biometrics Branch, C&FR, NINDB
Institute for Glaucoma Research, Inc.
Heart Disease Control Program, DCD, BSS

Man Years

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

Project Description:

This is a reservation-wide survey undertaken to obtain descriptive data on the frequency distribution of intraocular pressure among American Indians to augment that obtained in study NDB (CF) - 65 E 1256. Ocular tensions of each subject were measured using the Goldmann applanation tonometer in the sitting and supine positions, the Schiötz tonometer and the Maklakov tonometer as modified by Posner and Inglima. These tonometers include all those ordinarily used for the collection of survey data throughout the world. Measurements of blood pressure by standard methods were obtained on each patient in the sitting and in the supine position.

OBJECTIVES: (1) to obtain, under field survey conditions, a comparison of the performance of Schiötz, Goldmann, and Maklakov (modified) tonometers. (2) to collect additional data on the frequency distribution of intraocular pressure among American Indians. and (3) to compare measurements of ocular pressure and changes in ocular pressure resulting from changes in posture, with measurements of blood pressure obtained with similar changes in posture.

METHODS EMPLOYED

The field investigation was undertaken as a two-and-a-half week mass screening program. Examination facilities were established at two different

sites on the reservation. Data were obtained on approximately 435 examinees of age 25 and over, and are presently being analyzed with regard to the above objectives.

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 66 E 1316
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Hyperacute Allergic Encephalomyelitis. Electron Microscopic Observations.

Previous Serial Number: None.

Principal Investigator: S. Levine

Other Investigators: A. Hirano
H. M. Zimmerman

Cooperating Units: New York Medical College Center for Chronic Disease
Montefiore Hospital and Medical Center

Man Years

Total: 1/32
Professional: 0
Other: 1/32

Project Description:

In the hyperacute form of EAE, an inflammatory exudate of plasma-like fluid, neutrophils and mononuclear leukocytes appeared in the extracellular and subarachnoid spaces of the spinal cord before the advent of parenchymal necrosis. Leukocytes emigrated from vessels through gaps between endothelial cells. Plasma escaped from the lumen through the gap concomitantly with the leukocyte. The primary ultrastructural event in EAE has, however, not yet been identified.

Honors and Awards: None

Publications:

1. Levine, S., Hirano, A., and Zimmerman, H. M.: Hyperacute Allergic Encephalomyelitis. Electron Microscopic Observations. The American Journal of Pathology 47:209-221, 1965.

Serial No. NDB (CF) - 66 E 1317
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Fine Structure of Cerebral Fluid Accumulation. IX. Edema Following Silver Nitrate Implantation.

Previous Serial Number: None.

Principal Investigator: A. Hirano

Other Investigators: H. M. Zimmerman
S. Levine

Cooperating Units: Montefiore Hospital and Medical Center
New York Medical College Center for Chronic Disease

Man Years

Total: 1/2
Professional: 3/8
Other: 1/8

Project Description:

MAJOR FINDINGS

Implants of silver nitrate in the rat brain produced local necrosis and widespread edema of white matter associated with increased permeability of the blood-brain barrier. Electron microscopic examination of the edematous white matter demonstrated pools of moderately electron dense fluid which infiltrated the basement membranes, filled the perivascular spaces and extended into the distended extracellular spaces. The texture of the fluid was indistinguishable from blood plasma. The perivascular ring of astrocytic vascular feet had discontinuities, with direct communication between vascular basement membrane and extracellular fluid. In severely edematous areas, blood vessels floated in pools of edema fluid, completely stripped of attached glial processes. In the distal portion of the involved white matter, myelinated and nonmyelinated nerve fibers were usually well preserved. Occasionally, however, there was separation of outer loops and outer myelin lamellas with extension of edema fluid between the major dense lines of the myelin sheath.

Honors and Awards: None

Publications:

1. Hirano, A., Zimmerman, H. M., and Levine S.: The Fine Structure of Cerebral Fluid Accumulation. IX. Edema Following Silver Nitrate Implantation. The American Journal of Pathology 47:537-548, 1965.

Serial No. NDB (CF) - 66 E 1318

1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Fine Structure of Cerebral Fluid Accumulation. X. A Review of Experimental Edema in White Matter.

Previous Serial Number: NDB (CF) - 65 E 1249.

Principal Investigator: A. Hirano

Other Investigators: H. M. Zimmerman
S. Levine

Cooperating Units: Montefiore Hospital and Medical Center
New York Medical College Center for Chronic Disease

Man Years

Total:	1/2
Professional:	3/8
Other:	1/8

Project Description:

METHODS EMPLOYED

Cryptococcal polysaccharides were implanted into the rat brain, and their selective spread in the white matter was studied with light and electron microscopy, after the whole brain was fixed in situ by perfusion.

MAJOR FINDINGS

The cryptococcal polysaccharides in fluid were observed as electron-dense reticular substance with uranyl acetate or lead hydroxide stains. It originated from the region of the implant and moved a considerable distance in the white matter by way of the extracellular space in the acute stage. The location of fluid changed considerably as the lesion aged. The fluid flow was reduced in three to four days, and the fluid was exclusively localized within the glial cells after one week.

In the early phase of fluid spread, and in areas close to the implant, the extracellular space contained a second type of fluid. The latter was thought to be of hematogenous origin. Fluid of this type was the essential element of the pool of fluid produced in response to the intracerebral implantation of silver nitrate.

This research was presented at the Annual Meeting of the Federation of Western Societies of Neurological Sciences held on Sunday, March 7, 1965 at the Mirador Hotel, Palm Springs, California.

A further study was presented at the Workshop Symposium on Brain Edema, Vienna, September 11-13, 1965 following the 8th International Congress of Neurology.

Honors and Awards: None

Publications:

1. Hirano, A., Zimmerman, H. M., and Levine, S.: The Fine Structure of Cerebral Fluid Accumulation. X. A Review of Experimental Edema in White Matter. In Workshop Symposium on Brain Edema, September 11-13, 1965. Vienna, Austria, Springer-Verlag (in press).

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Determining autoimmunity in chronic neurological diseases
by the use of peripheral lymphocytes

Previous Serial Number: None

Principal Investigators: J. A. Brody
R. Huntley

Other Investigators: None

Cooperating Units: Arctic Health Research Center

Man Years

Total: 1/16
Professional: 1/16
Other: 0

Project Description:

Many chronic diseases are thought to involve hypersensitivity and hyperimmune states. There is a great accumulation of literature which suggests that diseases such as multiple sclerosis, the Guillian-Barre syndrome, slow virus diseases, and degenerative diseases of muscle are the result of a person's nervous tissue becoming sensitized to foreign protein. Recently it was shown that peripheral lymphocytes of a sensitized person will undergo transformation when cultured in the presence of the specific antigenic stimulus. This provides an in vitro method for the study of cellular immunity and hypersensitivity. To date although the lymphocyte transformation has been intensively studied, there is no simple means of quantitating the response. Our investigations have been directed toward growing lymphocytes in microculture and then stimulating them with various antigens. Since we are using microcultures we can have numerous controls and approach the problem of transformation on a statistical basis. We have succeeded in growing lymphocytes in microculture and are currently exploring various chemical and radiographical methods for quantitating our reactions.

Honors and Awards: None

Publications:

1. Brody, J. A. and Huntley, R.: Human lymphocytes cultured in Microplates. Nature 208(5016):1232, 18 December 1965.

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Incidence of ALS and PD among Guamanians living in the States

Previous Serial Number: See also NDB (CF) - 62 E 961

Principal Investigators: J. A. Brody
K. M. Chen

Other Investigators: T. S. Elizan
C. C. Plato

Cooperating Units: None

Man Years

Total:	8/16
Professional:	1/16
Other:	7/16

Project Description:

OBJECTIVES

ALS and PD occur with a phenomenally high incidence on Guam. It is not clear through our studies whether these diseases occur on Guam because of inherited or environmental factors. We are attempting therefore to document the incidence of ALS and PD among Guamanians who are no longer residing on the island. During the initial phase we have canvassed all villages on Guam to learn of people who have migrated away. In most instances those Guamanians who reside in the States maintain ties with their families on Guam. We are now contacting Guamanians living in the States in order to verify our data and possibly to learn of other Stateside dwelling Guamanians.

Should we find that the incidence of ALS and PD is markedly lower among Guamanians living in the States, we would have a clear indication that some exogenous factor present on Guam is the cause of these diseases. If, on the other hand, we find that the incidence does not change, we will be left with the problem of determining whether the etiology of ALS and PD is genetic or some form of exposure very early in life.

Honors and Awards: None

Publications: None

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

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Japanese B Encephalitis studies on Guam

Previous Serial Number: None

Principal Investigators: J. A. Brody
C. J. Gibbs, Jr.
K. M. Chen

Other Investigators: None

Cooperating Units: Laboratory of Slow, Latent, and Temperate Virus
Infections, C&FR, NINDB

Man Years

Total: 9/16
Professional: 1/8
Other: 1/2

Project Description:

A Japanese B encephalitis epidemic occurred on Guam in 1947 and 1948. Subsequently there was evidence that the virus completely disappeared from Guam which is almost unprecedented in ARBO virus ecology. Recently a very efficient mosquito vector of Japanese B encephalitis, the Culex tritaeniorhincus has appeared in large numbers on Guam.

OBJECTIVES

Our studies are divided into three major areas. (1) The long-term neurological and psychological effects of Japanese B encephalitis on individuals who suffered from clinical illness and particularly among individuals who suffered from subclinical or inapparent infections. (2) Serological studies of Japanese B encephalitis antibodies to determine their persistence and to evaluate the most sensitive methods for detection. (3) To determine if the virus completely disappeared on Guam and if not to study the ecology of this agent in an apparently hostile environment.

METHODS EMPLOYED

A large number of sera from humans and animals are being screened against Japanese B encephalitis virus and other viruses of the Group B complex. We have limited evidence that a Group B ARBO virus is still present on Guam.

Honors and Awards: None

Publications: None

Serial No. NDB (CF) - 66 E 1322
1. Collaborative & Field Research
2. Epidemiology Branch
3. Agana, Guam

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Ear infections and hearing loss on Guam

Previous Serial Number: None

Principal Investigators: K. M. Chen
J. A. Brody

Other Investigator: E. L. Eagles

Cooperating Units: None

Man Years

Total: 9/16
Professional: 1/16
Other: 8/16

Project Description:

OBJECTIVES AND METHODS EMPLOYED

As a contribution to geographic pathology we are investigating the incidence of hearing loss and acute otitis media among Guamanians. Similar studies have been conducted in Alaska and through the National Health Survey in the States. Our data will be comparable to these studies. We are conducting hearing tests on all school children in the villages of Yona, Talofofo, and Merizo. In addition for one year we are doing a prospective study on all children born since 1961 in these three villages to determine the incidence of otitis media.

The hearing testing has been completed and the field studies of otitis media are in their sixth month. In general we are encountering very little pathology.

Honors and Awards: None

Publications: None

ANNUAL REPORT
For Period July 1, 1965 through June 30, 1966
Perinatal Research Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

I. GENERAL STATEMENT

SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS

The analysis of data from the Study has, for the most part, covered in scope thus far, associations between events and factors observed during pregnancy, labor and delivery, and observations of outcome made on the growing child up to one year of age such as perinatal death, low birthweight, neonatal morbidity, indices of mental, gross and fine motor development at 8 months of age, as well as gross neurological damage at one year of age. Perinatal insult during the first trimester of pregnancy (rubella, toxoplasmosis, cytomegalovirus infections) has been related to outcome; in addition, relationships of maternal medical conditions such as diabetes, convulsive disorders, organic heart disease, bronchial asthma to outcome have been investigated; prior pregnancy loss, demographic variables (income, occupation, education, race, maternal age, parity, etc.) have also been related to outcome; obstetrical conditions including uterine dysfunction, uterine dystocia, cesarean section, premature rupture of the membranes, early symptomatology, incompetent cervix, have been correlated with birthweight, Apgar, bilirubin level; neuropathological and EEG studies on children have been studied in relation to prenatal and subsequent events; the predictive value of specific findings in the mother has been explored in relation to birthweight and the predictive value of a number of prenatal and postnatal factors in relation to mental, motor and physical development in the first year of life; specific pediatric conditions such as hyaline membrane disease, neonatal pneumonia, congenital heart disease, primary endocardial fibroelastosis, mental retardation, cerebral palsy and neonatal seizures have been studied in relationship to prenatal and postnatal factors. With the utilization of data at various chronological endpoints, e.g., 3 years, 4 years and 7 years, it will be possible to extend the scope of prenatal associations to encompass neurological deficit more clearly and specifically defined, as well as subtle neurological damage which cannot be diagnosed early and whose diagnosis is possible only with a multidisciplinary approach utilizing parameters from pediatric-neurology, psychology, speech, language and hearing and socioeconomics.

In addition to many publications and presentations on a number of occasions, the Project has itself held two Scientific Meetings so far, one in 1963 and one in 1966. Much of this work has been referred to in the separate reports by the various Sections.

Presentations and Publications by the Chief (In addition to listings in Individual Project Reports)

Mid-Eastern Regional Meeting, AAMD, Marriott Twin Bridges Hotel, Washington, D. C., October 29, 1965. Presentation on "Present Status of the Collaborative Project".

University of Arkansas, Little Rock, Arkansas, February 10, 1966. Participation in Post-Graduate Seminar on Perinatal Problems in Mental Retardation.

International Symposium on Mental Retardation, Dublin, Ireland, March 30, 1966. Presented paper entitled "Obstetrical Complications and Mental Handicap".

Conference on Prevention of Mental Retardation Through Control of Infectious Diseases, Cherry Hill, Pa., June 9-11, 1966. Present position paper entitled "The Role of Infectious Diseases in Mental Retardation".

Berendes, H. W.: Chapter 5. The Structure and Scope of the Collaborative Project on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood. Research Methodology and Needs in Perinatal Studies. Springfield, Illinois, Charles C. Thomas, 1966.

ADMINISTRATIVE ACCOMPLISHMENTS

The Project produced to date, 250 frequency tabulations and listings, 75 sets of cross tabulations and 40 special study tabulations. These three packages constituted the major portion of PRB feedback to institutions on the masses of data collected and available.

The total number of women registered in the Study as of March 31, 1966 is 59,095; 52,417 have been delivered; 37,069 births have been studied at one year of age; 12,109 (excludes cases of Boston and Pennsylvania receiving screening examinations only - these would be about 9,000 additional cases) at three years of age; 12,281 at four years of age. On December 31, 1965, the obstetrical intake of the current phase of the study terminated.

The available data derived from 1,630,418 Study forms have been transferred into 3 3/4 million punch cards, 111 card formats and converted into computer tape files.

The backlog in editing or coding is at a normal level and consists of 101,206 forms in coding and 145,084 in editing. A total of 29,220 forms are waiting to be sent to punch. The total number of brain specimens available for study is 1,066. The total number of virology specimens available for study is 277,031.

In line with the growing interest in devising an explicit plan for data analysis, the production of a cohort-based data file becomes paramount. PRB is attempting to complete its file on all registrants through 1964. Final registrants through 1965 will be added next year. This will hopefully have terminated by August 1966 and the current data file updated by October 1966, at which time this package of data will be utilized for subsequent analyses. To tap data on one cohort is more efficient, economical and productive than segmented analysis of a variety of cohorts. Such a data file will then be updated annually.

The problem of maintaining adequate control of data collection remains alive especially with the introduction of the 7 Year Battery of Tests. Elaborating on previous attempts, the creation of a unit is contemplated within PRB to be the focal point of such an activity. Based on adequate pretesting of this battery, a plan will be devised to maintain regular annual check-up on the completeness and consistency of the data collected and to safeguard, in addition, the poolability of data despite personnel turnover in data collectors at the various institutions.

The availability of computer facilities via contract or other sources has become of less concern than in the past. With the future development of NIH computer facilities at Computation and Data Processing Branch, the addition of programmers on PRB staff and completing the updating of the cohort-based data file, our computer needs and resources for data analysis will be met.

An explicit plan for analyzing the masses of data from the Project is again being seriously considered. The five previous attempts (Ad hoc Hypotheses Committee of 1962, the Program Committee of the First Scientific Meeting of 1963, the Data Analysis Task Forces of 1964, the Executive Board since 1965 and the Program Committee of the Second Scientific Meeting of 1966) have further emphasized the need of PRB for informal consultation with appropriate experts on a multidiscipline basis to come up with a plan to delineate the scope of major study proposals and be flexible enough to allow for a variety of specific individual study interests.

The 7 Year Battery of Tests ($7-7\frac{1}{2} + 3$ months), currently being finalized, is being more thoroughly pretested than previous tests and a system for its quality control is being devised. The components of this battery are: (a) Pediatric-Neurological Exam (almost adopted) — Pediatric-Neurological Manual (almost adopted), (b) Vision Screening Test (already adopted), (c) Psychology Tests (already adopted), (d) Socioeconomic Tests or FHH-9 (already adopted), (e) The 8 Year SLH Instrument (bordering the 7 year battery endpoint) is still being designed and will incorporate the Pure Tone Screening Test, (f) The Diagnostic Summary encompassing final multidisciplinary diagnosis of child based on all available post-natal data is currently being designed.

The Publications Review Board continues to perform efficiently. It has reviewed 134 manuscripts and/or abstracts since its start in September 1962

(83 this FY '66) and has, in cooperation with the Executive Board, prepared the program and agenda of the Second Scientific Meeting.

The Project Directors' Committee, as well as the Executive Board, (meeting separately every two months) continues to function as a mechanism for decision-making on a Project-wide basis. PRB, as an integral part of these two units, continues to prepare their agendas, schedule their meetings, write up their meeting summaries, and implement as well as coordinate their decisions in a sustained manner to insure cooperation with the Collaborating Institutions and liaisonship with PRC and the Extramural Program of NINDB. However, this pattern of decision-making deserves revision in order to make possible a more effective scientific and administrative leadership.

Mr. Frank Barden, a recognized historian and science writer, is writing the official statement on the history of this complex Project. It is anticipated that this document will be finished by the end of FY '67.

PRB has become a unit which, in addition to its varied roles and functions, has succeeded in developing workable retrieval systems for literature (4,975 selected reprints), data and specimens. Data can be retrieved from protocols, inventory files, data files, computer tapes, punch cards, etc.

Considerable time was spent on the preparation of background information for use by the special Gordon Ad Hoc Review Committee. PRB has been site-visited by the Committee at large and every Collaborating Institution similarly visited by an outside task force on behalf of the Committee. The final report will be submitted to Dr. Shannon by May 31, 1966.

II. PROBLEMS

PRB and PRC

The relationship between PRB and PRC is courteous but not as effective as can be. PRB communicates with PRC routinely on changes in program development and upon request, but PRC decisions are implemented across institutions with program implications discovered by PRB after the fact.

PRB and Principal Investigators and Project Directors

The interest of the Principal Investigators in the Project has been formal and remote. Various attempts to kindle more interest have failed. Decision-making in administrative and professional matters continues to be informal, non-focalized and non-binding. In the interest of fostering the spirit of collaboration, PRB explicitly encourages participation by the Project personnel in data analysis. The sense of commitment to the research endeavor within the scope of jointly established procedures and agreements leaves room for improvement and is a challenge to administrative creativity. No administrative mechanism can be devised as a substitute for personal initiative. Despite the operating mechanism

of PDC and the Executive Board, the assignment still remains for PRB to activate and keep afresh the scientific and collaborative interest of the personnel at all levels.

Abnormal EEG

In 1964, Dr. Peter Kellaway produced, with PRC support, an EEG Primer on the normal neonate. The Primer on abnormal EEG is still expected. PRB is currently active with EEG specialists in devising a plan for future ancillary EEG studies.

12 Year Follow-Up

Despite the fact that the PRC and the Project Directors' Committee have jointly and independently expressed, on a number of occasions, their interest in following the Project children to age 12, a decision on this issue is yet to be made pending the submission of the report of the special Gordon Ad Hoc Review Committee and the clarification of certain NIH policies regarding budgeting of research programs and research development. Two of the Collaborating Institutions (Oregon and Minnesota) have already received financial help from the Office of Education to pilot a study for assessing school performance as an aid to diagnosing neurological deficits, on a design previously made to explore for the Project as a whole, the feasibility of such a follow-up at all. NIH review mechanism has disapproved its grant application but the Office of Education has accepted it and expressed its desire to fund similar requests from other collaborators. PRB is contacting the Office of Education to insure coordination of such requests for potential use by the Project.

Budget Cut in FY '67

The Project Directors' Committee, as well as PRB have been concerned about the pending reduction in the budget for FY '67. Several meetings and discussions were held and a number of suggestions and recommendations made. As it is difficult to assess on a cost-performance basis the expense incurred by various facets of the Project (per patient or by phase or per protocol) it is almost impossible to recommend intelligently a budget reduction which will be fairly distributed across institutions and of minimum impact on the quality of research. PRB has been requested to cooperate with the NINDB Extramural Program in preparing a budget for FY '67, less in amount by \$650,000 than the proposed budget (natural attrition in expenses due to phasing out of OB intake notwithstanding).

Sample Maintenance

The follow-up of the sample under study throughout the various endpoints envisaged until age 7 is also a problem of serious concern. With the production and updating of the cohort-based data file, the magnitude of sample attrition can be realistically assessed. It is not enough to know the proportion of the sample who did not appear on schedule for a specific examination, or its general characteristics, but it is

perhaps more important to know the proportion out of this "delinquent" sample which can be located, how to contact it and what specific information to gather on it. It has been difficult thus far (a) to know in a uniform and standardized fashion the magnitude of the sample maintained and/or lost, by the institution and for the Project as a whole; (b) to establish the role of PRB and of the Collaborating Institutions in the follow-up. Now that the OB intake has ceased and the Project children are maturing during FY '67 into age 7, the attrition rate should and could be determined and depending on its size, plans would be devised to reduce it through school and institutional follow-up, etc....and to gather maximum research data on the sample salvaged.

Virology, Pathology and Neuropathology

Ever since the beginning of this Study, the centralized collection of these specimens has been intended with the sole objective of getting them adequately processed and properly analyzed on a concurrent basis. A number of administrative and professional attempts have been tried thus far to meet this objective but have not as yet been fully rewarding. Nevertheless, a specific plan will soon emerge, based on the experience gained so far, which will outline realistically the resources (personnel, antigens, space and equipment) required to analyze the backlog of brain specimens, as well as of the virology specimens (before deterioration) for selected specific studies which might contribute to diagnosis of neurological deficits in various infants.

III. PROPOSED FUTURE OBJECTIVES

Several attempts have been made to outline plans for the future. These have involved Principal Investigators, Project Directors, a number of consultants and PRB Section Heads. Budget limitations, changes in NIH fiscal policy, and the expectation of the report by Gordon's Ad Hoc Review Committee have dampened further efforts to come out with concrete plans. It is expected that final decision, regarding at least the follow-up of children to age 12, will soon be made early in FY '67.

A Third Scientific Meeting is contemplated in the fall of 1967, after which a synthetic review of all the up-to-date findings from the Study will be made to formulate the basis for intelligent and concrete research planning. The use of contracts rather than grants, animal experiments, controlled clinical studies, the follow-up of selected high-risk pregnancies, etc., will all fall in proper perspective within such an overall evolving plan.

A. SECTION ON OBSTETRICS
Report for the Period July 1, 1965 through June 30, 1966

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS

A. Scientific Activities

Drug Abstraction

The problems involved in the abstraction of drug information from a nonstructured record, which was not specifically designed for the collection of pharmacologic information, were discussed in two sessions with Dr. S. Katsh, consultant, and professor of pharmacology at the University of Colorado Medical School. Preliminary information shows that the number of drugs reported during pregnancy depends on several interrelated factors: the time of registration of the mother for prenatal care, race, age, and parity, the prior medical history, intercurrent epidemic diseases, and the pharmacologic-therapeutic concepts of the obstetric staff of the individual collaborative institutions. Such differences have to be carefully evaluated in all future drug studies. It was suggested that statistics on possible effects of drugs reported during pregnancy should not only be designed for the evaluation of a single, specified drug, but comparisons should always be made between a number of different drugs prescribed for the same diagnostic category of clinical conditions. For several drugs (antihistamines, steroid hormones, sedatives, and anesthetics) pilot studies have been initiated to evaluate the significance of the different factors specified.

Special Studies (See individual project reports)

1. Maternal Deaths in the Collaborative Project.
2. Evaluation of the Possible Teratogenic Effect of Antihistaminic Drugs Taken During Pregnancy.
3. Estrus, Ovulation and Menstruation; A Critical Historical Review.
4. The Length of the Premenstrual Phase by Age of Women.
5. Obstetric Factors in Twin Pregnancies.
6. Down's Syndrome and the Incidence of Infectious Hepatitis During Pregnancy.
7. Distribution of Abortions by Chronologic and Gynecologic Age of the Gravida.
8. The Span of Human Gestation.

9. Ourselves Unborn.

Papers 1, 3, and 4 were read at scientific meetings and have been accepted for publication. Paper 9 was compiled as a selected bibliography of Dr. G. W. Corner's publications on the anatomy and physiology of primate reproduction. It was distributed at a NIH lecture given by Dr. Corner on the occasion of the 50th anniversary of his initial study on the corpus luteum.

Small sample tests have been studied for a comparative evaluation of the differential effects of maternal chronologic versus gynecologic age on the course and outcome of pregnancy. The preliminary findings are very interesting and will be used in the final design of the study based on the completed sample of all obstetric study cases.

B. Administrative Activities

Editing and coding of obstetric forms: OB-2, 3, 4, 5, 6, 7, 8, 9, 10, and 42; editing of OB-42, 43, 51, 55, and 60; and abstracting of OB-32, 33, 15, and labor and delivery drugs. Correspondence with collaborating institutions for clarification and amplification of information requested in the study protocol. Editing of incomplete and backlog cases (2,450) identified through the record file inventory.

Assistance to collaborators and perinatal research staff in the design and preparation of research papers. Consultation and cooperation with other agencies and individuals on specific problems in the perinatal research area: National Institute of Child Health and Human Development, National Center for Health Statistics, Division of Vital Statistics, Food and Drug Administration, Margaret Sanger Research Bureau for Planned Parenthood, Current Medical Terminology (AMA), Paris Research Group on psychoprophylactic preparation for labor and delivery.

Professional staff seminars:

Dr. N. Eastman discussed the classification of maternal deaths.

Dr. L. Hellman reported on technical and statistical problems involved in perinatal research studies.

Dr. D. Baird talked about socio-economic factors involved in the course and outcome of pregnancy.

The Section was also host to Dr. G. W. Corner, former director of the Department of Embryology, Carnegie Institution of Washington.

Staff members lectured on the physiology of the menstrual cycle, the population explosion, oral contraceptives, and exchange transfusion of the erythroblastotic fetus. A scientific film on the incompetent cervix was also shown.

II. PROBLEMS

With the termination of the obstetric data collection in the Collaborative Project, the routine operation of the Section (coding, editing and abstracting of obstetric records) will be achieved, after the record files have been completed. The Section is now looking forward to making use of the experience and knowledge of the project data gained during the past years for a thorough analysis of the information collected. The function of the Section will therefore shift into three main areas:

1. special studies involving small numbers of cases in which complete case review and hand abstraction is required;
2. pilot studies on cases selected at random to identify variables and ranges, to test definitions and codes, and peculiarities of the hospitals in reporting observations. The results of such pilot studies will serve as a basis for the design of comprehensive studies for data retrieval by computer;
3. systematic analysis of the completed obstetric data following a structured, logical program.

III. REMARKS

So far, the obstetricians from the collaborating institutions have not been deeply involved in the complete analysis of the obstetric data collected. All efforts will be made to engage the collaborators in all phases of the study of the obstetric data.

Administrative possibilities must be explored to invite obstetric coordinators from collaborating institutions to join the Section on specified assignments for periods of from three to four weeks.

Personnel and space: no change.

B. SECTION ON PEDIATRIC NEUROLOGY
Report for Period July 1, 1965 through June 30, 1966

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS:

- A. The development of the Seven-Year Pediatric Neurologic Examination has continued, and in October of 1965, the examination form and manual were presented to the Project Directors as a pretest examination and were approved. The Section prepared the examination for Study-wide pretesting and it was distributed to all projects in January 1966. After each Project Director and his respective staff have pretested the examination, the form and manual will be revised in accordance with their recommendations, and a final protocol will be readied. The Seven-Year Examination has been designed by a group of pediatric neurologists to be a thorough and detailed physical examination for the identification of both neurological and non-neurological abnormalities in Study children between the ages of seven years, and seven years-six months. This examination represents the medical culmination of the lengthy and detailed longitudinal study. A great proportion of the value of the data already collected on each Study patient will depend on the accurate and specific identification of abnormality at seven years. The pretest form contains some 127 items recorded on 10 pages, and is accompanied by a 24-page instruction manual. By the end of this fiscal year, the Project Directors will have had experience with pretesting the examination and will be ready to finalize it. The Project Directors have been encouraged to perform examinations and re-examinations by different medical personnel on the same Study child in order to check interobserver reliability. However, the problem has always existed as to a means of measuring differences between institutions. To estimate this, a special pretest was completed at the Children's Hospital, Washington, D.C. Six neurologically abnormal children were examined, each by several pediatric neurologists from different Study hospitals. Results of this special pretest by examiners from different Study institutions examining the same patient will be incorporated into the general Study-wide pretest examination report.
- B. During the development of the Seven-Year Pediatric-Neurologic Examination, it became apparent that a standardized visual screening test would be needed. A modification of the Massachusetts Visual Screening Test was selected for Study pretesting. Within this eye-screening examination would be a test for visual acuity with and without glasses, a check of excessive farsight, a check of muscle balance, and a test of color vision. Study children failing this screening test will be sent to an ophthalmologist for examination. A Study form was devised for recording the ophthalmologist's findings. This form requests specific information that will be needed in the evaluation of Study children with visual abnormalities. The Section participated in a Study-wide workshop held at Johns Hopkins on November 5, 1965, on the introduction of the visual screening pretest.

- C. Work has continued on the development of the Seven-Year Diagnostic Summary. This form will be a precoded summary on which diagnoses and events occurring from 60 weeks of age to the seven-year level will be recorded. This summary will be completed at the collaborating institution. The diagnoses and historical events of importance will be recorded on this precoded form and will be readily available for data analysis. The section for recording neurological diagnosis was considered the most difficult, and the Study consultants, after much discussion and deliberation, have agreed on organization and definition of this part of the summary.
- D. In preparation for the Scientific Session, a Basic Document concerning the Project's development was prepared, under the editorship of this Section. Following the Second Scientific Session of the Collaborative Project, the Section will compile and prepare a compendium of the meeting. This compendium will consist of summaries of papers presented and discussion following the presentations at the Scientific Session. This reference document will be available for distribution throughout the Project.
- E. Section members have presented papers in a number of scientific meetings.
1. "Relationship Between Heart and Great Vessel Anomalies and Selected Perinatal Factors", 93rd Annual Meeting, American Public Health Association, October 18-22, 1965.
 2. "Prognostic Significance at One Year of Age of Early Signs of Neurological Abnormality for Premature Infants with Birthweights of 1000-2000 Grams", Eleventh International Congress of Pediatrics, Tokyo, Japan, November 7-13, 1965.
 3. "Predictability of Neonatal Defects by Statistical Methods", Tenth Anniversary Congress of the Illinois Division for Maternal and Child Health, Peoria, Illinois, February 16-18, 1966.
 4. "Perinatal Factors and Congenital Heart Disease", The Teratology Society Sixth Annual Meeting, Corpus Christi, Texas, May 24-28, 1966.

In addition, this Section made several presentations at the Second Scientific Meeting of the Collaborative Project held in the Statler-Hilton Hotel, Washington, D.C., March 24 and 25, 1966. Papers presented there were entitled:

1. "Perinatal Factors in Congenital Heart Disease."
2. "Effects on the Child of Convulsive Disorder in the Mother, Progress Report."
3. "Mortality and Morbidity Among Infants Weighing 1000-2000 Grams."

Members of the Section have participated as co-authors on a number of other papers presented at various scientific meetings.

- F. The Section on Pediatric Neurology has compiled two reviews in various steps towards publication. One review is of the hypothesis concerning the continuum of reproductive casualty. The other is a review of the Apgar mortality and morbidity data thus far presented by the Project.
- G. Intake of pediatric protocols has continued and as of the end of March 1966, some 835,419 forms have been received. During the last year coding and data processing have continued for the PED-1, PED-2, PED-4, PED-5, PED-6, and PED-8, in addition to the PED-10 and PED-11. New coding structures were designed for PED-12, working with the Section on Statistical Procedures.

During this fiscal year, the backlog of PED-3 has been coded, in addition to the PED-14 and the PED-12. The coding and the processing of the PED-12 represent a milestone in the progress of the Collaborative Project in that now specific diagnoses at one year of age are available for data analysis. This gives the collaborators a diagnostic endpoint on which to base many of their studies. As the obstetrical intake of this Study was discontinued as of January 1, 1966, there will be a diminished rate of intake of certain pediatric forms during this period. The last of the infants should be born into the Study sometime in early fiscal 1967, so the pediatric forms PED-1 through PED-8 will be discontinued at that time. In addition, within four months the PED-10 or four-month examination will be discontinued. This represents a marked decrease in data input for the Section. The one-year examination, PED-11, will discontinue late in fiscal 1967 or early fiscal 1968. However, some 3,000 Study patients will reach the age of seven years in the first half of fiscal 1967, so as the neonatal and four-month series of examinations are discontinued and their input ceases, the input of seven-year examinations will begin. These forms will need detailed review by the Section's editing unit and design of coding structures. Workshops will be conducted with the collaborating institutions to standardize data reporting and to clarify and solve specific problems encountered with the seven-year examination and summary.

- H. This Section has continued to participate in a number of projects. Topics which are now under study are:
 - 1. Apgar Scores.
 - 2. Retrospective Georgetown Study.
 - 3. Study of Socioeconomic, Medical and Genetic Factors in Major Congenital Malformations.
 - 4. Hyaline Membrane Disease.
 - 5. Maternal Rubella and Pregnancy Outcome.

6. Toxoplasmosis: Serological and Clinical Studies.
7. Early Signs of Neurological Abnormality in Newborn Infants.
8. An Investigation into the Relationship Between Congenital Heart and Great Vessel Anomalies and Selected Perinatal Factors as Recorded in the Collaborative Perinatal Research Project.
9. Maternal Seizures and Subsequent Development of the Child.
10. Sudden Unexpected Death.
11. Prematurity.

In addition, this Section continues to serve and assist collaborators in their studies, both as consultants and in terms of actual participants for case study reviews.

II. PROBLEMS:

During fiscal 1966, the entire backlog of PED-12 has been coded and made available for data analysis. This protocol was introduced late in the Study and the Section received a large backlog over an 18-month period. At the present time, about 25% of these forms have been reviewed by our editing unit and the narrative material abstracted. In order to obtain the information on the other 75%, it will be necessary to complete the Pediatric editing of these forms even if this is limited to abstracting the narrative information. Present staffing of the Section does not allow for a rapid solution to this problem.

The proportion of Study children who are located and actually return for their seven-year battery is of extreme importance. Rapid reporting to the Section of the successful completion of the seven-year examinations must be made so that intensified sample recovery procedures can be instituted should the recovery proportion prove to be low. A procedure should be initiated whereby each collaborative institution notifies the PRB immediately on completion of the seven-year examination. The PRB would then be able to quickly evaluate the number of registered children successfully followed up by each institution and Study-wide. If the PRB must wait for the arrival of Study examinations which are often received months after the examination, an effective evaluation of this problem may be thwarted.

III. PROPOSED FUTURE OBJECTIVES:

The Section anticipates a smooth introduction of the seven-year examinations and diagnostic summary. Workshops and site visits with the collaborating institutions will be held to facilitate this introduction. The influx of these new examinations will offset the decrease in the neonatal series of protocols. Input of PED-20's, PED-29's, PED-11's, and PED-12's

will continue at about the same rate in fiscal 1967. The Section must be prepared for rapid and intense effort should there be indications that sample maintenance has failed to insure follow-up of a high proportion of Study children at seven years. The Section plans to participate heavily in data analysis, not only on studies within the Section, but also with various members of our collaborating institutions. The Section will continue to assist and encourage our collaborators in the area of data analysis.

IV. REMARKS:

Personnel: The Section has projected a need for five additions to the Section staff in fiscal year 1968. This includes three professional positions, based on the anticipated need for qualified professional personnel to develop and carry out data analysis, and two clerk-typists to handle the anticipated additional typing and clerical duties. Space will be needed to house this additional personnel.

C. SECTION ON BEHAVIORAL SCIENCES
Report for period July 1, 1965 through June 30, 1966

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS

The 8-Month Psychological Examination Data from the 8-Month Psychological Examination is being used, with increasing frequency, in studies initiated by COLR obstetricians, pediatricians, and psychologists. Current emphasis of most of these studies is on the relationship or association of various types of perinatal insult to scores achieved on the mental and motor scales of children at 8 months of age. At the recent Scientific Symposium of COLR studies, five studies in which this examination played a major role, were authored and presented by COLR psychologist and COLR physician-psychologist teams.

The 3-Year Speech, Language and Hearing Examination Editorial evaluation of incoming scoring records indicates that data collection is proceeding according to manual and on schedule. Close coordination in the correction of errors and discrepancies continues to be exercised. Currently, variations among Collaborative Institutions with regard to incidence rates of normal, suspect and abnormal cases on this examination are a source of concern and are being carefully studied.

The 4-Year Psychological Examination Editorial evaluation of incoming 4-year examination records continues to be exercised, indicating that no further changes in test administration procedures were required. The examination is fully operational and coding and processing of data is proceeding without major obstructions or delay. Data from the 4-Year Psychological Examination was referred to in two studies presented by psychologists at the recent Scientific Symposium, despite the fact that this examination has been implemented fairly recently in the COLR study. Currently, variations among Collaborative Institutions with regard to incidence rates of normal, suspect and abnormal cases on this examination are a source of concern and are being carefully studied.

Maternal Intelligence SRA data have been collected since fiscal year '65. To date, one research study conducted by psychologists at the University of Oregon and presented at the recent Collaborative Study Scientific Symposium involved use of this test. As the COLR Study progresses, particularly in investigations of the association and influence of maternal intelligence upon the intelligence of the Study children this test will probably receive greater emphasis.

The 7-Year Psychological Battery Coordination with the COLR Pediatric-Neurological and Speech, Language and Hearing subcommittees resulted in additional refinement and avoidance of duplicating elements in this examination. Trial administration of the examination involving the collection of 129 cases from 12 Collaborative Institutions was completed.

Operational reports of the results of the trial administration were approved by the COLR psychology subcommittee, the COLR committee of chief psychologists. The selection of tests for the 7-Year Psychological Examination was completed by the committee of chief psychologists, was presented to Project Directors for approval, and was adopted as a Project protocol. The 7-Year Psychological Examination battery consists of the following tests: Wechsler Intelligence Scales for Children, Bender Visual Motor Gestalt Test, Wide Range Achievement Test, Goodenough-Harris Drawing Test, Auditory Vocal Association Test of the Illinois Test of Psycholinguistic Abilities and Tactile Finger Recognition Test.

The 8-Year Speech, Language and Hearing Examination In several meetings during this fiscal year, the COLR speech, language and hearing subcommittee devoted their efforts to clarification of the rationale underlying the objectives of the examination and the construction and/or procurement of reliable, valid and standardized tests. A draft examination battery is in the process of being completed; a preliminary draft of the examination was presented at a special meeting with eleven expert consultants who were not associated with the COLR Study. The recommendations and criticisms of the latter group are being considered carefully. Contact with several of these "outside" consultants is being maintained for further advice or counsel.

Evaluation of Performance and Data Collection This section routinely carried out editorial evaluations, coding, and processing of data reviewed on the 8-month, 3-year and 4-year examinations. Collaborative Institutions were contacted and advised of corrections in the data wherever applicable. The specific details of data intake and processing are as follows:

The 8-Month Psychological Examination As of March 31, 1966 there were 34,390 8-Month Psychological Examination forms received by the Section since the inception of formal data collection in the Collaborative Study. Of this number 31,806 forms had been sent to punch; punching and return of these cases to the section's files are pretty much completed. The planned backlog of forms awaiting processing prior to being sent to punch, as of March 31, 1966 is 2,584. During the period of July 1, 1965 through March 31, 1966 5,437 8-month examinations were received at PRB and a projected total of approximately 7,200 records is anticipated by June 30, 1966. The general quality of incoming records is very good and there are virtually no problems concerning modification or revision or scoring contemplated for this examination.

The 3-Year Speech, Language and Hearing Examination As of March 31, 1966, 11,575 3-year examination forms had been received by the Section since the implementation of the 3-year examination. Of this number, 5,086 had been sent to punch. The backlog of forms to be processed is 6,489 which will subsequently be reduced pending termination of collection of data on the 8-Month Psychological Examination. During the period July 31, 1965 through March 31, 1966,

2,973 3-year examinations were received by the Section; the anticipated number of forms for the complete period, July 1, 1965 through June 30, 1966 (the current reporting period) is approximately 4,000.

The 4-Year Psychological Examination As of March 31, 1966, a total of 11,882 4-year examinations had been received at the Section since the inception of the 4-year examination. Of this number, 7,839 had been sent to punch. The backlog of forms awaiting coding and processing is 4043. During the period July 1, 1965 through March 31, 1966, 3,953 4-year examinations were received; the projected number of forms to be received for the period July 1, 1965 through June 30, 1966 is approximately 5,300.

Special Studies (1) Serial No. NDB-(CF)-63 PR/BS 1169, "Antecedents and Correlates of Retarded Motor Development". This study was withdrawn because of the departure of Dr. Shectman, Principal Investigator, from the Collaborative Study and her subsequent unavailability.

(2) "Evaluation of the Gross and Fine Motor Functions of Children Eight-Months of Age in Relation to the Neurological Results of the Examination of the Children at One Year of Age", Serial No. NDB-(CF)-63 PR/BS 1166. This study has not been processed as yet because of low priority and delay in acquiring computer time and facilities.

(3) "Indexes of Socio-Economic Status of Study Mothers in Relation to Outcomes of Pregnancy", Serial No. NDB-(CF)-63 PR/BS 1168. This study was withdrawn since the essential data were collected and reported by the Section on Epidemiology and Genetics.

(4) "Explorative Study for the use of a Speech and Language Screening Examination for 3-year old children in the Home Situation", Serial No. NDB-(CF)- PR/BS 1167. This study is still being processed by Dr. Fiedler in Collaboration with a pediatrician at Boston Children's Medical Center; the study will probably be completed by early fiscal year '67.

(5) "An Evaluation of the Offspring of Unwed Mothers in Terms of the Complications of Birth and Delivery, Results on the 4-Month Examination, 8-Month Examination and One-Year Examination, as Compared to a Matched Group of Married Mothers and Their Offspring", Serial No. NDB-(CF)-63 PR/BS 1171. This project was withdrawn since data bearing on this problem were collected and presented by the Section on Epidemiology and Genetics.

II. PROBLEMS

A. Recruitment of Professional Personnel

Efforts to recruit a speech, language and hearing specialist for the Section on Behavioral Sciences were continued, but without success.

An additional developmental psychologist is also required but as yet has not been recruited. The nature of the responsibilities and the relatively low remuneration for these positions may be responsible for difficulty in recruitment.

Inspectional site visits as an additional measure for quality control and evaluation of the data have not been accomplished adequately because of the shortage of professional personnel in the Section.

B. Space Facilities

With the expected influx of records on the 7-Year Psychological Examination and the 8-Year Speech, Language and Hearing Examination additional filing cabinets are required.

III. PROPOSED FUTURE OBJECTIVES

1. One of the uses of the 7-Year Psychological Battery is the identification of specific learning problems of those children considered to be mildly retarded, that is with I.Q.'s ranging from 50 to 75 especially upon entrance into school at grade 1, 2 or 3. The Wide Range Achievement Test, as part of the 7-year, provides information of value in regard to the academic performance, at least initially, of children in the Study identified as being deviant, neurologically and psychologically.
2. The 8-year examination, among other aspects of the child's communicative behavior, will furnish identification of specific problems children have (reading or writing deficiencies, dyslexias, dysgraphias etc.) the relationship these problems may bear to data collected on these children during the first 7 years of the Project's existence will be explored.
3. Identification of specific deficiencies uncovered in earlier speech, language and hearing examinations in relation to the psychological data obtained on the children as well as performance on the 8-Year Speech, Language and Hearing Examination. Additional studies would deal with the relationship or association of various kinds of perinatal insult to the psychological and speech, language and hearing examinations.
4. Research studies designed to determine the relationship between performance on the 8-Month Psychological Examination in relation to the performance of the same child on the 3-Year Speech, Language and Hearing Examination as well as the 4-Year Psychological Examination. An efficient predictor of future problems and difficulties in the child's life, e.g.; the 8-Month Psychological Examination, would be of great benefit in early identification and treatment of aberrant children.

IV. REMARKS

Personnel

With the development and implementation of the 7-Year Psychological and the 8-Year Speech, Language and Hearing Examinations, it is anticipated that at least one perhaps two additional statistical clerks will be required for processing of the data on to punch cards and subsequently on to tape. In addition one professional speech, language and hearing specialist and a developmental psychologist may also be required to handle the additional workload imposed by the implementation of these two examinations and for associated research and quality control activities. The need for professional personnel to carry out the special site visits will also be increased since this is a routine on-going operation.

Space

Additional space is also required for files and records in terms of the increased workload.

D. SECTION ON INFECTIOUS DISEASES
Report for period July 1, 1965 through June 30, 1966

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACHIEVEMENTS

LARGE-SCALE SEROLOGICAL TESTING

Three types of studies are being conducted:

1. Broad serological approaches to identify the viruses which are infecting the study population.

Over 68% of the study patients were found to be immune to rubella during the nationwide 1964 epidemic. A total of 2.6% had clinical rubella (serologically confirmed) and 1.2% had sub-clinical infections.

Over 35% of the study population has had experience with Toxoplasmosis. This was correlated with age of the women and race. There is some indication of an association between frequency of antibody and housing density as well as poor mental and motor performances.

2. Intensive studies of important infectious agents.

Intensive studies of rubella reveal acute rubella in the mother and chronic infection of the fetus and child. These children are infectious.

Cytomegaloviruses were isolated from 7 of 200 pregnant women studied at delivery. It was also isolated from 1 of the children of these 7 women.

3. Selected studies of pregnancies with abnormal outcomes and matched controls.

Selected studies of abnormal and matched controls have revealed several viruses which occur in significant frequency in patients with abnormal pregnancy outcomes. The Coxsackie B viruses are being studied with particular interest.

Research on the development of antigens for use in the study of viral infections of pregnant women in the Collaborative Perinatal Research Study has resulted in the production of prototype quantities of 120 antigens.

A total of over 62,855 blood samples were submitted from July 1, 1965 through March 31, 1966, making a total of 326,037 on hand. The quality and quantity of the samples were over 90% satisfactory. This includes sera received from the study under the direction of Dr. Margaret Jones of the University of Southern California in Los Angeles and Dr. Paul McCallin, Kaiser Permanente Hospital, Honolulu, Hawaii.

STUDIES IN EXPERIMENTAL ANIMALS

1. Pregnant monkeys in Puerto Rico and Bethesda, Md.

Studies of pregnant monkeys infected with rubella virus were conducted in collaboration with the Laboratory of Perinatal Physiology, NINDB, San Juan, Puerto Rico. Animals were inoculated early in pregnancy. These showed evidence of infection. None of the animals showed congenital malformation.

2. Ferrets were found to develop experimental rubella when inoculated by the intranasal route. The drug Amantadine was found to moderate the infection. Newborn ferrets developed chronic infections while adult animals had a limited infection.

ISOLATION OF AGENTS AND CHROMOSOME STUDIES

1. Rubella was isolated from more than 60% of fetuses obtained at therapeutic abortion for maternal rubella. Congenital infection resulted in the development of complement fixing antibody in most cases by 6.8 months of age.

2. Chromosome studies.

VOLUNTEER AND VACCINE AND SPECIAL STUDIES

1. Several studies were conducted with volunteers at the Petersburg Federal Reformatory. Intradermal inoculation of rubella virus produced clinical rubella.

2. Epidemics of mumps, chicken pox and rubella were investigated in collaboration with the Arctic Health Research Center. These studies provided greater understanding of these diseases. Additional studies of rubella were also conducted in Los Angeles (family studies); Puerto Rico (epidemics in medical students); Hawaii (use of gamma globulin); Japan (effect of epidemics).

3. The small lot of rubella convalescent gamma globulin is being studied in Hawaii in a controlled investigation of the efficacy of gamma globulin for rubella.

VACCINE DEVELOPMENT PROGRAM

A total of 10 contracts for the development of rubella vaccine development have been awarded and project officers are assigned by the Section on Infectious Diseases. Coordination and direction of the program is given by the project officers and the Section provides laboratory checks of materials prepared under the contracts as well as extensive animal studies of immunogenicity.

II. PROBLEMS

LACK OF FUNDS FOR MATERIALS NECESSARY FOR STUDIES

Because of the reduction in the research and Development Contract funds for virus reagents this year, it has not been possible to prepare many of the important antigens for testing. For example, only a few milliliters of CF antigen for rubella could be prepared. No cell pack antigens for the Adenoviruses or Herpes Simplex could be provided. This has resulted in a restriction of testing performed.

INSUFFICIENT PERSONNEL

We are severely limited by the lack of sufficient positions for technicians, a secretary, Serum Center personnel, and statistical clerks. A minimum of six additional positions are necessary for efficient utilization of serum and antigen. This is particularly necessary because an increasing percentage of sera have demonstrated marked deterioration. Only through efficient utilization of procedures which are available will it be possible to obtain information concerning the exposure of patients to viruses and the importance of this exposure to the production of abnormal infants.

VARIABLE ANTIGENIC PREPARATIONS

It is apparent that the biological systems involved in producing the antigens are frequently not reproducible. Frequent changes in procedures, changes in tissue or special methods of concentrating antigenicity are necessary to obtain a new lot of antigenic material. In other cases anticomplementary effects must be eliminated or the antigens may have to be remade. The production of antigens requires individual research and development.

LIMITED STUDIES

Two factors which are limiting testing at the present time are the incomplete serum collection during the first two years of the study which resulted in numbers of patients for whom few if no specimens are available; and incomplete identification of abnormal pregnancy outcomes which restricts identification of serum specimens for serological testing.

III. PROPOSED FUTURE OBJECTIVES

ADDITIONAL PERSONNEL

Every attempt will be made to obtain six new positions in Fiscal Year 1967 which are absolutely necessary for our study. Two of these positions will be for laboratory technicians for emergency testing of sera which are undergoing loss of activity; two positions will be for assistance to unpack and make available the back log of over one year of serum collection (160,000 vials). Two positions will be used for analysis of the back log of test data and secretarial assistance to correlate with data being punched from the Obstetrical and Pediatric records.

ANTIGEN PRODUCTION

Additional funds for reagents will be sought so that antigens for rubella,

salivary gland virus, varicella and new viruses will be prepared and incorporated in the testing program. Several new bacterial and protozoal antigens will be added to the routine testing of the study sera.

ANTISERA PRODUCTION

Prototype sera will be prepared for 10 viruses for control testing.

LARGE-SCALE TESTING

Serological studies involving 100,000 specimens from pregnant women in the Collaborative Perinatal Research Study will be continued. The tests will include antigens for Flu A, Herpes Simplex, Mumps, Salivary Gland Virus and Rubella. Increased investigations of cross reactions with serological tests will be conducted. Supplemental sampling of the study population will be necessary to include patients who register early in the first trimester of pregnancy so that documentation of virus experience in this period will be possible.

CYTOGENETIC STUDIES

The new laboratory for cytogenetic studies will be expanded to work in conjunction with the infectious disease studies of abortion. This laboratory will include one additional technician and an animal handler.

NEUROLOGICAL DISEASES AND EYE DEFECTS

A new laboratory for the study of defects of the central nervous system and eyes which are due to infectious agents will be developed. This laboratory will use the combined techniques of infectious diseases and fluorescent microscopy.

SPECIMEN COLLECTION

Specimen collection will be expanded to include tissue samples from placentas, abortuses, at the Kaiser Hospitals in Los Angeles and Honolulu, so that the isolation of viral agents may be utilized to confirm and extend the serological findings and simultaneous cytogenetic studies will be performed. Special fetal specimens will be studied from patients with abortion.

EXPERIMENTAL ANIMALS

Further investigations will be conducted with experimentally infected pregnant and nonpregnant animals to provide direct information on routes of infection, pathogenesis, and possible methods of prevention of defects in the fetus due to infectious agents. Experimental animal studies will continue in pregnant monkeys at the Laboratory of Perinatal Physiology, San Juan, Puerto Rico, and with pregnant monkeys obtained under contract arrangements. Viruses under study will include rubella, mumps, and salivary gland virus.

GAMMA GLOBULIN STUDY

The efficacy of the use of gamma globulin for the prevention of rubella will be extended using titered gamma globulin in controlled studies in Honolulu, Hawaii.

VACCINE STUDIES - RUBELLA VIRUS

Further studies on inactivated and attenuated vaccines for rubella will be pursued. These will include animal inoculation of vaccine materials to determine antigenic potency as well as possible administration to volunteers using safety tested material.

PAPERS PRESENTED AT SCIENTIFIC MEETINGS BY SECTION PERSONNEL

1. "Comparison of Serological Methods for Rubella" presented at the American Public Health Association Meetings on October 19, 1965, by Dr. Sever.
2. "Recent Experience with Rubella Infections" presented at the American Academy of Pediatrics on October 22, 1965, in Chicago and at the Tenth Anniversary Congress of the Illinois Division for Maternal and Child Health on February 17, 1966, in Peoria, Illinois by Dr. Sever.
3. "Virology: Implications of Infections in Pregnancy and Newborns" presented at the Chicago Medical Society Meetings in Chicago on October 12, 1965, by Dr. Sever.
4. "Congenital Rubella and Teratogenic Effects in Ferrets" presented at the American Federation for Clinical Research on December 3, 1965, by Dr. Fabiyi. Also presented at the Collaborative Study Meetings in March held here.
5. "Cytomegalovirus and the Normal Pregnant Female" presented at the Western Section of the American Federation for Clinical Research held in Carmel, California, on January 26-30, 1966, by Dr. Hildebrandt.
6. "The Congenital Rubella Syndrome CSF Antibody and Isolation Studies in Adults and Children" presented at the Western Section of the American Federation for Clinical Research in Carmel, California, on January 26-30, 1966, by Dr. White.
7. "Congenital and Acquired Rubella" presented at the Annual Clinical Conference of the Chicago Medical Society on February 27 - March 2, 1966, by Dr. Sever.
8. "Viral Diseases and Damaged Children" presented at the Milwaukee Academy of Medicine on March 15, 1966, by Dr. Sever.
9. "Toxoplasmosis: Serological and Clinical Studies of 23,000 Pregnant Women"- Collaborative Study Meetings in Washington, D.C., on March 24, 1966, by Dr. Sever.

10. "Viral Infections in 30,000 Pregnancies" presented at the Collaborative Study Meetings in Washington, D.C., on March 24, 1966, by Dr. White.

E. SECTION ON PATHOLOGY
Report for the period July 1, 1965 through June 30, 1966

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS

Automated Biologic Image Processing

Collaborative research with the Artificial Intelligence Group of the National Bureau of Standards on the plans and specifications for a general purpose device began on a formal basis on July 1, 1964. The objective of the general purpose device is to provide for the conjoined human-computer analysis, synthesis and description of biological images. The results of the work thus far with respect to the general purpose device itself are described in the attached individual project report (Serial No. NDB (CF)-65 PR/P 1278) and for the purpose of reporting is summarized into the following four categories:

Language Processing

Aims toward development of a language which can be used by neuro-pathologists for interrogating a system for processing photomicrographs and other microscopic images. The desirable properties of such an interrogation language have been described and two such languages were exhibited in NBS Technical Notes.

Picture Processing

A number of picture processing investigations have been initiated and include: the use of the above languages to determine certain geometric properties of cells in photographs; the study of texture in images; a study of images which have a codified system of natural language descriptions; creation of synthetic photographs of tissue sections by juxtaposing objects within such photographs; and, the use of COMIT programming language for dealing with two-dimensional objects.

Direct Access Interrogations Systems

This work has been performed to investigate the use of interrogation systems in which a computer is provided with immediate access facilities to many simultaneous users. Dr. Lipkin was able to determine that with an on-line interrogation system, languages of such size that their rules could otherwise not be learned or memorized, can conveniently be used. This task is being expanded to study comparable problems on the ANFS-Q-32 computer at the System Development Corporation in Santa Monica, California.

Miscellaneous

This category represents the organization by R.A. Kirsch of a session on pictorial information processing and included the presentation of a paper by Lipkin, Watt and Kirsch, "Analysis, Synthesis and Description of Biological Images," January 31, 1966.

Project Material and Related Data Processing

Shipping of brains, duplicate autopsy slides, and PATH-I, II and III forms of project deaths from collaborating institutions has continued according to prescribed procedure with minimal administrative difficulties. Project brain accessions have increased over the past year due to discontinuation of local processing by one collaborative institution. Procedures and methods for brain and placenta processing and study have not changed from previous reports. A study of the statistical validity of heart weight as a predictor of organ weight entitled "Predictors of Organ Weight at Autopsy - A Multivariate Analysis; I. Heart Weight" has been finished and was presented by Dr. Lipkin on March 24, 1966 at the Second Scientific Meeting (Serial No. NDB (CF)-66 PR/P 1344). Additional organs including lung, kidney, adrenal, liver, thymus, etc., are now being considered. An analysis of pulmonary hyaline membranes in the neonatal period determining the relationships of the presence and quantity to a number of factors has been completed by Drs. Fujikura and Froehlich, entitled "The Influence of Race and Other Factors on Pulmonary Hyaline Membranes," and presented at the Second Scientific Meeting, March 24, 1966 (Serial No. NDB (CF)-66 PR/P 1279).

Laboratory Procedures

During the past year, the section has greatly expanded its laboratory research projects. The Electron Microscope Laboratory has modified and improved a number of technical procedures covering all areas of tissue processing and include the use of a new irrigating buffer solution, perfusing technique simplifications, improved embedding procedure and media, a new glass knife marking instrument, and development of new section re-embedding molds. Electron microscopic facilities are now being used to augment a number of light microscopic investigations within the section.

A cell perfusion chamber for study of stain progressivity has been developed and microspectrophotometry investigations using the chamber are under way using a microspectrophotometer on loan from NCI. This investigation along with the study of absorption curve characteristics in DNA stains will be expanded to the microspectrophotometer under development by Dr. Lipkin in the near future.

Several new histological staining methods have been employed and added to the methodological armamentarium of the laboratory and the senior technicians. These include special methods for fluorescence microscopy and electron microscopy.

Special Studies

Corneal Graft Using Human Amnion

There is a close resemblance, histologically and physiologically between the cornea and the amnion. The remarkable similarity of their collagen fibrils are shown by electron microscopic examination. These are important facts supporting the possibility of corneal amnion graft. A pilot study was done between Dr. Fujikura in collaboration with Dr. William Green of the Ophthalmology Branch, NINDB, using human amnion grafts on rabbit cornea. Five adult rabbits (New Zealand) were treated and the corneal grafts were examined microscopically after 58 days. Immunological reaction developed making it clear that the human amnion is unsuitable for corneal grafts. However, it was interesting to note that the human amnion grafts were still alive in the rabbit cornea at the end of 58 days. The human amnion shows metamorphosis which resembles that of adult connective tissue. It is interesting to know that immature type (histologically) of tissue such as the amnion and the cornea have a strong resistance and adaptation to host immunological reaction.

Pulmonary Studies

The detailed study of incidence and histological classification of pulmonary hyaline membranes was expanded by Drs. Fujikura and Froehlich to include 434 neonatal deaths out of 27,407 single live births. The influence of race, neonatal mortality rate, birthweight, postnatal age and associated pulmonary histological findings were statistically analyzed and accepted for publication in the American Journal of Obstetrics and Gynecology. (Serial No. NDB (CF)-65 PR/P 1279). An additional study is in progress to show relationships of hyaline membranes and acute chorioamnionitis and the results are to be compared with the incidence of this acute infection in the general study population and in neonatal deaths not showing hyaline membranes. It is also planned that a combined clinical and morphological study will be made in collaboration with other members of the Hyaline Membrane Task Force.

The incidence of intrauterine pneumonia and its relationship to race, birthweight and associated placental infection was studied by Drs. Fujikura and Froehlich. Pulmonary sections of 512 infants dying within the first 48 hours of life out of 36,212 single live births have been reviewed and the information accepted for publication in the American Journal of Obstetrics and Gynecology. (Serial No. NDB (CF)-66 PR/P 1340).

Placental and Cord Studies

The incidence of single umbilical artery (SUA) and its relationship to congenital malformations, maternal age, race, gravidity, associated maternal disease, infant sex, birthweight and placental weight have been analyzed on 26,539 placentas by Drs. Froehlich and Fujikura and published in the American Journal of Obstetrics and Gynecology. (Serial No. NDB (CF)-65 PR/P 1281). A continued study of surviving SUA cases with particular emphasis on the neurologic course of these infants, a more detailed analysis of associated placental and cord abnormalities, and the addition of new cases of SUA to the series is contemplated.

A study of placental shape in relation to race, birthweight, gestational age and placental weight is being conducted by Drs. Fujikura, Froehlich and Lipkin in an effort to test the hypothesis that the definitive shape of the placenta is established early in pregnancy and changes little in the last five months of gestation. Preliminary analysis shows that placenta tended to be rounder with increasing birthweight and placental weight. (Serial No. NDB (CF)-66 PR/P 1341).

They are also investigating the association between cord length and two main indices of embryonic growth, namely birthweight and gestational age. It is contemplated that this study be enlarged to include analysis of its association with other indices of growth such as crown-rump length, crown-heel length, placental weight, as well as other placental findings such as type of cord insertion, placental shape, etc. Besides the observation that mean cord length showed increase with increments of gestational age and birthweight, other possible effects on cord length such as single umbilical artery, cord entanglement, cardiovascular and other malformations, etc., will be investigated. (Serial No. NDB (CF)-66 PR/P 1342).

In cooperation with Drs. Tuncer and Ueda at the University of Buffalo, Dr. Froehlich has developed a rapid simple method of detecting neutrophilic infiltration in the umbilical cord and is studying the relationship between neutrophilic infiltration of the cord and perinatal infection. They have been able to show significant correlations between prolonged membrane rupture and positive imprint tests and have projected the possibility of using this method as a simple emergency diagnostic tool in perinatal infections. (Serial No. NDB (CF)-65 PR/P 1280).

Classification of Abortions

A simplified anatomic classification of abortions has been devised by Drs. Fujikura, Froehlich and Driscoll (Boston) to give a more uniform method of reporting abortions based on the morphology of products recovered and requires neither a special interest in embryology nor an undue expenditure of time. This classification has been accepted for publication in the American Journal of Obstetrics and Gynecology. (Serial No. NDB (CF)-66 PR/P 1343).

Predictors of Organ Weight

In assessing the deviation from normality, Dr. Lipkin has been evaluating crown-rump length, crown-heel length, gestational age and body weight at autopsy for practical utility as predictors of heart and other organ weights. He has devised, using multivariate analysis, a simple equation with enveloping confidence limits for predicting the logarithm of heart weight as a functioning of log body weight. This information was presented at the Second Scientific Session of the Collaborative Study, March 24-25, 1966 and will be expanded to include other organs. (Serial No. NDB (CF)-66 PR/P 1344).

Epidemiological Studies

Two-hundred and seven cases of sudden unexpected deaths in the Collaborative Study material have been identified and are being studied by Drs. Fujikura, Drage and Froehlich to determine the possible influence of sex, race, birthweight, gestation, placental weight, placental infection, autopsy findings, maternal diseases, type of feeding, Apgar score and socio-economical factors. (Serial No. NDB (CF)-66 PR/P 1345).

Dr. Froehlich is also collaborating with Dr. S. Mitchell (NHI) and with the Section on Infectious Diseases in a project to make an epidemiological assessment of primary endocardial fibroelastosis. Their findings through October 1, 1965 on seven cases of this rare lesion were presented at the Second Scientific Session of the Collaborative Study, March 24-25, 1966. (Serial No. NDB (CF)-66 PR/P 1346).

Individual Case Reports

Dr. Leventhal has collaborated with his former associates at the Massachusetts General Hospital on the clinical and neuropathological report of a case of Marchiafava Bignami disease. This was presented to the American Neurological Association in June 1965 and is being prepared for publication. (Serial No. NDB (CF)-66 PR/P 1347).

An intensive study of clinical and autopsy material from a project case of an infant with multiple congenital anomalies was undertaken by Dr. Leventhal and presented at the Second Scientific Session of the Collaborative Study, March 24, 1966 under the title "Embryologic Analysis of Multiple Congenital Anomalies." (Serial No. NDB (CF)-66 PR/P 1348).

Neuropathologic material received through the Pathology Department of the Clinical Center and in consultation from the University of Oregon relative to a large kinship with familial Alzheimer's disease was processed for routine and special fluorescence microscopic studies and photography. Dr. Leventhal served as neuropathological consultant and collaborating co-author on a clinical and pathological report being prepared for publication. (Serial No. NDB (CF)-66 PR/P 1349).

Primate Studies

The relationships of birthweight, gestational age, sex and type of delivery in Rhesus monkeys is being studied by Drs. Fujikura and Niemann from timed matings in the breeding colonies of the Perinatal Physiology Laboratory, Puerto Rico. Single live births of which 179 were delivered vaginally and 292 by cesarean section with lengths of gestation ranging from 135 to 171 days have been included in this study. They have demonstrated that mean birthweight in males was consistently higher at each gestational interval than in females and that there was a wide variability in birthweights roughly constant regardless of gestational age, sex and type of delivery. (Serial No. NDB (CF)-66 PR/P 1350).

In another study conducted by the Perinatal Physiology Laboratory in Puerto Rico, Dr. Fujikura is investigating the placental pathology occurring after ligation of a major vessel in the interlobar area of the Rhesus monkey placenta. (Serial No. NDB (CF)-66 PR/P 1351).

As a comparison to the Perinatal Project material, Drs. Carleton and Lipkin are studying the brains of the primate perinatal period according to the protocol established for the processing of whole brains coming from the Collaborative Study. This will serve as a laboratory control for evaluating technical procedures used in the Project's protocol and, in addition, after the neuropathological findings have been tabulated their relationships to data extensively collected on each animal during its perinatal period will be evaluated. (Serial No. NDB (CF)-66 PR/P 1352).

Electron Microscopy Studies

During this period of this report a study of ultrastructural changes in an experimentally produced traumatic brain lesion has been initiated by Dr. Carleton and Mr. Priester, and has progressed through a series of seven phases including an evaluation of the methodology, reproducibility of wounding, a light microscopic study, EM atlas of early and late changes, thick serial section series, and the adaptation of large epoxy section technique to the investigation. The information obtained from these phases is now being applied to an expanded series for a final analysis of the process of brain healing. It is anticipated that in the future this study will be extended to include other factors related to brain repair such as host age, host species, infection and other experimental conditions. (Serial No. NDB (CF)-66 PR/P 1353).

The electron microscopic laboratory program has recently been expanded to provide ultrastructural examinations of other research projects within the section including neonatal lung responses to chemical stimulation and placental artery study.

Automated Microspectrophotometry Employing the LINC Computer

The need for a computer controlled microspectrophotometer was indicated in the last annual report.

In December 1965 this section obtained the loan of a LINC computer from the LINC Evaluation Board. The loan is for a one year period and was made in order to demonstrate the utility of the LINC in reducing professional time in data gathering in microspectrophotometry, the on-line compensation for inherent instrumental errors, and the use of the computer for on-line result computation and display.

The requisite optical devices have already been fitted with stepping motors and a precision differential photomultiplier amplifier power supply combination has been built. Many of the requisite subroutines have been written. At this writing, the only missing component is the stepping motor controlled digital stage which is in process of construction. (Serial No. NDB (CF)-66 PR/P 1354).

Using microspectrophotometric techniques, three major factors influencing the quantitation of DNA staining are under investigation by Drs. Carleton, Lipkin and Spencer. The factor of Feulgen stain fading with storage was completed last year and is being published with a statistical analysis by Mr. Weiss and Miss Jackson. An initial investigation of Feulgen DNA absorption curves in cells from each embryonic germ layer and different species has been completed. Valid conclusions could not be made, however, since significant instrument variations were encountered. This study will be amplified using the microspectrophotometer under development by Dr. Lipkin. A preliminary evaluation of progressivity in whole cell Feulgen staining has yielded interesting results and will be expanded using recently developed techniques. (Serial No. NDB (CF)-66 PR/P 1355).

Other Activities

Intramural

Dr. Lipkin has served as contract officer for the Collaborative Program with the Artificial Intelligence Group of the National Bureau of Standards as described in the individual project report.

Dr. Leventhal has served as neurological and neuropathological consultant to various training research and service activities of the Medical Neurology Branch, NINDB, and the Pathologic Anatomy and Medicine Branches, NCI.

Non-Government

Dr. Leventhal has served as consultant in neurology at D.C. General Hospital and clinical instructor in neurology at Georgetown University.

American Academy of Cerebral Palsy

The laboratory has served as a repository center for the Brain Registry of the American Academy of Cerebral Palsy. This relationship has been strengthened by the interest of Dr. Preston Robb, Chairman of the Brain Registry. A small number of brains of unusual interest have been added to the collection. A case of Krabbe's Globoid Leukodystrophy was given intensive study by a variety of histochemical techniques. Detailed report of this case was submitted to the Academy and to the donating physicians.

II. PROBLEMS

The problem of adequate personnel for coding, punching and editing of PATH-I, II and III forms has been a major one. Under existing conditions, part-time help has been borrowed for a limited period. The task of routine editing of all incoming PATH-I and II forms and of processing PATH-III forms remains understaffed.

In order to fully implement the project's commitments and research programs of the section, success in recruitment for an additional neuropathologist, programmer, as well as one medical editor or statistical clerk in the next several months is essential.

The need for animal space as outlined in last year's annual report has become even more acute with the expansion of electron microscopic, cytophotometric, and light microscopic animal research investigations. No solution to this problem is presently apparent and productivity of this aspect of our research programs is severely curtailed by this lack of small animal facilities. In addition to this problem of animal space a room used for professional office space and general laboratory procedures had to be relinquished March 1, 1966. Short term arrangements have been initiated but remain inadequate and totally unsatisfactory for current and projected laboratory efforts.

III. PROPOSED FUTURE OBJECTIVES

The Section on Pathology will continue to concentrate on the studies of Project material in ever-increasing detail.

The development of a special purpose neuron counting device which would greatly speed the accumulation of microscopic data is proceeding as previously outlined and presents no basic engineering or programming difficulties.

Pursuit of the general purpose device along the lines previously established with particular emphasis upon areas such as machine generated three-dimensional reconstructions will be continued.

Established research projects will be pursued as detailed in the individual reports.

IV. REMARKS

Personnel

A new histological technician will begin training in April to fill a vacancy in the laboratory. Recruitment for a neuropathologist to fill the position left by the transfer of our biochemist is in progress. A statistician has been transferred from the section to the Section on Statistical Analysis.

F. SECTION ON EPIDEMIOLOGY AND GENETICS

Report for the period July 1, 1965 - June 30, 1966

I. SUMMARY OF SCIENTIFIC AND PROFESSIONAL ACCOMPLISHMENTS

The major research activities of the Section on Epidemiology and Genetics during the last fiscal year have been directed towards the completion of the numerous projects which were initiated in the genetic and socioeconomic areas. Availability of computer facilities made this possible and, undoubtedly, the anticipation of the Second Scientific Meeting provided a stimulus for the preparation of these studies for presentation and publication. A summary of them will be given below.

Physically the Section has adequate space for the professional and clerical staff and also for the storage of records. The present space division is flexible enough so as to accommodate the expanding volume of files to its maximum anticipated growth and also provide office space for the additional professional personnel which will be joining this Section during the summer of 1966.

The Section has continued to receive, collect, coordinate, and code information on the GEN 5-8 and SE-1 forms. During this time about 9,000 each of GEN 5-8 and SE-1 forms have been received and an equal number coded, checked and sent to punch.

This Section which has always prided itself for the completeness of its records, has joined the effort to inventory all forms due in its files and bring the entire cohort up-to-date. It is anticipated that this mopping-up operation will be completed during the coming year.

The determination of zygosity of twins born to Study mothers continues. The objective of this operation is to determine the zygosity of all twins born to the Study mothers so that anyone wishing to engage in twin research may have the material available. Unfortunately, the Collaborating Institutions are derelict in obtaining the required finger and palm prints and blood samples from the twins. As a result, the number of twin pairs for whom zygosity has been determined is far below the actual surviving number of like-sexed twins born to the Study.

The development of the socioeconomic index to describe and categorize our population has been completed. About 38,000 cases have now been scored by two scoring systems, one based on the scores which have been developed by the U.S. Bureau of the Census and the other based on scores which have been developed from the cumulative percentage distribution of the component parts of the index in our population. This has made possible a number of useful comparisons of our total population and that of the various Institutions to themselves and to

the United States population, by a number of demographic characteristics. It appears that the population of the Perinatal Research Study is not as skewed towards the lower socioeconomic brackets, when compared to the United States population, as we originally thought it would be. A paper describing the methodology of the socioeconomic index and the first findings was presented at the Second Scientific Meeting of the Collaborative Study in March 1966, in Washington, D.C.

The study of the relation of several socioeconomic variables with birthweight has also been completed. By sequential multiple regression analysis it was shown that although the socioeconomic variables used appeared to have an influence on birthweight this was erratic and unpredictable. On the other hand it was found that race had a constant effect which equalization of racial differences in socioeconomic factors failed to remove. It appears that white babies are about 130 grams heavier than Negro babies; Puerto Rican babies have an innate average weight which approximates that of either white or Negro babies, depending upon what adjustment is made for family income. A paper reporting the results of this study was also presented at the Second Scientific Meeting of the Perinatal Research Study.

A third completed study deals with sequential aspects of spontaneous abortions. The findings from this study are that the age trend apparent in women with spontaneous abortions is not an artifact, as some investigators claim, but represents real age-parity effect. These findings have also been presented at the Second Scientific Meeting of the Perinatal Research Study.

Another paper presented from this Section at the Second Scientific Meeting of the Perinatal Research Study was concerned with methodology in relating pregnancy outcome to prior pregnancy record. The special methodology which was developed for this study is a modified life table procedure.

The first stage of a long-range study of the population on dynamics of Tay-Sachs' disease was completed early during this period. The problem here was to determine why Tay-Sachs' disease is about 100 times more frequent among Ashkenazi Jews than among other Jewish groups and non-Jewish populations. A controlled study of the fertility of the presumed Jewish heterozygote suggested that the Jewish heterozygote may have a measure of selective advantage (higher fertility) than the Jewish homozygous normal, and that the rise of the TSD gene among the Ashkenazi Jews may have taken place by this mechanism during the centuries of the diaspora. This study is now continued to determine what selective factor may have enhanced the fertility of the Jewish heterozygote in the East European communities.

Among studies which have progressed considerably during the past year is the study of genetic basis of neonatal reflexes; the required computer work for this study has been completed and the cases are now undergoing hand review. Progress has also been made on the study of

the effects of a number of genetic, socioeconomic and medical factors on major congenital malformations; a first-stage regression analysis for these variables has been completed and a more refined analysis is in progress. A computer program is being written for a study of the effects on family size of fetal loss due to Rh immunization.

Dr. Naylor, the population geneticist of this Branch, has been instrumental in designing the analysis of many of these studies, often doing the programming himself. In addition, Dr. Naylor has been interested in problems of efficient test statistics for use with epidemiologic data and in theoretical points of biometric analysis.

The Second Scientific Meeting of the Collaborative Study

The Second Scientific Meeting of the Collaborative Study which was held in Washington, D.C. during the last week of March, 1966, was in a sense a testing ground of the work of PRB during the last three years. This Section participated with four papers, mentioned earlier, presenting the results of projects in both the genetic and socioeconomic areas. Dr. Kathrine French, of the University of Oregon, and Dr. V. Elving Anderson of the University of Minnesota joined Drs. Myriantopoulos and Naylor in the analysis of the material and the preparation of the reports. The reception and comments which the scientific audience gave our papers provided a sense of deep satisfaction and accomplishment on our part.

Workshop for the FHH-9

A training workshop for the new form, FHH-9, was held at the Metropolitan Hospital, New York City, at the end of February, 1966. The FHH-9 is intended to bring up-to-date the genetic and socioeconomic information which was collected prenatally and will be administered with the 7-year battery of tests. The workshop was attended by a large number of interviewers from the participating Institutions and proved to be a great success. During the didactic and practice parts of the workshop no apparent serious difficulties were encountered. It was the consensus of opinion that the form was well designed and thoughtfully put together with respect to both content and mechanics and that the manual was lucidly written as to explain all the aspects of the form and its use very clearly. The form has already been released for use immediately by all Institutions.

Personnel Recruitment

Two new professionals will be joining this Section during the summer of 1966. Our long search for a medical sociologist ended successfully in the recruitment of Dr. Glen Bartlett who holds a M.D. degree from Stanford University and in addition a Master's Degree in sociology from the same institution. Dr. Bartlett, who is now interning at Strong Memorial Hospital in Rochester, New York, is expected to stay

with us for at least two years. He is very interested in problems of medical sociology and will be engaged in the utilization and analysis of our socioeconomic data.

Also joining us for two years will be Mr. Etan Markowitz, who trained in mathematics and statistics in California Institute of Technology, and is now completing a degree in population genetics at the University of Wisconsin. Thus, slowly but surely this Section is acquiring professional staff of high caliber and necessary qualifications to cope with the analysis of the collected data and to implement our expanding program.

II. PROBLEMS

The most critical problem facing this Section still is the lack of trained professionals among the Collaborating Institutions to participate in the analysis of the genetic and socioeconomic material. Although the Collaborating Institutions have on their staff a plethora of obstetricians, pediatricians, and psychologists they do not have a single geneticist or sociologist. This fact was mentioned in last year's annual report but the situation has not changed since then. It appears that this Section will have to bear the burden of analysis alone. The addition of another geneticist and a medical sociologist to the staff of this Section will ease the problem considerably but the amount of material available for analysis requires the participation of several more professionals. Our efforts to open our genetic and socioeconomic material to selected graduate students who would benefit from well collected data for these projects has not yet materialized but hopefully progress will be made in the future.

III. PROPOSED FUTURE OBJECTIVES

In last year's annual report it was pointed out that one of the most important readjustments which will have to be made as the intake of further cases ceases, is that of systematic shift of positions from semi-professional and clerical, which now constitute the majority in each Section, to professional and related technical positions. During the past year this policy has been implemented by this Section. Semi-professional personnel lost by attrition are being replaced by two professionals, a medical sociologist and a geneticist. It is anticipated that this conversion will continue slowly during the next year and will eventually taper off to a functional balance of professional and non-professional personnel.

In the research area the plans are to complete the research projects now in progress; to utilize the results of the first analysis in order to identify those areas in which specific genetic problems lie; and to investigate those problems by a variety of approaches.

IV. REMARKS

A. Personnel

The personnel of the Section on Epidemiology and Genetics consists at present of the following: Professional, Dr. Myrianthopoulos, Head, Dr. Naylor, Geneticist, Mrs. Vincent, Senior Nurse Officer and Field Worker, Miss Martin, Statistician; other: one secretary-stenographer, one clerk-typist, seven coding clerks and one part-time statistical assistant.

As mentioned earlier a medical sociologist, Dr. Glen Bartlett, and a population geneticist, Mr. Etan Markowitz, will join this Section for two years, during the summer, 1966.

B. Activities of the Section Head

The Head of the Section, Dr. Myrianthopoulos, in addition to his formal duties, has carried out independent investigations on the genetics of neurological disorders. Dr. Myrianthopoulos has maintained his affiliation with George Washington University as an Associate Professor of Neurology and Director of the Genetic Counseling and Research Center. In this capacity, he gives a series of lectures in the medical school and conducts genetic counseling upon referral by individual physicians. Dr. Myrianthopoulos is also a member of the faculty of the Graduate Program at NIH where he gives a course in Human Genetics.

During August, 1965, Dr. Myrianthopoulos attended the Mendel Centennial celebration in Brno and Prague, Czechoslovakia. The celebration was sponsored by the Czechoslovak Academy of Sciences and UNESCO and was held in commemoration of Mendel's discovery of the laws of heredity which he presented in a little-noticed paper 100 years ago. The celebration was attended by over 1,000 scientists from all over the world including a 100-strong delegation of Russian scientists. During this trip Dr. Myrianthopoulos lectured on the genetic aspects of the Collaborative Study before the Medical Faculty of the University of Frankfurt, Germany, and discussed with Professor K.-H. Degenhardt various problems pertaining to the new Collaborative Study of congenital malformations which is getting under way in Germany under his direction. From Frankfurt, Dr. Myrianthopoulos proceeded to Aberdeen, Scotland where for two weeks he worked in the Obstetric Medicine Research Unit of the University of Aberdeen with Professor Raymond Illsley, who is in charge of the socioeconomic studies. The Unit is justly famous for its perinatal studies under the direction of Sir Dugald Baird. Dr. Myrianthopoulos also had a series of conferences with Professor Neville Butler who made a well-known study of perinatal mortality in Great Britain during 1958, and now is in the

midst of conducting a 7-year follow-up. Details of Dr. Myrianthopoulos's activities abroad can be found in his extensive trip report.

Dr. Myrianthopoulos attended the Second International Symposium on Sphingolipidoses which took place in New York in October, 1965, and delivered a paper on his work on the population dynamics of Tay-Sachs' disease; Dr. Myrianthopoulos also participated and presented papers at the following scientific sessions:

Second Scientific Meeting of the Collaborative Study, Washington, D.C., March, 1966. Dr. Myrianthopoulos presented paper "A Socioeconomic Index for the Population of the Collaborative Study." Drs. Naylor and Myrianthopoulos presented paper "The Relation of Selected Socioeconomic Factors to Human Birthweight."

Seminar of the Department of Preventive Medicine, University of California School of Medicine, San Francisco, California, April, 1966. Dr. Myrianthopoulos presented lecture "Epidemiology and Genetics in the Collaborative Study."

American Academy of Neurology, Philadelphia, Pa., April, 1966. Dr. Myrianthopoulos presented lecture "Recent Advances in Neurological Genetics."

American Population Association, New York, N.Y., April, 1966. Dr. Myrianthopoulos presented paper "Socioeconomic Background and Fertility of an Experimental Population."

American Neurological Association, Washington, D.C., June, 1966. Drs. R.P. Mackay and Myrianthopoulos will present paper "Multiple Sclerosis in Twins and Their Relatives. A five year follow-up."

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G. SECTION ON SYSTEMS DESIGN AND PROCEDURES
Report for Period July 1, 1965 through June 30, 1966

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS

This year was again one of considerable accomplishment for the Section on Systems Design and Procedures. It was the second year in which substantial data processing facilities were available to the study and, while the method of use of such facilities was less than fully desirable, a substantial amount of data processing was accomplished.

The services of the contract facilities were readily available to the Branch during the year. In addition, card punching and some specialized services came from the Computation and Data Processing Branch, DRS. However, due to the small number of programmers available at PRB and to the limits of available software at NIH, these additional services were extremely limited.

The Section has continued to develop its own programming capacity. The latter has increased from 4 to 6 programmers. As these new people are trained in programming and achieve greater capacity to perform, it is assumed that the data processing capability of CDPB and other computers will be more fully utilized.

The Section has continued to update, edit and add to the data file of the study. In the previous year there were 91 different card formats accounting for approximately 2½ million punch cards on computer tape. At the present time there are 111 different card formats in the file representing almost 3 million punch cards on tape. In addition, another 700 thousand punch cards containing study data were produced during the year and are now awaiting update into the tape files. It is to be expected that by June 30, 1966, almost all of these cards will have been added to the data file, thus producing a data file of about 3½ million punch card equivalents.

Extensive efforts were made during the year to correlate the inventory file systems to the data file and physical record systems of the study, corresponding with each of the hospitals in order to resolve any differences between the inventory record, data file record and physical file. This has included a complete review of the inventory files to determine availability of records. All records found not available per the inventory file have been checked against the physical file and those found missing from both files have been reported to each of the hospitals in order to identify the reason for non-submission, or to obtain submission or resubmission of the file if it was previously sent but not received.

The Section has continued to produce cross tabulations of various kinds for new files of the study such as the 3 year and 4 year examinations, has continued to produce frequency distributions of various kinds based on new data or cross tabulations for data of interest to the medical, statistical and other professional staff of the Branch or the collaborating institutions.

Coding has continued at a regular pace and has, in general, kept up with the work flow of the Branch. Of the approximately 2 3/4 million forms currently received, over 90% of the obstetric forms have been processed and about 80% of the pediatric forms. Forms in the behavioral area and socio-economic and genetic area present comparable percents.

While a continued effort is being made to collect or account for all missing forms, in general, the data provided have been sufficient to permit meaningful analysis. This can be readily seen in the scope of the collaborative study scientific session held on March 24 and 25, 1966. Of the 51 papers presented at the scientific session, the tabulations for 22 of these studies were prepared directly by the Section based on the study designs presented using the major data files and were provided to the authors and the Statistical Section for analysis and review. For 11 additional studies the initial selection of cases was made from the entire study file through use of computer facilities and the study was produced on a case analysis basis using the study files by the Statistical Analysis Section and the respective authors. Thus in toto the computer facilities were used wholly or in part for 33 of the 51 studies produced for the scientific session.

II. PROBLEMS

The most serious problem in the operation of the Section has continued to be, as for last year, the lack of assurance of continuity for data processing facilities. It is still necessary to obtain the needed data processing facilities via contracting or other sources. Contracts for a period exceeding one year are not available to us. Therefore, the generality this imposes upon the Section's approach to data processing (that is in the creation of data files, systems design and retrieval procedures) limits the full development of the computer as a research tool. These systems must be maintained in such a manner that a new contractor could assume them for continued production of work for SSDP each year. This precludes specialized summarizations of files or taking advantage of peculiar features of a given computer since this would automatically preclude many proposed contractors from bidding on the new year's proposal.

The short range nature of systems work under these conditions means continual failure to take the best advantage of computer facilities. So long as the facility within SSDP is not large enough to assume the full programming burden, or CDPB's facilities are not adequate in terms of machine availability (that is machine and program availability) we must continue in this manner. However, it is hoped that within the next several years much of this problem will be alleviated by the Section's own programming capacity and greater stability in the files resulting from completion of intake of gravida and the birth of all study children.

The last gravida was registered in December of 1965 and it is expected that the last child will be born in September of 1966. This greater stability of the data files will make it possible to create more fixed files and summary files for the purposes of studies and still maintain generality. This will in part alleviate some of the problems we have been facing.

III. PROPOSED FUTURE OBJECTIVES

We will continue to process the data records coming in and add these to the existing data files and data systems. The development of research proposals is expected to continue at approximately the same level but the types of studies will continue to increase both in scope and depth of analysis as more and more information is obtained concerning the problems under investigation. This has already been observed in the use of more refined statistical tools for analysis in studies completed during the year.

It is expected that by the end of the next coming fiscal year period the programming capacity of the Section will increase to a level that we can make substantial use of either the Bureau of Standards facilities or the facilities available at NIH and maintain and keep up to date our own data files. If we are thus able to offer a contractor an established, organized data file as a base to which all proposals for contract facilities can be made our efficiency will grow. However, this is dependent on the capacity of the Section's programmers and availability of equipment.

It is hoped that the Computation Branch's acquisition of the IBM 360, model 65, will permit adequate use of specialized data files, remote systems, etc. that will make it possible for the increased use of data in the study with faster turn-around. This also would make it possible to develop more sophisticated systems for data storage and retrieval.

H. SECTION ON PROJECT SERVICES

(I) Medical Literature

Report for Period July 1, 1965 through June 30, 1966

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS

The selected listing of journal literature and references on congenital malformations has been discontinued.

Special service for providing quick acquisition of needed literature from the NIH Library, which is not available at the PRB Reading Room, continues. During the past year, Medical Literature Services procured from NIH-NLM Library 106 books and journals for the use of PRB staff. In addition, there were 3,174 photocopies of reprints procured, as requested, by the various sections as shown below:

Office of the Chief	917
Section on Behavioral Sciences	251
Section on Epidemiology and Genetics	23
Section on Infectious Diseases	22
Section on Obstetrics	1,018
Section on Pathology	141
Section on Pediatric-Neurology	198
Section on Systems Design & Procedures	36
Section on Statistical Analysis	16
Medical Literature	528
Miscellaneous	24

PRB staff borrowed approximately 432 text books and journals from the Reading Room. About 800 persons visited the library.

During the past year, 35 new books and 9 new subscriptions to journals have been added to our shelves. The Reading Room now has a total of 514 books and 67 journals, including 6 abstracting periodicals. Our locator file has a total of 847 books giving information on medical and technical books available in PRB offices other than the PRB Reading Room.

Medical Literature Service continues to prepare two bibliographies:

1. Thyroid function and its relationship to fertility, pregnancy outcome (e.g., prematurity and mental retardation), and neonatal, mental and physical development.

2. Updating a listing of references representing clinical and laboratory research financially supported by the Collaborative Project on Cerebral Palsy, etc., NINDB.

The other bibliographies have been discontinued.

MLS reprint collection and retrieval system - There are over 3,300 reprints already on file. These are indexed and cross-indexed and a system for their utilization by PRB staff is already under way. This depository is made up of photocopy requests, bibliography requests, etc.

II. PROBLEMS

In January 1966, the Medical Officer in charge was transferred to the Office of the Chief to assist in more substantive literature review and digestion. In April 1966, a Supervisory Clerk was assigned to the section. The section consists of the following:

Supervisory Clerk

2 Scientific Reference Analysts (1 part-time, 1 full-time. The full-time analyst replaced the biologist who transferred to another branch.

2 Library Assistants (1 part-time, 1 full-time)

2 Clerk-Typists (1 part-time, 1 full-time. The full-time clerk-typist position is open and we are recruiting for a replacement).

Additional space is no longer a problem.

III. PROPOSED FUTURE OBJECTIVES

MLS will continue to be a supportive service to expedite for PRB staff, their needs in literature retrieval.

H. SECTION ON PROJECT SERVICES
(11) Information Service
Report for Period July 1, 1965 - June 30, 1966

An Information specialist and an editorial assistant have been assigned by the Institute Information Officer to assist the Perinatal Research Branch primarily in the areas of paramedical and public information.

Second Scientific Meeting

Because of broad interest in the Second Scientific Meeting of the Collaborative Project (March 24-25, Washington, D. C.) the Institute information staff, as well as members detailed to PRB, assisted with press arrangements. NINDB Information Office prepared a press kit (containing basic release, six backgrounders, special report by NINDB's director, statistical tables of the Project, and a copy of the Collaborative Reporter) arranged a press briefing prior to the meeting, and set up a press room with interviews during the meeting. Reporters covering the proceedings represented the Washington Post, Time, Changing Times, the National Observer, Drug Research Reports, Science Service, Medical World News, Modern Medicine, Medical Tribune, Antibiotics News, OB-GYN Observer and Baltimore station WBAL-TV. The Information Office also handled nearly 100 slides for scientific presentations and arranged for two scientific exhibits on brain pathology. Blake Cabot Associates taped the discussions as background for a proposed scientific film series based on NINDB-centered perinatal research. "Infections and Birth Defects," a new film describing the procedures of NINDB's Section on Infectious Diseases, was shown twice at the meeting. This 20-minute color film was produced by the Information Office, NINDB, and the Medical Arts and Photography Branch, DRS.

The Collaborative Project Reporter

The 8- to 12-page Collaborative Project Reporter is issued three times each year to Study staff (1600 copies). During this reporting period, issues 34, 35, 36 carried brief articles on Project Directors meetings, an outline of the program in genetics, abstracts of several major papers from the Study, resume's of articles about the Project in the popular press, and other items. Extra copies of the Reporter help in answering certain inquiries from press and public.

General Distribution of Information Materials

During the past year about 10,000 pamphlets and reports, including Institute-prepared folders on disease topics were mailed from the PRB Information Service. A 34-page brochure on the Project and related research was published by the Institute including 14 illustrations from participating medical centers. This was distributed to the special mailing list of 954 names maintained by PRB Information Service at request of these individuals interested in the Project.

Public and Press Inquiries

Hundreds of public and press inquiries were handled during the year, including correspondence from about 300 individuals and associations on topics pertinent to the program. The November 1965 issue of Parents magazine carried an article on the Study employing some background materials supplied by PRB. NIH "News Service to Professional Journals" for October 1965 distributed a 5-page illustrated feature on Project work to about 60 journals. Another pertinent article on the Study was drafted for and bylined by the Institute Director at the request of AMA's Today's Health magazine for early publication.

Other Work

During the past year, the PRB Information Service has obtained art, photographic prints and/or slides on about 400 charts and graphs of Project data. A newly assigned activity is the distribution and inventory control of sets of the Project basic document, composed of a synopsis and forms and manuals. These are being requested and used by other groups throughout the world. The sets are composed of 7 parts. 532 of the sections have been distributed during the past month.

The Information Officer is transferring to another Institute at NIH. A replacement may be considered.

I. SECTION ON STATISTICAL ANALYSIS
Report for period July 1, 1965 through June 30, 1966

SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS

During the past year, the Section on Statistical Analysis performed its various functions in connection with the following studies:

1. The association of maternal age and parity to pregnancy outcome and certain obstetric complications.
2. Cesarean section in labor.*
3. Spontaneous premature rupture of the membrane.
4. Fetal hazard after rupture of the membranes.*
5. The effects of uterine dysfunction and mechanical dystocia.*
6. The epidemiology of neonatal seizures.*
7. Predictors of organ weight at autopsy - a multivariate analysis.*
8. Prior pregnancy loss and present infant outcome.
9. The placental weight/birthweight relationship.*
10. The effects of the duration of labor on the fetus.
11. Complications of pregnancy in relation to the duration of labor.
12. Dilatation curves.
13. The assessment of infant motor and mental development.*
14. Early symptomatology and pregnancy outcome.*
15. The serological and clinical study of toxoplasmosis.
16. Neonatal pneumonia in liveborn infants.*
17. Organic heart disease in pregnancy.*
18. Reproductive wastage in bronchial asthma.*
19. The incompetent cervix.*
20. The relationship of certain demographic variables to the conditions and complications of pregnancy.*

*See footnote on next page

21. The prediction of birthweight - a multivariate analysis.*
22. Neonatal serum bilirubin levels and subsequent neurologic deficits.
23. Measures of fetal heart rates in relation to pregnancy outcome.
24. Marginal sinus rupture.
25. The effects of birth injuries.
26. Placental anomalies.*
27. Early signs of neurologic disorders.
28. Glomerulonephritis in pregnancy.
29. Retinal hemorrhages in the newborn.
30. The effects of maternal diabetes.
31. Certain socio-cultural parameters in relation to pregnancy outcome.

*Denotes that one or more members of the section were authors of papers, currently published, in press, or in preparation, arising from these studies.

PROBLEMS

The major problem of the moment is the incompleteness of the Collaborative Project data file. Certain studies are severely hampered by missing data, and the use of certain mathematical techniques is restricted because of the necessity to estimate unknown parameters.

The Section understands that the data file will be brought up to date in July, 1966; this should permit a strengthened approach to many of the studies undertaken by the Collaborative Project.

FUTURE PLANS

In all probability, the soundest source material developed in the course of the Collaborative Project lies in the several thousands of "repeat" cases - that is, the women who have been under observation in study hospitals during two or more pregnancies.

For one thing, these cases permit an evaluation of the reliability of interview, and in some instances, clinical data. More importantly, these cases provide a sample in which, in large measure, each woman is her own control.

The Section advocates strongly that these cases be used as the basis for a wide variety of studies, and proposes to proceed, so far as it is able, to develop and undertake studies involving these pregnancies.

The Section is currently engaged in abstracting material from case records for particular studies of various conditions and complications of pregnancy. This undertaking serves several purposes:

It provides data that are sounder than can be obtained from a computer scanning of screening forms, since diagnoses are verified; it permits the obtaining of fairly complete data; it allows close definition of variables; and it is generally more economical than comparable operations performed by computer.

The Section suggests, and is prepared to participate in, the review of case records to provide a close definition of conditions and complications of pregnancy and of outcomes, so that suitable cohorts may be obtained for studies of etiologies and relationships.

REMARKS

The Section on Statistical Analysis, during the past year, continued its research into the devising and application of mathematical methodologies to Study data, as has been discussed in earlier reports. It should be pointed out that the complexity of a technique does not insure its utility; and that the nature of the data of the Collaborative Project makes the cross-classification the most utilitarian tool. While some of the more sophisticated approaches to Project data have been applied, and have, within limits, produced useful results, the extraordinary number of variables measured in the Study requires, as a necessary first step, the continued development of frequency distributions and cross-classifications.

1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Epidemiology of Infectious Diseases in the Collaborative Study on Cerebral Palsy, Mental Retardation, and other Neurological and Sensory Disorders of Infancy and Childhood.

Previous Serial Number: Same

Principal Investigators: Dr. John L. Sever
Dr. David Fuccillo
Dr. Robert J. Huebner

Other Investigators: Dr. Akinyele Fabiyi
Dr. Gary Gitnick
Dr. Lon White
Dr. Richard Hildebrandt
Anita Ley
Flora Wolman
Renee Traub
Mary Ruth Gilkeson
Collaborative Institutions in the Perinatal
Research Study

Cooperating Units: NIAID, Laboratory of Infectious Diseases
(Dr. Robert J. Huebner)
NICHD, (Dr. Lon White)
MBA - Dr. Gabriel Castellano
Cooperating Institutions in California
(with the Section on Infectious Diseases)

Man Years:

Total: 10
Professional: 2.5
Other: 7.5

Project Description:

Objectives:

To utilize new serologic techniques in an intensive study of viruses, protozoa, PPLO, and bacteria to determine their role in the production of birth defects and related abnormalities. To develop, wherever technically possible, the

serologic methods necessary for a large scale investigation of the natural course of the disease as caused by infectious agents. To supplement the serologic approach with direct isolation of the microorganisms involved and appropriate immunological studies.

Methods Employed: Serologic techniques, including complement fixation tests, hemagglutination-inhibition, hemadsorption, viral neutralization and tissue culture neutralization tests are now available for the identification of over 130 viral, protozoal, and bacterial infections.

The first phase of this project has been the production and standardization of antigens and antisera for the performance of the serological tests. The viral agents produced under the research and development contract with Microbiological Associates, Inc., in consultation with the Virology Sections, LID and NINDB, together with the direct purchase of viral reagents constitutes the materials utilized for the serological investigations. These reagents are essential for the continuation of the studies.

The second phase of this project has been the development and utilization of an integrated laboratory facility, employing and training technicians capable of handling large scale testing and development of new serological methods.

The third phase of the project is now to utilize the extensive serological data available from the tests in conjunction with the clinical information available from the Perinatal Research Study to determine the possible relation between the infecting agents and the outcome of the pregnancy or abnormalities of the individuals.

Major Findings: A total of 120 reactive antigens have been developed. Approximately three-fourths of the antigens have been thoroughly evaluated and are now being applied in routine testing of study sera. The development and maintenance of large quantities (1,000 ml) of satisfactory antigens for 45 viruses is an integral part of the investigation being carried on by the study. The other antigens are receiving intensive developmental work and 20 of these antigens are under test for specificity. Specific control antisera have been prepared from 85 microorganisms. In addition, to provide improved safety, extensive work has been conducted on the inactivation of the live virus antigens.

The serological studies are being conducted in accordance with three major study designs:

First, epidemiological studies to determine the frequency of virus experience among study populations. Specimens from representative patients at study hospitals are being tested for evidence of antibody. By testing these specimens, it is possible to establish the frequency of antibody and change in antibody titer to each virus. The data for each hospital is then analyzed in relation to other information from the Collaborative Perinatal Study, and relation to other information from the Collaborative Perinatal Study, and epidemiological

data concerning the occurrence of abnormal pregnancies and children.

The second and most active category of study design involves the selection of special pregnancy outcomes for intensive testing. Studies of this type have involved, for example, the testing of sera from all study patients for antibody to toxoplasmosis and special investigations of rubella. Other studies have included specific patients who have had unusual obstetrical histories or defective children for whom special serological testing is indicated. Intensive studies are now being conducted utilizing antigens for Influenza A, Mumps, Cytomegaloviruses, Herpes Simplex and Rubella.

Third, studies are designed to obtain maximum data concerning the virus experience of patients with abnormal pregnancy outcomes, and patients who are "matched controls". The results of this type of study are then analyzed in terms of differences in frequency of antibody among the abnormal and matched controls. The matching of the patients include factors which are known to influence virus experience, such as time of the year during which the specimen was obtained, race, age, number of living children in the family, and geographic location of the patients. These studies are conducted when a sufficient number of abnormal of a particular type have been identified so that statistical analysis might establish valid information. The initial studies were directed at abnormalities which are relatively frequent, such as abortions, stillbirths, and neonatal deaths. The less frequent abnormalities or those which cannot be recognized in infancy or early childhood are being studied as greater numbers of these patients are identified in the Collaborative Study population.

The data from the three types of study design outlined above is analyzed with the assistance of statisticians in the Section, Branch, Institute, and Institutes. All data is analyzed daily for the reproducibility of the testing itself. A minimum standard of 90% reproducibility is required for all testing.

Studies to date have demonstrated information concerning the extent of virus experience in the study population. These investigations indicated at least 22% of the pregnant study population tested had evidence of exposure to one or more of the 120 agents which have been studied. The study of the effect of the 1964 epidemic of rubella on the Collaborative Study Population is being continued with continued analysis of clinical data. Other studies are directed towards investigations of prevalence of virus experience in the population under surveillance. In addition, specific studies of infection among patients whose pregnancy terminated in an abnormal outcome are also being conducted. Studies are being directed toward abnormal pregnancy outcomes which can be recognized shortly after birth, such as abortions, stillbirths, and neonatal deaths and congenital malformations (including congenital heart defects). Several viruses have been found to occur in high frequency in specific patients with abnormal pregnancy outcomes. Particular emphasis in these studies relate to the Coxsackie B viruses.

Serum specimens from pregnant women participating in the Collaborative Perinatal Research Study at 12 hospitals located throughout the United States were

tested for antibody to toxoplasmosis . The tests employed a micro modification of the hemagglutination technique of Jacobs and Lunde. Of patients studied, 38.4% demonstrated titers of 32 or greater, 11.7% had titers of 256 or greater, and 2.2% had titers in the range considered to be "suspect" for recent or active toxoplasmosis. Significant increases in antibody during pregnancy were identified for approximately 2% of the patients and for one tenth of this group, there was evidence for primary exposure. Antibody titers were correlated with the age and race of the patients. There was a suggestion of a relation between high antibody titers in the mothers and prematurity as well as low mental and motor performance in the children. Studies are being conducted on the association between multiple sclerosis and antibody to measles virus. In addition, several other viruses were tested in the clarification of the role, if any, in the etiology of multiple sclerosis. Complement fixation, hemagglutination inhibition and neutralization tests are conducted with the cerebrospinal fluid and sera of multiple sclerosis patients and controls.

Collaborating Studies:

The Cooperative Perinatal Study (Oakland, California) is collaborating in a serological study of the role of infection in the perinatal period. This study now involves over 17,000 patients and provides valuable first trimester blood specimens which are essential for studies of abortion and congenital malformation.

The Perinatal Study (Los Angeles Kaiser Hospital) also cooperates in the serological and virological study of perinatal infection and has provided sera and other specimens for over 6,000 patients.

Significance to the Program of the Institute:

The use of the micro serological techniques for a large group of new viruses provides an opportunity to investigate the course of human disease caused by viruses which are either difficult to isolate or are resistant to evaluation because the clinical effects are delayed until a long time after infection has subsided. This is particularly true in the case of birth defects. The application of this tool for analysis is providing valuable information on the epidemiological aspects of virus infections.

Proposed Course of the Project:

The serological program will be expanded in terms of antigenic materials and the collection of sera. New study arrangements for investigation of viral and genetic causes of abortions are being developed.

As additional abnormal pregnancy outcomes are reported, these will be added to existing studies on abortions, stillbirths, neonatal deaths, and congenital malformations.

The initial "leads" obtained by the present serological testing are being

explored in detail with the use of expanded serological investigations as well as other techniques of virology and by new study designs involving the intensive sampling of particular groups. It is already clear that supplemental sampling will be necessary for patients who register early in the first trimester of pregnancy and for those who have had repeated abnormal pregnancies. Arrangements are being developed for obtaining full sets of serum specimens and data for patients who abort or have stillbirths. Specimen collection for virus studies has been expanded to include tissue samples from placentas, abortuses, stillbirths, and neonatal deaths so that direct isolation of viral agents is included to confirm and extend the serological findings.

The existing research and development of virus diagnostic reagents will continue in the following 5 main areas: (1) The development of 120 virus antigens and testing of specificity; (2) the development of 60 specific control antisera for use in the serological studies; (3) the development of improved methods of storage of antigens and antisera prepared under this contract; (4) the preparation of specific antigens which require special developmental work each time they are prepared because of variation in the biological systems used; and (5) the preparation of histological sections of tissues prepared by the Section on Virology.

Other studies will be conducted in cooperation with the Epidemiology Branch, NINDB. More intensive serological studies will be initiated on the role of viruses in the etiology of infectious mononucleosis.

Honors and Awards: None

Publications:

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1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS - NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Clinical Investigations in Human Volunteers and other Populations of Virus Effects and Production of Prototype Human Antisera and Vaccines

Previous Serial Number: Same

Principal Investigators: Dr. John L. Sever
Dr. Gary Gitnick
Dr. Akinyele Fabiyi
Dr. Robert J. Huebner

Other Investigators: Dr. Richard J. Hildebrandt
Dr. Lon White
Dr. David Fuccillo

Cooperating Units: NIAID, Laboratory of Infectious Diseases
(Dr. Robert J. Huebner)
NICHD (Dr. Lon White)
NINDB, Epidemiology Branch
(Dr. Jacob Brody)
Bureau of Prisons, Department of Justice
Mr. Myrl Alexander, Director
Dr. Charles E. Smith, Medical Director
Petersburg Reformatory

Chanute Air Force Base
United States Air Force Hospital
Chanute, Illinois
Captain John R. Baringer

Naval Medical Research Unit No. 4
Great Lakes Hospital
Great Lakes, Illinois

Dr. Paul McCallin, Honolulu, Hawaii
Dr. Dolores Mendez-Cashion, San Juan, Puerto Rico
Dr. Janet Hardy, Baltimore, Maryland
Dr. Sheldon B. Korones, Memphis, Tennessee
Dr. Frank R. Lock, Winston-Salem, North Carolina
Dr. Dwayne M. Reed, Anchorage, Alaska

Man Years:

Total: 4.5
Professional: 1.0
Other: 3.5

Project Description:

Objectives:

To study the effects of specially selected viruses on human volunteers and other populations to determine the pathogenicity of these agents (as rubella), the immunity which is developed. To develop prototype human antisera for controlled virus studies as in above and project (Serial No. NDB(CF)-62 PR/ID 972). To study the efficacy of prophylactic and therapeutic materials for the prevention and control of infectious diseases.

Methods Employed:

Human volunteer studies are conducted in collaboration with the Federal Bureau of Prisons. These studies are reviewed and approved by the Clinical Research Committee and the Medical Board of the National Institutes of Health. Additional study arrangements were developed for studies of epidemics and vaccines.

Major Findings:

Several new volunteer studies were initiated to study: 1) the efficacy of killed vaccines for rubella 2) the value of Amantadine for the control of rubella. These studies are now being analyzed.

Investigations of virgin epidemics in Alaska provided valuable data on the spread of epidemics of mumps, the clinical findings in the disease.

A joint study of rubella convalescent gamma globulin is in progress during the epidemic of rubella in Hawaii and Puerto Rico. Combined clinical and serological data will be available.

Significance of the Project:

Volunteer studies provide a valuable contribution to the study of infectious agents and the development of specific immune sera. The studies of epidemics and affected individuals have permitted the clarification of disease patterns which are important to the prevention and control of infections of significance in the perinatal period and later.

Current studies of gamma globulin should provide valuable information on the usefulness of this material.

Proposed Course of the Project:

Additional studies are necessary on the value of new vaccines for the prevention of rubella. In addition, quantities of specific human sera are necessary for investigations of cytomegaloviruses and other agents. Further investigations of epidemics of agents important in the perinatal period will be conducted.

Further studies of titered gamma globulin are to be conducted to establish optimal conditions for the use of this material. In addition, the effectiveness of new chemotherapeutic agents is being studied. Other studies with viruses being investigated in the Collaborative Perinatal Research Program will be continued.

Honors and Awards: None

Publications:

Sever, J. L., Huebner, R. J., Castellano, G. A., Sarma, P. S., Fabiyi, A., Schiff, G. M., and Cusumano, C. L.: Rubella Complement Fixation Test, SCIENCE, 148: 385-387, April 16, 1965.

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Korones, S. B., Ainger, L. E., Monif, G. R., Roane, J., Sever, J. L., and Fuste, F.: Congenital Rubella Syndrome: Study of 22 Infants, Am. J. Dis. Child., 110:4, 434, October 1965.

Schiff, G. M., Sever, J. L., and Huebner, R. J.: Experimental Rubella, Clinical and Laboratory Findings, A. M. A. Arch. Intern. Med., 116:537, October 1965.

Schiff, G. M., Smith, H. D., Dignan, P. S., and Sever, J. L.: Rubella: Studies on the Natural Disease, Am. J. Dis. Child., 110:366, October 1965.

Avery, G. B., Monif, G., Sever, J. L., and Leikin, S. L.: Rubella Syndrome after Inapparent Maternal Illness, Am. J. Dis. Child., 110:444, October 1965.

Sever, J. L., Huebner, R. J., Fabiyi, A., Monif, G. R., Castellano, G., Cusumano, C. L., Traub, Renee, Ley, Anita C., Gilkeson, Mary R., and Roberts, Jean: Antibody Responses in Acute and Chronic Rubella, Proc. Soc. Exp. Bio. Med., To be Published.

Monif, G. R. G., and Sever, J. L.: Hepatic Dysfunction in the Congenital Rubella Syndrome, Brit. Med. J., To be Published.

1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Experimental Animal, Tissue Culture, Histopathological and Serological Investigations of the Role of Viruses and Other Micro-Organisms in the Perinatal Period.

Previous Serial Number: Same

Principal Investigators: Dr. John L. Sever
Dr. Richard J. Hildebrandt
Dr. Robert J. Huebner

Other Investigators: Dr. Akinyele Fabiyi
Dr. Gary Gitnick
Dr. Lon White
Renee Traub
Flora Wolman
Pernell Crockett
Jean Roberts
Nannette Ratner
Frank West

Cooperating Units: NIAID, Laboratory of Infectious Diseases
(Dr. Robert Huebner)
NICHD (Dr. Lon White)
NINDB, Laboratory of Perinatal Physiology, San Juan,
Puerto Rico (Dr. Ronald E. Myers, Dr. Gilbert W.
Meier)
NINDB, PRB, Section on Pathology
(Dr. Toshio Fujikura, Dr. Lewis Lipkin)

Man Years:

Total: 12
Professional: 3.0
Other: 9.0

Project Description:

Objectives:

- A. Infect pregnant and non-pregnant animals of a number of different

species with various viruses and other microorganisms to determine the effect of these agents on the animals and the fetal tissues.

B. Attempt to recover infectious agents responsible for abnormal fetal development.

C. Correlate A and B with histopathological findings.

D. Correlate A, B, and C with serological findings.

Methods Employed:

An investigation of the role of viruses and other microorganisms in the perinatal period by the combined use of experimental animals; tissue culture techniques; histopathological studies; and serological testing:

A. Pregnant and non-pregnant animals of various species including monkeys are being infected artificially with viruses and other microorganisms and are being observed for evidence of diseases and/or effect on fetal tissues. Some of these animal studies have been conducted in cooperation with the Laboratory of Perinatal Physiology.

B. Virus isolation investigations utilizing new tissue culture techniques to study the location of virus infection produced (part A) and to recover viruses from tissues of experimental animals.

C. Collaborative histopathological studies are conducted on specimens obtained in A and B.

D. Extensive serological studies are conducted with the many viral antigens developed for the collaborative study (Serial No. NDB(CF)-57 PR/ID 402) and new antigens with materials being studied in A, B, and C.

Major Findings:

Animal investigations with pregnant ferrets demonstrated that the inoculation of a tissue culture grown rubella virus early in gestation produced runting and abortion. Neutralizing antibodies developed in all inoculated animals. Virus was recovered from the infected animals from the 12th day through the 20th day.

The availability of this animal model system for experimental rubella may be of particular value in the development of vaccines for rubella and the mechanism of pathogenesis of the disease. In addition, studies with newborn ferrets were undertaken to determine the response of this mammal to experimental rubella infection. Tissue culture propagated rubella virus (RV) was inoculated intramuscularly into groups of 2 day old puppies. A chronic infection was produced which lasted 6 to 8 weeks. This infection is markedly similar to the chronic infection found to occur in man.

Similar studies are now under way with mumps and herpes simplex. New isolation facilities are available for these animals.

Significance to the Program of the Institute:

The broad program of experimental animal, tissue culture, histopathological investigations compliments the strict serological approach being used with human sera (Project Serial No. NDB(CF)-57 PR/ID 402) and presents a balanced investigation of the role of viruses and other microorganisms in the perinatal period. It provides a means of integrated direct experimental investigation of microorganisms which may contribute to perinatal pathology.

The information already gained on rubella, through these studies, opens the possibility of the development of much more information on this disease and its teratogenic effects; possible development of a vaccine; and the production of hyperimmune rubella gamma globulin.

Proposed Course of the Project:

Further studies of experimental rubella and other infections of ferrets are in progress. Investigations with a new vivostatic drug are being initiated in these model systems. Vaccine studies are also being conducted in experimental animals.

Salivary gland virus, herpes simplex, mumps, and PPLO's are also being studied in experimental animal models.

Honors and Awards: None

Publications:

Sever, J. L., Monif, G. R. G., Cusumano, C. L., Schiff, G. M., and Huebner, R. J.: Clinical and Subclinical Rubella Following Intradermal Inoculation of Rubella Virus into Human Volunteers, Am. J. of Epid., to be published.

Monif, G. R. G., and Sever, J. L.: The Pathogenesis of the Teratogenic Effect of Rubella Virus, Obstet. & Gynec., to be published.

1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Isolation of Infectious Agents from Tissues and Chromosomal Studies.

Previous Serial Number: Same

Principal Investigators: Dr. John Sever
Dr. Jack Singer
Dr. Richard Hildebrandt
Dr. Robert Huebner

Other Investigators: Linda Carter
Blanche Anderson
Renee Traub
Jean Roberts
Flora Wolman
Pernell Crockett

Cooperating Units: NICHD (Dr. Jack Singer, Linda Carter)
National Naval Medical Center
(Departments of Obstetrics and Pathology)

Man Years:

Total: 6.0
Professional: 1.5
Other: 4.5

Project Description:

Objectives:

To utilize new techniques for the isolation of infectious agents from fetal and adult tissues; and to conduct studies of the chromosomes of aborted and term fetal tissues as combined efforts to obtain direct information on the causes of abnormal pregnancy outcomes and pathologic findings.

Methods Employed:

Tissue specimens from fetuses and adults are being obtained from collaborating institutions and physicians. These specimens are being

studied intensively in laboratory techniques including tissue culture and histopathology and in some cases, electron microscopy.

Major Findings:

Rubella virus was isolated from several children with cytogenetic abnormalities and cytomegaloviruses were isolated from 2 of 40 fetuses studied. These studies are being extended.

Significance of the Project:

Direct isolations of agents from involved tissues provide valuable data on the probability role of the agent as the cause of the defects. Considerable new information has recently been obtained in this way for rubella.

Proposed Course of the Project:

A full time cytogeneticist will be brought into the Section for expanded studies of the genetic aspects of defective pregnancies. New studies are needed concerning the role of mumps in endocardial fibroelastosis.

Awards and Honors: None

Publications:

Monif, G. R. G., Avery, G. B., Korones, S. B., and Sever, J. L.: Postmortem Isolation of Rubella Virus from Three Children with Rubella Syndrome Defects, Lancet, 723-724, April 3, 1965.

Monif, G. R. G., Sever, J. L., Schiff, G. M., and Traub, R. G.: Isolation of Rubella Virus from Products of Conception, Am. J. Obstet. and Gynec., 91: 8, 1143-1146, April 15, 1965.

Monif, G. R. G., and Sever, J. L.: The Pathogenesis of the Teratogenic Effect of Rubella Virus, Obstet. & Gynec., to be published.

Monif, G. R. G., and Sever, J. L.: Chronic Infection of the Central Nervous System with Rubella Virus, Neurology, 16: 111, 1966.

Korones, S. B., Ainger, L. E., Monif, G. R. B., Roane, J., Sever, J. L., and Fuste, F.: Congenital Rubella Syndrome: New Clinical Aspects with Recovery of Virus from Affected Infants, J. Pediat., 67: 2, 166-181, August, 1965.

1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

FHS - NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Toxoplasmosis: Serological and Clinical Studies.

Previous Serial Number: ~~SB~~ (CF)-65 PR/PN 1270

Principal Investigator: John L. Sever, M.D., FRB, NINDB

Other Investigators: Joseph S. Drage, M.D., FRB, NINDB

Cooperating Units: Perinatal Research Branch, NINDB

Man Years:

Total: 2/12

Professional: 1/12

Other: 1/12

Project Description: This study relates rises in antibody titer to abnormal pregnancy outcomes. Within the Pediatric Neurology Section, a hand review was completed on 128 cases that had shown various degrees of titer rise. Within the group of 47 patients with titer elevations of greater than 4096, or significant increases in antibody titer, five were found to have definite toxoplasmosis and ten were suspected of having toxoplasmosis. The ten included six with motor retardation, two pregnancies resulted in stillbirths, and two in neonatal deaths. The sera from ten of the remaining 32 apparently normal children were tested for antibody to toxoplasmosis and one was found to have a high titer. Study in progress.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS - NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Maternal Rubella and Pregnancy Outcome.

Previous Serial Number: NDB (CF)-65 PR/PN 1271

Principal Investigator: John L. Sever, M.D., FRB, NINDB

Other Investigators: Karin B. Nelson, M.D., FRB, NINDB
Mary R. Gilkeson, FRB, NINDB

Cooperating Units: Perinatal Research Branch, NINDB

Man Years:

Total: 2/12

Professional: 1/12

Other: 1/12

Project Description: Study continues, in conjunction with the Section on Infectious Diseases, on the consequences of the rubella epidemic of 1964. Analysis of pediatric outcome through the first month of life of children born to women in the Study who had experienced clinical rubella or were exposed to it in the first trimester, was completed and published. Among the data reported were exposure and incidence rates in this pregnant group, the incidence of congenital anomalies recognizable through the first month of life, occurrence of the newly described chronic infection of the newborn, evidence relating to the occurrence of inapparent infection in twice as many women as suffered clinical disease, the efficacy of gamma globulin prophylaxis, and the limitation of that efficacy by the fact that fewer than half of the women were aware of the exposure which preceded their clinical illness.

Plans are now in progress for the second phase of this work, which will be the follow-up at the age of one year of the children of the 6161 women who were enrolled in the Study over the period of the epidemic. Because some of the abnormalities associated with congenital rubella cannot be identified in the neonatal period, it is anticipated that this follow-up will materially alter estimates of risk derived from the previous report. For instance, cataracts, hearing defects, and microcephaly may not be evident in the neonate, and in fact may only be developing during the first month of life. Congenital heart disease may be over or under diagnosed in the neonate. Examining the same sample at one year should permit the identification of these abnormalities.

Use will be made in this analysis of the one-year diagnostic summary and the psychological test scores at eight months of age, so that the occurrence of visual and hearing defects, of congenital abnormalities, of poor performance on psychological testing, can be compared for one-year olds whose mothers did, and for those whose mothers did not, have evidence of rubella experience by clinical history or serological testing. It is hoped by this means to arrive at a more accurate estimate of the toll in life and health taken by rubella.

Honors and Awards: None

Publications:

Sever, John L., M.D., Nelson, Karin B., M.D., and Gilkeson, Mary R.: Rubella Epidemic, 1964: Effect on 6,000 Pregnancies. Amer. J. Dis. Child. 110:4, 395-407, Oct. 1965.

1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Rubella Vaccine Development Program

Previous Serial Number: None

Principal Investigators: Dr. John L. Sever
Dr. Akinyele Fabiyi
Dr. David Fuccillo
Dr. Lon White
Dr. Gary Gitnick
Dr. Robert J. Huebner

Other Investigators : Renee Traub
Flora Wolman
Pernell Crockett
Nannette Ratner

Cooperating Units : NIAID, Laboratory of Infectious Diseases
(Dr. Robert J. Huebner)
NIAID, Vaccine Development Branch
(Dr. Daniel Mullally)
NICHD (Dr. Lon White)

Man Years:

Total: 8.5
Professional: 2.0
Other: 6.5

Project Description:

Objectives:

Collaborate with the Vaccine Development Branch, NIAID, in studies on the development of killed and attenuated live rubella virus vaccines. Provide project surveillance for contracts awarded by the National Institutes of Health for rubella vaccine work. Provide intramural guidance, support, competence, and laboratory studies as well as conduct volunteer studies in conjunction with this program.

Methods Employed:

The Vaccine Development Branch, NIAID, is supporting 10 contracts relating to research on the development of rubella vaccines. Eight contracts are with major pharmaceutical manufacturers and two are materials and services research and support contracts.

For this program contract, Project Officers are assigned from the Section; reference specimens, sera and testing is provided; experimental animal studies are conducted; and volunteer studies are performed with candidate materials.

All studies are conducted in conjunction with the Vaccine Development Program, NIAID.

Major Findings:

New methods for increasing the titer of rubella virus have been developed by varying physical conditions of growth. Inactivation studies using formalin, ultraviolet and beta-prone are in progress. Reference virus pools and antisera have been prepared and distributed. Cell pack antigens are being developed. Candidate attenuated strains are being tested.

Significance of the Project:

The prevention of rubella in pregnant women would eliminate the fetal damage caused by this infection.

Proposed Course of the Project:

The rubella vaccine development program will be expanded under the Vaccine Development Program. Testing of virus seed pools, candidate oral vaccines, killed vaccines, and subparticle vaccines will be conducted in our laboratories and in the volunteer program at the Petersburg Federal Reformatory.

Honors and Awards: None

Publications:

Fabiyi, A., Sever, J. L., Ratner, N., and Caplan, B.: Rubella Virus: Growth Characteristics and Stability of Infectious Virus and Complement Fixing Antigen, Proc. Soc. Exp. Biol. and Med., to be published.

Sever, J. L., Meir, G. W., Windle, W. F., Schiff, G. M., Monif, G. R. G., and Fabiyi, A.: Experimental Rubella in Pregnant Rhesus Monkeys, J. Infect. Dis., 116: 21, 1966.

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: An Instrument For The Conduct Of A Retrospective Study Of Seizures, Cerebral Palsy, Mental Retardation And Other Neurological And Sensory Disorders Of Infancy And Childhood.

Previous Serial Number: Same

Principal Investigators: Z.A. Shakhashiri, M.D.
Leonard V. Phelps

Other Investigators: Lenore Bajda, M.D.
John R. Day, M.D.
Blanche L. Vincent, S.N.O.
Zula C. Meekham, B.S.N.
Rose R. Tortorella

Cooperating Units: Georgetown University Hospital, Retarded Children's Clinic, Selected Maternity Hospitals and Physicians in Metropolitan Washington.

Man Years:

Total:	3.10
Professional:	2.00
Others:	1.10

Project Description:

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

Methods employed: Recognized damaged outcomes of pregnancy, such as seizures, diplegias, hemiplegias and choreoathetoids are to be studied and related to defined perinatal or postnatal events. These outcomes were selected because they were construed to be related to or manifestations of or involved in the biological or psycho-sociological mechanism underlying the following hypotheses; (1) anoxia, (2) toxic influences on the brain, (3) metabolic influences, (4) trauma to the head,

(5) infection of the brain, (6) dehydration of the child, (7) genetic or familial patterns and (8) socioeconomic status.

Proposed Course of the Project: The pretest of the Instrument is now continuing in progress. More than 300 clinic records of mentally retarded children at Georgetown Hospital, Retarded Children's Clinic have been medically abstracted and constitute the partial completion of data concerning the damaged child. Socio-economic information has been collected for the families of the damaged cases, sib and non-sib controls. Part of the abstraction of the maternity and nursery records covering the 300 damaged cases, sib and non-sib controls has been completed.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Revision and Expansion of Previous Project entitled A Commentary on the Appropriateness of The Use of Certain Tabular Data, For Formulating Generalizations Concerning Populations in the Same Cities As Those In Which The Collaborative Study on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood Is Being Conducted.

Previous Serial Number: Same

Principal Investigators: Z. A. Shakhshiri, M.D.
Leonard V. Phelps

Other Investigators: None

Cooperating Units: The Census Bureau and the National Center for Health Statistics cooperated in the furnishing of necessary statistical information for the United States and cities.

Man Years:

Total:	1.60
Professional:	1.00
Other:	.60

Project Description:

Objectives: To expand and revise the original project so that the statistical information previously made available would be provided for a more extended time period and in a more detailed fashion; the purpose to formulate generalizations concerning populations in the same cities as those in which the Collaborative Study on Cerebral Palsy, mental retardation and other neurological and sensory disorders of infancy and childhood is being conducted.

Methods Employed: The statistical information included both numeric tables and charts for 11 of the cities in which the Collaborative Study is being conducted to cover both the Collaborative Study population and the population of the city in which each Study hospital is located. It included the number of live births, deaths under one year and death rates per 1,000 live births; deaths under 28 days of age, 1-11 months and corresponding death rates for 1,000 live births; stillbirth-neonatal death rates per 1,000 live births; live births of 2,500 grams or less per 1,000 live births; for Study hospital population, deaths and death rates of infants under 24 hours of age and also

under 7 days of age; these data are not available for the cities. Number and percent distribution of the female population 15-44 years of age in the cities (1960 Census) and number of women registered in the Collaborative Study. All of this information is shown according to color, white and non-white. Some tables are included for sex and color.

These data constitute a ready reference of statistical information pertaining to the infant death rates, numbers of deaths and percent distributions by color. This effort might warrant publication as soon as all tables are studied and appropriate literature reviewed.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Cesarean Section in Labor

Previous Serial Number: None

Principal Investigator: Gilbert J. Vosburgh, M.D.
Columbia University, New York

Other Investigators: M. J. Gates, M.D., Columbia University
H. Berendes, M.D., PRB, NINDB
J. Deutschberger, PRB, NINDB
N. Lipko, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years: Total: .05
Professional: .04
Other: .01

Project Description:

Data are presented on the pregnancies and their outcomes of 459 women delivered by primary Cesarean Section undertaken after the onset of labor. The cases are grouped according to indication, and comparisons are made among groups.

Except in one instance, every fetal death occurred in a case in which the indication for the section was a placental and/or a cord condition, as, for example, premature separation, previa, or cord prolapse. Neonatal losses, on the other hand, were related to a wider range of indications, but occurred with greatest frequency among those cases in which the indication was fetal distress.

The children born of the pregnancies under study were classed as to whether or not they suffered fetal distress. Those cases with fetal distress showed a higher rate of newborn morbidity than those without. Of seven children who had definite neurological abnormalities at one year of age, all but one were in the fetal distress group.

Specific neurological findings for these children are discussed in relation to fetal distress, its severity, and associated maternal conditions.

Honors and Awards: None

Publications:

Westphal, M.C., and Joshi, G.B.: The interrelationship of birth weight, length of gestation, and neonatal mortality. Clin. Obstet. Gynec. 7: 670-686, Sept. 1964. (This publication resulted from Research Project NDB (CF) 63 PR/0 which was completed during January 1, 1963 - June 30, 1964 annual report period.)

Serial No. NDB (CF)-66 PR/OC 1369

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS--NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Spontaneous Premature Rupture of the Membrane

Previous Serial Number: None

Principal Investigator: R. S. Sappenfield, M.D.,
Charity Hospital, New Orleans, La.

Other Investigators: J. P. Mule, Charity Hospital, New Orleans, La.
H. Berendes, M.D., PRB, NINDB
W. Weiss, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	.05
Professional:	.04
Other:	.01

Project Description:

Review of the data from the literature and from Charity Hospital in New Orleans suggested that an increased rate of perinatal morbidity and mortality is associated with the occurrence of spontaneous premature rupture of the membrane during pregnancy. This study describes an attempt to confirm and better define this association and to delineate factors related to increased incidence of premature rupture.

The operational definitions chosen for this study included those patients with premature rupture of the membrane before or after one hour preceding the onset of labor. This definition was chosen in relation to difficulties of establishing the onset of rupture and the onset of labor. Patients with induced labor followed by spontaneous or artificial rupture of the membrane and artificial rupture followed by spontaneous or induced labor were removed from further consideration because the interference made it impossible to define their status as to the natural occurrence of spontaneous premature rupture. Other limitations of this study will be presented.

In order to consider birthweight and gestation time when considering the association of spontaneous rupture (premature) to pregnancy outcome, a formula of:

No. with premature rupture
No. delivered

within the various gestational or birthweight groups was used. This formula allows the influence of other possible causes of premature labor or low birthweight to be demonstrated along with the influence of premature rupture.

When the associations with the increased occurrence of premature rupture were studied, a formula of:

$$\frac{\text{No. with premature rupture occurring during a given gestational period}}{\text{Total number of pregnancies exposed to that period of gestation}}$$

was used.

This last rate is believed to be a better estimate of the incidence of premature rupture and removes the effects of other possible causes of premature labor.

In view of the data received thus far from the Collaborative Study, this allows us to describe the following relationships:

a. The relative degree of association of spontaneous premature rupture of the membrane to low birthweight and short gestation, thus indicating the relationship to "high risk" infants.

b. The association of spontaneous premature rupture with congenital anomalies or abnormal presentation in the infant.

c. The consistency of the above associations among the different population groups by race, maternal age, parity and other maternal complications.

d. An estimate of the possible advantage if premature rupture could be prevented and if prevention of premature rupture could prevent premature labor.

e. The relationship of rate of occurrence of spontaneous premature rupture to gestational interval.

f. The effect of race, parity, maternal age and other maternal complications or factors associated with these variables on the rate of occurrence of spontaneous premature rupture at various gestational intervals.

g. To indicate field in which study of the etiology of premature rupture is likely to be effective.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Fetal Hazard After Rupture of the Membranes

Previous Serial Number: None

Principal Investigator: Frank Shubeck, M.D.,
University of Oregon, Portland, Oregon

Other Investigators: R. C. Benson, M.D., University of Oregon
W. W. Clark, Jr., M.D., University of Oregon
H. Berendes, M.D., PRB, NINDB
W. Weiss, PRB, NINDB
J. Deutschberger, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.6
Professional:	0.5
Other:	0.1

Project Description:

Extended duration of the interval between the rupture of the membrane and delivery has been suspected of increasing the hazard to the fetus.

The hypothesis suggests that the extended interval increases the degree of neutrophilic infiltration and infection of the placenta, thereby increasing mortality and morbidity in the product of the pregnancy.

This study reports on the associations of the length of this interval, for spontaneous and artificial ruptures, with pregnancy outcomes such as perinatal mortality, birthweight, infection and respiratory abnormality in the newborn; characteristics of the gravidae, such as race, age, parity, duration of labor, and prior pregnancy outcome are assessed in relation to the duration of the interval, as are complications of pregnancy such as vaginitis, urinary tract infection, fever and neutrophilic infiltration of the placenta in the mother.

Most of the associations are demonstrated to be strongly linearly associated with the duration of the membrane rupture.

The association of duration of interval with increasing percentage of cases with neutrophilic infiltration is particularly strong: each increase of one hour in duration of rupture is related to a rise of 0.4 percent cases with infiltration. Neutrophilic infiltration, in turn is associated with increased rates of stillbirth and respiratory abnormality for infants of similar birthweight.

Honors and Awards: None

Publications:

Shubeck, F., Benson, R. C., Clark, Jr., W.W., Berendes, H., Weiss, W., and Deutschberger, J.: Fetal hazard after rupture of the membranes. J. Obstet. & Gynec., to be published.

Serial No. NDB (CF)-66 PR/OC 1371

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Epidemiology of Neonatal Seizures

Previous Serial Number: None

Principal Investigator: W. W. Clark, Jr., M.D.,
University of Oregon, Portland, Oregon

Other Investigators: H. Berendes, M.D., PRB, NINDB
A. Kantor, PRB, NINDB
J. S. Nemore, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.5
Professional:	0.3
Other:	0.2

Project Description:

The Collaborative Study has thus far identified 96 infants with documented seizures in the neonatal period. Approximately 40% of these were of unknown origin. Of the 55 cases with known origin, approximately half are associated with birth insult, 30% with infection, and 10% each with developmental CNS malformation or metabolic disease.

The prognosis of these infants is bleak. In excess of 30 percent are premature by gestation, and though males are strongly predominant, a similar prematurity rate is found when birthweight is the criterion. Almost 30 percent do not survive the neonatal period; another 8.5 percent die before one year, and another 4 percent are dead by age 5. Some 7 percent of the infants have maximum serum bilirubins of at least 20 mg percent, and more than 1/3 have one-minute Apgar scores of 0 to 3.

Morbidity persists in the survivors at later examinations. At eight-months, their rate of low mental and motor scores is more than twice that of the Study population; at one-year the rate of neurologic abnormalities is 9 times the normal rate.

The characteristics and conditions of pregnancy of the gravidas of infants with convulsions imply increased risk of pregnancy outcome. While the proportions of Negroes and whites are similar to those of the Study

population, mothers of infants with convulsions are significantly older and of higher parity. They have a several-fold increase in extended labors, in their rates of placenta previa, abruptio placentae, and prolapsed cord. Four times as many of these women are cesarean sectioned, or delivered by breech, as would be expected.

Within the limitations of the number of cases available for study, the variables described above are examined by etiological source of the convulsions.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Birthweight-Placental Weight Relationship --
A Statistical Analysis

Previous Serial Number: None

Principal Investigator: A. Sedlis, M. D.,
New York Medical College, New York, N. Y.

Other Investigators: H. Berendes, M.D., PRB, NINDB
H. S. Kim, M.D., New York Medical College, New York,
N. Y.
W. Weiss, PRB, NINDB
J. Deutschberger, PRB, NINDB
E. Jackson, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.3

Project Description:

Objectives:

To investigate the nature of the birthweight-placental weight relationship among various races, sexes, and mortality groups.

To develop a graphical aid to the physician which would permit him to determine, for an individual case, the degree to which a placental weight, for a given birthweight, differed from expectation.

Methods Employed:

The placental weights and birthweights of some 17,000 cases were analyzed. Statistical variance analyses were used to compare the placental-birthweight relationships of Negroes and white, of males and females, and of those infants who died stillborn, or neonatally, or who survived the neonatal period.

Major Findings:

The differences in mean placental weights between Negroes and white, and

between the sexes are explained by differences in the mean birth weights between the groups.

The placental-birthweight relationships between the races and the sexes are similar in all aspects -- slopes, intercepts and curvilinearity.

When the data for the races and the sexes are combined, the placental-birthweight associations for the stillbirths and the neonatal deaths are found not to be significantly different for infants of similar birthweight. The mean placental weights between the groups are not demonstrably different; weight relationships for both groups are best expressed by straight lines rather than curves, and the two lines are parallel.

The placental-birthweight association for those infants who survived the neonatal period, on the other hand, is best represented by a line which is slightly, but demonstrably curved -- concave upward.

The mean placental weights of both death groups are greater than that of the survival group, for infants of the same birthweight in the range 2000-4000 grams.

The "normalcy" of an observed placental for a specified birthweight, or for those of an unusual group (those, for example, whose mothers were toxemic), can be measured by means of a graph constructed from the data of the Collaborative Project Study.

Honors and Awards: None

Publications:

Sedlis, A., Berendes, H., Kim, H. S., Weiss, W., Deutschberger, J. and Jackson, E.: The Birthweight-Placental Weight Relationship -- A statistical analysis. Journal Developmental Medicine and Child Health, London, England, to be published.

Serial No. NDB (CF)-66 PR/OC 1373

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Effects of the Duration of Labor on the Fetus

Previous Serial Number: None

Principal Investigator: Kenneth R. Niswander, M.D.
University of Buffalo, Buffalo, New York

Other Investigators: Zekin A. Shakhshiri, M.D., PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.4
Professional:	0.3
Others:	0.1

Project Description:

Objectives: To determine the effect on the fetus of the duration of labor.

Methods: Prior work has identified certain complications of pregnancy (such as abruptio placentae, fetal and maternal dystocia, uterine dysfunction) that are not randomly distributed over the course of labor; these may account for the untoward effects found in conjunction with very short or very long labors. As a consequence, by holding these complications constant, it is likely that the effects of duration of labor per se can be measured.

Outcomes used were: Mortality, Apgar Scores at one and five minutes, and findings throughout the first year of life, both on neurological and psychological examinations. Birthweight and parity were used as controls (race has been shown in a prior study not to be related to duration of labor, and is therefore not an essential control).

Results: With certain complications equated out, there is no apparent untoward effect of duration. That is, if, apparently, there is no dysfunction or dystocia, the labor of long duration is relatively uncomplicated and does not produce immediate or long-lasting (throughout the first year of life) difficulties for the fetus.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Complications of Pregnancy in Relation to the Duration of Labor

Previous Serial Number: None

Principal Investigator: Kenneth R. Niswander, M.D.,
University of Buffalo, Buffalo, New York

Other Investigators: M. Westphal, M. D., University of Buffalo
H. Berendes, M. D., PRB, NINDB
J. Deutschberger, PRB, NINDB
W. Weiss, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.6
Professional:	0.4
Other:	0.2

Project Description:

Objectives:

Obstetricians have long accepted that either prolonged or fairly precipitous labor increases the hazard of perinatal death. The question as to whether the length of labor per se is responsible for the added hazard, or whether in fact certain complications of pregnancy are associated with the duration of labor, and the hazard is an effect of the complication.

This research then is designed to determine whether certain complications of pregnancy are randomly distributed over all durations of labor, or are closely associated with particular intervals.

Methods Employed:

Some 25 conditions and complications of pregnancy were measured against duration of labor, with factors such as parity and race held constant.

Major Findings:

Abruptio placentae is related to short labor, while uterine dysfunction, maternal dystocia, and fetal dystocia are closely correlated with excessively

long labor. The length of labor does not appear to be associated with conditions or complications such as placenta previa, prolapsed cord, marginal sinus rupture, or the presence in the gravida of toxemia, organic heart disease, asthma, bacteriuria, etc.

In a proper study, then, of the effects of duration of labor, those complications associated with particular intervals should be held constant, so as to avoid the possible bias of the effect of the complication.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Early Symptomatology and Pregnancy Outcome.

Previous Serial Number: None

Principal Investigator: Glidden Brooks, M.D., Brown University,
Providence, R. I.

Other Investigators: W. A. Reid, M.D., Brown University
R. H. Holden, Brown University
Z. Shakhashiri, PRB, NINDB
A. Kantor, PRB, NINDB
W. F. Crane, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.8
Professional:	0.6
Other:	0.2

Project Description:

The generalized complaints that a woman makes when she first seeks care in the course of her pregnancy may stem from an organic or a psychosomatic base. In either event, these symptoms should be evaluated to determine the extent that they are indicative of the outcome of her pregnancy.

This communication reports a study of 22 symptoms claimed or denied by each of 29,006 women on her entry into the Collaborative Project. These symptoms range from the fairly trivial, such as colds or back pain, to the potentially serious, such as convulsion or vaginal bleeding.

The 22 complaints are evaluated separately and in total against certain outcomes of pregnancy, such as perinatal mortality, birthweight, Apgar scores, and findings at a one-year neurological examination. Comparisons are made by race of gravida and by parity.

The proportions of whites and Negroes making each complaint are fairly close, except for a few symptoms; the total number of symptoms claimed by Negroes but not by whites, reflects the outcomes of pregnancy. Except for particular symptoms, primigravidas and multigravidas respond in like proportions; there are, however, differences between these groups in terms of the association

between the number of complaints and outcome.

Given symptoms are strong indicators. Vaginal bleeding, for example, is associated with a variety of poor outcomes, for all races and parities, and goes beyond a relationship with abortion and stillbirth only.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/SC 1376

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Organic Heart Disease in Pregnancy

Previous Serial Number: None

Principal Investigator: Kenneth R. Niswander, M.D.
Children's Hospital of Buffalo, Buffalo, New York

Other Investigators: H. Berendes, M.D., PRB, NINDB
J. Deutschberger, PRB, NINDB
M. Westphal, M.D. Children's Hospital of Buffalo,
Buffalo, New York
N. Lipko, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total	0.5
Professional	0.4
Other:	0.1

Project Description:

Reports in the literature have stipulated the risk of perinatal mortality in cases in which the pregnant woman is suffering heart disease. The consensus is that the risk is substantial even in asymptomatic cardiac patients, and increases readily with the severity of the disease.

In the investigation reported here, 327 pregnancies in which organic heart disease was authenticated were studied. Assessments were made of the outcomes of these pregnancies, both immediate and through the first year of life of the surviving infant.

The perinatal loss rate in these 327 cases was relatively high, but not as great as that found by earlier investigators. The severity of the disease, as judged by limitation of activity and by digitalis therapy, appears to be a factor in outcome; as other investigators have noted, however, proper prenatal care apparently produces a beneficial and salvaging effect on pregnancies complicated by heart disease.

While severe heart disease is associated with the production of low birth-weight children and of children who exhibit an increase in morbidity during the neonatal period, as evidenced by low Apgar scores, the long-term

prognosis for children who survive the perinatal period does not appear to be overly poor. The one-year status of children of mothers with heart disease suggests that serious neurological problems are indeed quite rare. Severe maternal organic heart disease certainly might lead to anoxic episodes in the fetus. The lack of a clear-cut demonstration of an effect on the central nervous system of surviving children as shown by these data thus far could be due to the limitations of a one-year neurological examination in identifying more subtle neurological deficits and/or effects on higher intellectual and perceptual functions which cannot be identified in young infants.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/OC 1377

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Reproductive Wastage in Bronchial Asthma

Previous Serial Number: None

Principal Investigator: Myron Gordon, M.D.
New York Medical College, New York, N.Y.

Other Investigators: H. Berendes, M.D., PRB, NINDB
A. Kantor, PRB, NINDB
N. Lipko, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.6
Professional:	0.5
Others:	0.1

Project Description:

Bronchial asthma is a medical disorder characterized by recurrent paroxysmal dyspnea due to an allergic reaction to a variety of substances. Studies of the effects of the disorder upon the course and outcome of pregnancy have been few and generally inconclusive. The Collaborative Research Study afforded a unique opportunity to examine the effects of bronchial asthma upon many aspects of the "Continuum of Reproductive Wastage".

Case Analyses of 369 records revealed 281 cases of asthma complicating pregnancy. In addition, there were 48 cases with a history of asthma but no manifestations or active treatment in this pregnancy; and 40 cases of acute bronchitis with broncho-spasm (asthmatic bronchitis). These 281 cases represent 0.9% of Core of 30,861 births and 4 of these 281 were twin births (1.4%), the remaining 277 births are single first-study pregnancies with bronchial asthma of varying severity.

TABLE 1REPRODUCTIVE WASTAGE AS OF 1 YEAR

	<u>ASTHMA</u>		<u>CORE</u>
	<u>No.</u>	<u>Percent</u>	<u>Percent</u>
Stillbirths	8	2.9	1.7
Neonatal Deaths	8	2.9	1.2
Infant Deaths	3	1.1	1.0
Abnormal neurologically	6	2.2	1.9
Total Wastage	25	9.1*	5.5
Total Cases	277		

*P = 0.02

Table I demonstrates significant differences in wastage between the asthma cases and the Core Study. It is apparent that this difference was mainly manifested in the perinatal mortality rates although the abnormal neurologically group participates in the trend.

Exploration of the factors possibly responsible for this observed difference in outcome reveals the following data:

(1) Race distribution in the asthma group is heavily Negro (48.7%), and Puerto Rican (22.7%).

(2) Overall prematurity rate by birthweight is increased in the asthma group to 17% compared to the Core of 11%.

(3) The Negro prematurity rate in the asthma group is increased to 23% compared to the Core of 14.6%.

(4) 13 of the 16 perinatal deaths were associated with low birth-weight and 10 of these 13 were Negro babies.

(5) 4 of the 6 neurologically abnormal children were Negro and 3 of the 6 were premature by weight at birth.

Thus asthma does exert a detrimental effect upon pregnancies in this population group; it seems to do so mainly through its effect upon the Negro patient.

Additional study of the health of the infants reveals that of the 251 who survive the neonatal period, 15 or 5.9% developed asthma and 48 or 15% developed other severe pulmonary disorders. Interestingly, 43 of these infants with complications are white and Puerto Rican, thus reversing the trend seen in reproductive wastage.

Serial No. NDB (CF)-66 PR/QC 1377

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/OC 1378

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Prediction of Birthweight - Multivariate Analysis

Previous Serial Number: None

Principal Investigator: Heinz W. Berendes, M.D., PRB, NINDB

Other Investigators: W. Weiss, PRB, NINDB
J. Deutschberger, PRB, NINDB
Z. Shakhashiri, M.D., PRB, NINDB
E. Jackson, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.7
Professional:	0.5
Others:	0.2

Project Description:

In the Collaborative Project, the birthweight of the infant is the best predictor, by far, of neonatal mortality and of almost every characteristic of morbidity in the Study.

Birthweight, then, merits intensive investigation. It is fortunate that this variable has attributes conducive to statistical treatment; it is always reported, it is measured with considerable precision and it is continuous over a wide range of values.

This communication reports on two aspects of a study of birthweight. The first is directed towards the identification of those characteristics and conditions of pregnancy which will, most precisely, estimate the birthweight of the infant. The variables studied range in time, relative to the period of pregnancy, from those available prior to the study pregnancy, to those which are measured in the stages of labor and delivery.

The identification of those variables, which, taken together, provide the means for estimating birthweight within narrow limits, may lead to increased understanding of the role of these variables in their association with birthweight.

The practical aspect of prediction - that is, the measure of the availability and contribution of important characteristics for predicting birthweight, by time sequence, during the period of pregnancy, is discussed.

On the basis of the prior examination of study data, a total of 38 variables were selected for a multiple regression study of birthweight.

The birthweights of the children of some 6600 whites and 8000 Negroes, who had at least one prior pregnancy, were evaluated separately by race.

Of the 38 considered, 21 variables and 30 variables, for whites and Negroes respectively, show significant association with birthweight. Despite the larger number of significant variables, the correlation with birthweight is less for Negroes than for whites.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Fetal Heart Rate in Relation to Pregnancy Outcome.

Previous Serial Number: None

Principal Investigator: Ralph C. Benson, M.D.,
Univ. of Oregon Medical School
Portland, Oregon

Other Investigators: F. Shubeck, M.D., Univ. of Oregon Medical School
H. Berendes, M.D., FRB, NINDB
W. Weiss, FRB, NINDB
J. Deutschberger, FRB, NINDB
Z. Shakhashiri, M.D., FRB, NINDB

Cooperating Units: Section on Statistical Analysis, FRB, NINDB

Man Years:

Total:	0.7
Professional:	0.5
Other:	0.2

Project Description:

Objectives: To identify a fetal heart rate factor which would be a sensitive indicator of fetal distress.

Methods Employed: Fine fetal heart rate factors, which include the lowest rates, the variability in rates, sudden drops in rate, and consecutive drops in rate were compared against indices of fetal mortality and fetal morbidity, as well as morbidity up to one year of age, with such factors as the birthweight, use of anesthesia, stage of labor and number of heart rate observations kept constant.

Major Findings: The fine fetal heart rate variables form a hierarchy in their degree of sensitivity as predictors of fetal distress. The significant associations of these variables appear to be exclusively with stillbirths and neonatal death rates, and with the five-minute Apgar score. Little association is noted with the other indices of morbidity studied.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

FHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Acute and Chronic Glomerulonephritis in Pregnancy

Previous Serial Number: None

Principal Investigator: Heinz W. Berendes, M.D., PRB, NINDB

Other Investigators: Z. A. Shakhashiri, M.D., PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total: 0.2

Professional: 0.1

Other: 0.1

Project Description:

Glomerulonephritis occurs relatively infrequently in pregnant women. This study is a close review of 50 cases in which the diagnosis was made in an attempt to determine the effect on the fetus.

About half of the children of these pregnancies were of low birthweight (under 2501 grams). This enormous rate indicates the severe stress to which the bulk of these children are subjected, and is reflected in the excessive perinatal mortality rate that occurs among them.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Complications of Pregnancy and Their Association with Change in the Placental Weight/Birthweight Relationship

Previous Serial Number: None

Principal Investigator: Frank Shubeck, M.D.,
University of Oregon, Portland

Other Investigators: R. C. Benson, M.D., University of Oregon
W. W. Clark, Jr., University of Oregon
H. Berendes, PRB, NINDB
W. Weiss, PRB, NINDB
J. Deutschberger, PRB, NINDB
E. Jackson, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.8
Professional:	0.6
Other:	0.2

Project Description:

The study of the relationships of placental weight to birthweight may provide insight into the mechanisms which compromise the fetus. This study of approximately 16,000 pregnancies examines the placental weight to birthweight relations in infants of mothers in various "high-risk" categories. This particular report is restricted primarily to placenta previa, a complication of pregnancy.

The cases were divided into six birthweight groups, resulting in 24 distinct placental weight to birthweight groupings.

For each of the birthweight categories, there was observed an increase in the percentage of placenta previa with relatively increasing placental weight. A statistical technique was used to determine whether or not the cases of placenta previa were concentrated in the groups of relatively heavier placental weights. This clustering was found in the two lowest and the highest groups.

There was no support for the hypothesis that the placenta previa cases had relatively larger placentas because of a reduced duration of gestation.

The degree of severity of placenta previa did not influence the relative size of the placenta.

The reduction in mean placental weight of 15 Gm. in the placenta previa group compared with that of all cases was not statistically significant. On the other hand, the reduction of 549 Gm. in birthweight of the placenta previa group and that of all cases was significant ($p < .0001$).

Thus, among the gravidas with placenta previa the apparent increased placental weight has been demonstrated primarily to be related to a very substantial reduction in birthweight.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/CC 1382

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Effects of Maternal Diabetes

Previous Serial Number: None

Principal Investigator: Luke Gillespie, M.D.
Boston Lying-In Hospital, Boston, Mass.

Other Investigators: A. Grimaldi, M.D.,
New York Medical College, New York
Zekin A. Shakhshiri, M.D., PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years (estimated)

Total:	1.4
Professional:	1.1
Others:	0.3

Project Description:

This study, which is in progress, is an attempt to make a definitive determination of the effect on the fetus of diabetes in the gravida.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Certain Socio-cultural Parameters in Relation to Pregnancy Outcome

Previous Serial Number: None

Principal Investigator: Zekin A. Shakhashiri, M.D., PRB, NINDB

Other Investigators: H. Berendes, M.D., PRB, NINDB
W. Weiss, PRB, NINDB
J. Deutschberger, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.4
Professional:	0.3
Other:	0.1

Project Description:

It is generally assumed that socio-cultural status is an indicator of pregnancy outcome, although there are few hard findings to bear out this suggestion. Moreover, data developed in this area almost invariably refer to the gravida, more than to the father of the child.

For this study, so-called socio-cultural variables are measured for both gravida and father of child, and are taken in combination with respect to outcome.

Other measurements, such as age of gravida and of father of child, are taken in combination in similar fashion and related to outcome. While these variables do not qualify as purely of a socio-cultural nature, they do fit in with the general study plan, and it is economical of effort to deal with them in the course of this study.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/OB 1327

1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Maternal Deaths in the Collaborative Project

Previous Serial Number: None

Principal Investigator: Jose G. Marmol, PRB, NINDB

Other Investigators: Rudolf F. Vollman, PRB, NINDB
Myron Gordon, New York Medical College
Sam P. Patterson, University of Tennessee
William A. Reid, Providence Lying-In Hospital

Cooperating Units: All institutions participating in the Collaborative Study

Man Years

Total:	0.6
Professional:	0.5
Others:	0.1

Project Description:

Objectives: To study all etiologic factors related to the maternal deaths reported in the Collaborative Project.

Methods: After identification of the cases by a numerical computer printout and by cross-checking with a previously established card file on maternal deaths, each case history was reviewed in detail: past medical history, past reproductive history, family planning, course and complications during study pregnancy, and labor and delivery. With the cooperation of the collaborative institutions involved, all efforts were made to secure missing information and to clarify equivocal information. Copies of post-mortem reports and/or death certificates were solicited.

Major Findings: For a total of 37,545 live births registered in the Collaborative Project since its beginning in 1959 through June 1965, 40 maternal deaths were reported. In all the cases a definite diagnosis of the cause of death was established. Sixteen of the 40 deaths were attributed to direct obstetric causes; all of these were Negroes. Among the direct obstetric causes of death, infections (6 cases) occupy the first place, followed by vascular accidents (4 cases), anesthesia complications (3 cases), hemorrhage (2 cases), and toxemia (1 case). No deaths related to placenta previa or abruptio placentae

were reported in the study. Nor were there any deaths due to ectopic pregnancy.

In the category of indirect obstetric causes of death (14 cases), four mothers died from cardiac diseases, three from pulmonary conditions, two from vascular diseases, two from lupus erythematosus, and one each from hepatic, metabolic, and sickle cell disease. Of the maternal deaths not related to pregnancy, five were due to accidents.

A minute review of each case of maternal death as to its preventability revealed clearly that a ceiling of therapeutic effectiveness in the reduction of maternal deaths is approaching. Therefore, the hypothesis was tested if, in the future, maternal mortality could be reduced by identifying non-pregnant women who are potentially high risk mothers, in whom pregnancy would be a life-endangering complication. Using the criteria for high risk mothers established by the Maternity and Infant Care Project of the New York City Department of Health, 28 of the 40 maternal deaths were classified as high risk mothers prior to conception. Of these 28 high risk mothers, only three wanted to become pregnant, 20 stated positively that they did not wish to become pregnant, for five the project record contains no information regarding their intention to conceive. It is felt that in this group of high risk women a major reduction in maternal mortality can be achieved through adequate family planning.

Paper read at the Second Scientific Meeting of the Collaborative Study, March 24-25, 1966, Washington, D. C.

Honors and Awards: None

Publications: Marmol, J.G., Vollman, R.F., Gordon, M., Patterson, S.P., and Reid, W.A.: Maternal deaths in the Collaborative Project. Obstet. Gynec. In press.

Serial No. NDB (CF)-66 PR/OB 1328

1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Evaluation of the Possible Teratogenic Effect of Antihistaminic Drugs Taken During Pregnancy

Previous Serial Number: None

Principal Investigator: James H. Tarver, Jr., PRB, NINDB

Other Investigators: Jose G. Marmol, PRB, NINDB
Rudolf F. Vollman, PRB, NINDB
Irene B. Ross, PRB, NINDB

Cooperating Units: Section on Systems Design and Procedures, PRB, NINDB

Man Years

Total:	1.8
Professional:	0.6
Others:	1.2

Project Description:

Objectives: Experimental evidence has been published that certain antihistamines of the meclizine group when given during the organogenetic phase of pregnancy produce a high rate of cleft palate malformations in rodents. Reports from the clinical literature on a possible teratogenic effect of antihistamines in human pregnancy are controversial, mainly due to lack of controlled drug studies. The information available in the Collaborative Study on specified antihistamine drugs will be analyzed by controlling for age, race, parity, and date of registration for prenatal care of the mother, chronic and acute diseases during pregnancy, obstetric complications during pregnancy, labor and delivery, and fetal outcome.

Methods: Of the large number of antihistamine drugs reported in the Collaborative Project, representatives of four different types of antihistamines (ethylene diamine, aminoalkylether, alkylamine series, meclizine) have been selected, based on the maximum number of cases available from a drug printout. It is assumed that any one of these antihistamines may be prescribed arbitrarily for similar, if not identical, conditions in pregnancy (nausea, vomiting, malaise). Therefore, by controlling for the variables specified above, a differential drug effect on the fetus may be measured directly, as expressed in the rates of abortions, stillbirths, and malformations.

Project is in progress.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/OB 1329

1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Estrus, Ovulation and Menstruation; A Critical Historical Review

Previous Serial Number: None

Principal Investigator: Rudolf F. Vollman, PRB, NINDB

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.4
Professional:	0.3
Others:	0.1

Project Description:

Objectives: To study some of the conceptual and methodological factors involved in the historic development of mammalian reproductive physiology during the past 100 years.

Methods: Review and analysis of approximately 800 publications on estrus, ovulation and menstruation, published between 1824 and 1932, together with a study of autobiographies and correspondence of the leading researchers in this field.

Major findings: A large fund of rather precise knowledge on mammalian reproduction was acquired on an empirical basis by animal breeders, long before systematic scientific analysis of reproductive physiology came into being. While the sperm cell had been seen under the most primitive microscope and with some crude experimentation its "assistance" in the process of fertilization had been guessed (Spallanzani), the mammalian ovum was searched for only after the concept of the cell theory had opened a new horizon. Within two generations the conspicuous morphologic features of the reproductive process (follicle formation, ovulation, fertilization, segmentation, tubal journey of the blastocyst, endometrial changes, the implantation process, and the formation of the placenta were discovered (Baer, Bischoff, Pouchet, Raciborski, Coste, Taruffi, Home, Barry, Lataste, Hertwig and others). The time interval between estrus and ovulation was measured in different species.

Ovulation was found to occur spontaneously in most mammals, with a few exceptions in which ovulation is induced through the mating stimulus. The phases of the estrus cycle were defined in relation to the corresponding morphologic changes in the ovary, the uterus and the vagina. With the discovery of the hormonal monitors and their connection with the pituitary-hypothalamic feedback system, the essential mechanism of the estrus cycle was defined. The confusion between menstruation and the estrus bleeding, and the absence, in women, of overt behavioral signs of sexual receptivity threw the discussion on the menstrual cycle into a sterile controversy. Only the results of the systematic study of the primate menstrual cycle made possible a logical approach to the analysis of women's menstrual cycle.

Paper read at the Annual Meeting of the American Association of Anatomists, April 6-8, 1966, San Francisco. An abstract will be published in the Anatomical Record.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/OB 1330

1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Length of the Premenstrual Phase by Age of Women

Previous Serial Number: None

Principal Investigator: Rudolf F. Vollman, PRB, NINDB

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.3
Professional:	0.2
Others:	0.1

Project Description:

Objectives: The ovulatory-menstrual cycle involves a multitude of morphologic, endocrine and neurologic mechanisms. It is therefore conceivable that the correlation of these functions is not fully established at menarche but requires a period of development during which it reaches optimal performance. Under the same premise, prior to menopause a gradual disintegration in the reproduction process may be expected. The length of the post-ovulatory phase in the menstrual cycle will be used to test this hypothesis.

Methods: Basal body temperature records of 546 women reporting on 12,751 menstrual cycles between menarche and menopause will be analyzed for age changes in the length of the postovulatory phase.

Major findings: Monophasic temperature curves have been observed most frequently immediately after menarche (45 percent of all cycles) with a consistently decreasing rate during adolescent development. Between 23 and 44 years of age, an average of only 2 percent of the BBT curves are monophasic. At age 45, the rate of the anovulatory cycles steadily increases toward menopause.

BBT curves, with short premenstrual phases of from three to ten days' duration, have been found in 44 percent of all cycles after menarche. The rate decreases in the course of adolescence to about 15 percent during the phase of maturity, with little change during the premenopause.

Pre-menstrual phases of 11 to 16 days' duration are infrequent immediately after menarche, but comprise about 80 percent of all cycles between ages 23 and 44 years, with decreasing rates during the premenopause.

These age changes in the ovulatory cycle do not seem to be directly correlated with female fertility; high conception rates during the adolescent phase contradict the low ovulatory rates during the same period.

Paper to be presented at the Fifth World Congress on Fertility and Sterility, Stockholm, June 16-22, 1966.

Honors and Awards: None

Publications: Vollman, R.F.: The length of the premenstrual phase by age of women. Proceedings of the International Fertility and Sterility Association. In press.

1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Obstetric Factors in Twin Pregnancies

Previous Serial Number: None

Principal Investigator: Rudolf F. Vollman, PRB, NINDB

Other Investigators: Wolfgang Tretter, Columbia-Presbyterian Hospital, N.Y.
Irene B. Ross, PRB, NINDB
Zula C. Meekham, PRB, NINDB

Cooperating Units: All institutions participating in the Collaborative Study Staff, Section on Obstetrics, PRB, NINDB

Man Years

Total: 2.61

Professional: 1.11

Others: 1.50

Project Description:

Objectives: The early diagnosis of a twin pregnancy still remains an important problem on which depend the prenatal care and the management of the labor and delivery. The study has two objectives:

1. to study the outcome of twin pregnancies in relation to the time the diagnosis was first established.
2. to make a comparison of the obstetric problems presented by the first versus the second twin and their effect on the fetal outcome.

Methods: With the help of a computer printout, an established case and card file, and an additional review of a current file on abortions and fetal deaths, the twins delivered in the Collaborative Project through December 31, 1965, have been identified. All case records were reviewed and additional information or clarification was solicited from the collaborating hospitals as needed. The mother's medical, family, and reproductive history, together with information on the course of the study pregnancy, intercurrent diseases, drugs, obstetric complications, labor and delivery, and outcome of pregnancy were abstracted. These data have been used to prepare a set of tabulations for the study of the variables specified above.

The study is presently being written for publication.

Honors and Awards: None

Publications: None

Serial No. NDB(CF)-66 PR/OB 1332

1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Down's Syndrome and the Incidence of Infectious Hepatitis During Pregnancy

Previous Serial Number: None

Principal Investigator: Rudolf F. Vollman, PRB, NINDB

Other Investigators: Jose G. Marmol, PRB, NINDB
Reba L. Goldman, PRB, NINDB

Cooperating Units: All institutions participating in the Collaborative Study

Man Years

Total:	0.6
Professional:	0.3
Others:	0.3

Project Description:

Objectives: In an Australian study, the epidemic occurrence of infectious hepatitis was related to an increase in the birth of mongoloid children approximately nine months after the peak of the epidemic. Perinatal Research Project data on mongoloids from study pregnancies are being analyzed to verify or reject this hypothesis.

Methods: The pregnancy histories of all mothers who delivered mongoloid children while in the project have been abstracted for reports of infectious hepatitis, icterus, and other "liver conditions" within one year prior to and during the study pregnancy. In addition, the record was screened for gamma globulin injections the mother might have received for treatment of hepatitis during the course of pregnancy.

A random control sample was established by selecting the next four cases delivered in the same hospital from which the mongoloid index case had been reported. The study is in progress and tabulations of the findings are being prepared.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/OB 1333

1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Distribution of Abortions by Chronologic and Gynecologic Age of the Gravida

Previous Serial Number: None

Principal Investigator: Rudolf F. Vollman, PRB, NINDB

Other Investigators: Annie W. Litz, PRB, NINDB
Jose G. Marmol, PRB, NINDB

Cooperating Units: All institutions participating in the Collaborative Study

Man Years

Total:	0.7
Professional:	0.4
Others:	0.3

Project Description:

Objectives: Information is accumulating which demonstrates that endocrine and morphologic conditions for optimal reproductive performance are reached only several years after menarche. The conventional association of pregnancy outcome by chronologic maternal age will be compared with the mother's gynecologic age, based on the age at menarche.

Methods: For study pregnancies terminating in abortion, a number of important maternal variables will be abstracted from the study record: race, chronologic and gynecologic age, marital status, gravidity and parity, length of the menstrual cycle, medical and obstetric complications of the study pregnancy, duration and outcome of the study pregnancy. These variables will serve as controls in the analysis of chronologic versus gynecologic maternal age. The study is in progress.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Span of Human Gestation

Previous Serial Number: None

Principal Investigator: Rudolf F. Vollman, PRB, NINDB
Jose G. Marmol, PRB, NINDB
Irene B. Ross, PRB, NINDB
Annie W. Litz, PRB, NINDB
Zula C. Meekham, PRB, NINDB

Other Investigators: Robert N. Rutherford, Seattle, Washington
A. Lawrence Banks " "
Wallace A. Coburn " "
Robert L. Welsh " "

Cooperating Units: None

Man Years (For PRB investigators only)

Total:	0.4
Professional:	0.3
Others:	0.1

Project Description:

Objectives:

1. To measure and compare the length of human gestation from the onset of the last menstrual period (LMP) and from conception.
2. To estimate the degree of variability in the length of gestation from LMP and from conception.
3. To analyze the association between birthweight and the length of gestation from LMP and from conception, respectively.
4. To study a possible association between the length of the menstrual cycle and the duration of pregnancy.
5. To measure the relationship between weight gain of the mother during pregnancy and the birthweight of the neonate.

6. To estimate the rate of early pregnancy losses in a sample of healthy young women.

Methods: At the time of the premarital examination, healthy women will be requested to volunteer for the study through the sequential series of their menstrual cycles and up to the termination of their first pregnancy. They will keep a menstrual calendar and take daily basal body temperature readings, report weekly body weights, illness, medication, travel, and a number of other factors that may affect ovulation and menstruation. In cases in which conception occurs, LMP will have been reported prior to the onset of pregnancy. The event of pregnancy will be diagnosed by a change in the basal body temperature and verified by sequential hormonal pregnancy tests.

The study is in progress and first findings will be reported in 1967.

Honors and Awards: None

Publications: None

Serial No. NDB-(CF)-63 PR/PN 1155
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Apgar Score - An Index of Infant Morbidity.

Previous Serial Number: Same

Principal Investigator: Joseph S. Drage, M.D., PRB, NINDB

Other Investigators: Charles Kennedy, M.D., Children's Hospital of
Philadelphia
Benjamin K. Schwarz, PRB, NINDB

Cooperating Units: Children's Hospital of Philadelphia, Philadelphia, Pa.
Perinatal Research Branch, NINDB

Man Years

Total: 3/12
Professional: 2/12
Other: 1/12

Project Description: This project relates Apgar scores taken at one and five minutes after birth and birthweight to certain indices of infant morbidity based on a neurological examination at one year of age. Outcomes at one year are defined in terms of overall neurological abnormality of muscle tone, locomotive retardation, and abnormalities of prehensile grasp. A general summary of the preliminary tabulation shows that this data gives support to previous reports in the literature that relate low birthweight to abnormal neurological outcome, not only in terms of overall diagnostic judgment, but in terms of specific judgments regarding locomotor development, muscle tone and prehensile grasp. In the design of this study and in terms of the definitions of neurological outcome at one year, this preliminary analysis of project data suggests that the five-minute score has value in predicting infant morbidity and that this predictive ability is independent of birthweight. Within the combinations of birthweight in five-minute score groups, abnormal neurological outcome is associated more strongly with the combination of low birthweight and low five-minute score. In general, the percent abnormal within each birthweight group is greater among those infants with scores of 0-3 than among those infants in the same birthweight group with scores of 7-10. It appears that while both birthweight and five-minute scores have predictive ability regarding neurological abnormality at one year, this predictive ability increases considerably when birthweight and five-minute scores are combined.

Honors and Awards: None

Publications: Drage, J.S., Kennedy, C., Berendes, H., Schwarz, B.K., and Weiss, W.: The Apgar score as an index of infant morbidity. Develop. Med. Child Neurol. 8: 141-148, 1966.

Drage, J.S., Berendes, H.: Apgar scores and outcome of the newborn. Pediat. Clin. N. Amer. To be published August, 1966.

Serial No. NDB-(CF)-63 PR/PN 1156
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Neonatal Hyperbilirubinemia and Subsequent Neurological Sequelae.

Previous Serial Number: Same

Principal Investigator: Janet Hardy, M.D., Johns Hopkins Hospital

Other Investigators: T. R. Boggs, M.D., Pennsylvania Hospital
Heinz Berendes, M.D., PRB, NINDB
B. H. Williams, M.D., PRB, NINDB
William Weiss, PRB, NINDB
Joseph S. Drage, M.D., PRB, NINDB

Cooperating Units: Johns Hopkins University, Baltimore, Maryland
Pennsylvania Hospital, Philadelphia, Pennsylvania
Perinatal Research Branch, NINDB

Man Years

Total: 2/12
Professional: 1/12
Other: 1/12

Project Description: Approximately 23,000 infants resulting from single live-births among women registered in the Collaborative Project between January 1, 1959 and June 30, 1964, have had one or more neonatal total serum bilirubin determinations performed and have been examined at 8 and 12 months of age. Correlations between the maximum observed neonatal total serum bilirubin level and subsequent neurologic deficit have been made. These correlations were made both before and after the exclusion of major congenital malformations including those of the brain, the spinal cord, the skeletal system, the eye, the kidney, the upper respiratory tract, the heart and the gastro-intestinal tract and syndromes such as mongolism, cretinism, gonadal dysgenesis, Pierre Robin's syndrome, etc. The exclusion of such congenital malformations did not alter essentially the relationships observed before their exclusion and therefore infants with such defects are included in the survey. The study cases included both Coombs positive and Coombs negative infants.

The most striking findings were those encountered on evaluating the Motor and Mental Scores (modifications of the Bayley Scale) of these infants at

their age of 8 months (\pm 2 weeks). There was a highly significant linear component to the regression lines of the associations between increasingly high total serum bilirubin levels and the incidence of infants performing in the lowest decile of their group as concerns motor and mental scores. Although these observations are most striking for the lower birthweight groups, they held for all birthweight groups and continued to hold after the birthweight groups were further controlled for the 5-minute Apgar score. Furthermore, the bilirubin-low mental and low motor score relationships were noted not to begin abruptly at the 20 mgm percent level but were observed to rise progressively and to become substantial at the 16 - 19 mg. percent concentration.

Honors and Awards: None

Publications: None

Serial No. NDB-(CF)-63 PR/PN 1161
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Prematurity.

Previous Serial Number: Same

Principal Investigator: Milton C. Westphal, M.D., Children's Hospital, Buffalo

Other Investigators: Edward H. Bishop, M.D., Pennsylvania Hospital
B. H. Williams, M.D., PRB, NINDB
Heinz Berendes, M.D., PRB, NINDB
Jerome Deutschberger, PRB, NINDB
Benjamin K. Schwarz, PRB, NINDB

Cooperating Units: Children's Hospital, Buffalo, New York
Pennsylvania Hospital, Philadelphia, Pennsylvania
Perinatal Research Branch, NINDB

Man Years

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

Project Description: This paper is based on data derived from approximately 15,000 children in the Collaborative Study of Cerebral Palsy. The results of three performance measures (8-month mental, 8-month motor and 12-month neurological) and three growth measures (weight, height, and head circumference at 12 months) were tabulated by birthweight in 500 gram increments and length of gestation in 3 week intervals. The relationships of the outcome variables to birthweight and length of gestation independently and in interaction, and to rate of intra-uterine growth were analyzed. Relationships to intra-uterine growth retardation were shown by measures having a linear relationship to birthweight, an inverse relationship to gestation when controlled for birthweight, and a significant birthweight X gestation relationship.

The results indicate that birthweight and length of gestation are independently and linearly related to growth, and that retarded intra-uterine growth is followed by poor growth during the first year of life. There is no relationship between rate of intra-uterine growth and the performance measures. Low birthweight is related to poor mental, motor and neurologic dysfunction, and short gestation is related to poor mental and motor

performance but not to neurologic dysfunction. Neonatal respiratory symptoms are related to low birthweight, short gestation, and poor mental, motor and neurologic performance but not to infant growth.

These findings emphasize the necessity of analyzing birthweight and length of gestation relationships both independently and in interaction in order to isolate the effects of birthweight, length of gestation and intra-uterine growth on outcome measures.

Honors and Awards: None

Publications: None

Serial No. NDB-(CF)-63 PR/PN 1162
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Apgar Scores.

Previous Serial Number: Same

Principal Investigator: Joseph S. Drage, M.D., PRB, NINDB

Other Investigators: William Weiss, PRB, NINDB
Benjamin K. Schwarz, PRB, NINDB
Charles Kennedy, M.D., Children's Hospital of
Philadelphia

Cooperating Units: Perinatal Research Branch, NINDB
Children's Hospital of Philadelphia, Philadelphia, Pa.

Man Years

Total: 3/12
Professional: 2/12
Other: 1/12

Project Description: This project will match the one and five-minute scores of each child and will relate the various combinations to outcome, considering the resuscitative procedure used. Such studies should sharpen the apparent relationship between the combined use of the one and five-minute scores and their relationship with outcome in terms of mortality and morbidity. In addition, prenatal factors will be related to scores and the scores in turn to outcome. Infants with low scores associated with a specific prenatal factor may be more strongly correlated to undesirable outcome than equally low scores unassociated with such a prenatal factor. In other words, the significance of a low etiology of the low score in terms of specific events or conditions during pregnancy, labor and delivery. Study in progress.

Honors and Awards: None

Publications: None

Serial No. NDB-(CF)-63 PR/PN 1163
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: An Investigation into the Relationship Between Congenital Heart and Great Vessel Anomalies and Selected Perinatal Factors as Recorded in the Collaborative Perinatal Research Project.

Previous Serial Number: Same

Principal Investigators: Lenore Bajda, M.D., PRB, NINDB
Sheila Mitchell, M.D., O.A.D.C.S., NHI

Other Investigators: H. Berendes, M.D., PRB, NINDB
J. Sever, M.D., PRB, NINDB

Cooperating Units: Laboratory of Technical Development, OADCS, NHI
Perinatal Research Branch, NINDB

Man Years

Total: 10/12
Professional: 9/12
Other: 1/12

Project Description: The primary objectives of the study are to assess the relationship between certain perinatal factors and congenital heart-great vessel anomalies.

Maternal parameters analyzed included age of gravida, parity, ABO blood group, prior pregnancy outcome, prior and current health status, current smoking pattern, and viral antibody status.

Secondary objectives include investigating relationships between early signs of abnormality and the existence of definitive congenital heart lesions thereafter as well as determining the existence of a congenital heart-great vessel anomaly in conjunction with mental retardation as recorded in the eight-month psychological examination, the one-year summary records, and four-year psychological examination.

Study data is obtained from Collaborative Study records received by PRB from the onset of the Study (January 1959) through December 1964. The Study group is drawn from a population pool of approximately 38,000 live and stillbirth reports received during this period.

Major findings: No significant findings were observed re maternal blood group distribution and smoking history. There was a definite preponderance of mothers over 35 in the C-V Study group. Controlling for race, and removing cases with chromosomal aberrations, there were more white mothers in the 35 and over age group than expected at the .05 level. This trend is also noted among Negroes. There was a greater than expected number of gravida with systemic disease complications amongst the mothers of the cardiacs.

Because the number of patients with each specific cardiac abnormality was small, specific associations between serological findings and clinical observations was not possible, although several interesting trends were noted. Further analysis with additional patients is under way.

Honors and Awards: None

Publications: None

Serial No. NDB-(CF)-63 PR/PN 1164
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Early Signs as Predictors of Death and Neurological Abnormality
Among Premature Infants Weighing 1000-2000 Grams.

Previous Serial Number: Same

Principal Investigator: Joseph S. Drage, M.D., PRB, NINDB

Other Investigators: Karin B. Nelson, M.D., PRB, NINDB
Heinz Berendes, M.D., PRB, NINDB

Cooperating Units: Perinatal Research Branch, NINDB

Man Years

Total: 3/12
Professional: 2/12
Other: 1/12

Project Description: A group of 720 single liveborn premature infants with birthweights of 1000-2000 grams has been prospectively studied relating the presence of specific neurological signs during the nursery period to neurological status at one year of age. Of the 720 infants, 485 were examined at one year, 159 died during the first year, and 76 were lost to follow-up. There were 101 neurologically abnormal infants among the 485 examined at one year. The first four PED-2's (Neonatal Pediatric Examinations) were reviewed for the presence or absence of early signs. Among the 720 infants, 78.2% received four or more PED-2 examinations, while 100.0% of the abnormal and 94.8% of the normals received four or more examinations. Many of the deaths were in the first 24 hours of life, and most of the infants receiving zero, one, or two PED-2 examinations were deaths. A positive early sign was defined as the occurrence, on at least one examination, of the specific early sign being studied. Specific signs studied included cry, suck, palmar grasp, traction response, Moro reflex, eye movement, muscle tone, local convulsions, general convulsions, highest serum bilirubin, Coombs' test, procedures of resuscitation, etc. Each of these signs was considered separately, and those showing significant association with death and abnormal outcome were then combined. When three signs were considered in combination, the more that were positive the greater the association of neurological abnormality and death, and of neurological abnormality alone.

Honors and Awards; None

Publications; None

Serial No. NDB-(CF)-65 PR/PN 1272
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Hyaline Membrane Disease.

Previous Serial Number: Same

Principal Investigator: James Drorbaugh, M.D., Children's Medical Center, Boston

Other Investigators: William Cochran, M.D., Boston Lying-In Hospital
B. H. Williams, M.D., PRB, NINDB
Toshio Fujikura, M.D., PRB, NINDB
Benjamin K. Schwarz, PRB, NINDB

Cooperating Units: Children's Medical Center, Boston, Massachusetts
Boston Lying-In Hospital, Boston, Massachusetts
Perinatal Research Branch, NINDB

Man Years

Total: 2/12
Professional: 1/12
Other: 1/12

Project Description: This study is evaluating maternal characteristics, socio-economic data, factors of pregnancy, labor, and delivery, in terms of respiratory distress syndrome (hyaline membrane disease) in the pregnancy outcome. Study cases identified as respiratory distress syndrome have been obtained from the PED-8 (Newborn Diagnostic Summary). This group of cases would include false positive; that is, cases carrying the diagnosis but without the x-ray and laboratory data to justify such a diagnosis. Identification of the false negatives must also be made; cases with the syndrome having laboratory and x-ray evidence of having the syndrome, but not reported on the PED-8, will be identified by reviewing all cases that have received oxygen therapy as reported on the PED-3. The definition of the respiratory distress syndrome is: Increased respiratory rate, retractions, cyanosis, decreased pH, increased CO₂, and x-ray evidence compatible with the diagnosis.

Once the Study cases have been satisfactorily identified and reviewed, correlations will be made relating maternal characteristics, factors of labor and delivery, and socio-economic data to the mortality and morbidity

of the offspring. Special interest has been shown in the neurological morbidity of those infants surviving the syndrome.

Study in progress.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/PN 1335
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Mortality and Morbidity Among Infants Weighing 1000-2000 Grams.

Previous Serial Number: None

Principal Investigator: Joseph S. Drage, M.D., PRB, NINDB

Other Investigators: Karin B. Nelson, M.D., PRB, NINDB
B. H. Williams, M.D., PRB, NINDB

Cooperating Units: Perinatal Research Branch, NINDB

Man Years

Total: 3/12
Professional: 2/12
Other: 1/12

Project Description: A group of 720 liveborn infants, with birthweights ranging from 1000-2000 grams, has been studied regarding outcome within the first year of life. This group of 720 infants represents approximately 2% of the group of 35,000 single live births from which they were drawn.

Within the group of 720 infants, 485 were examined at one year of age, and of these 101 were considered to have definite neurological abnormality. There were 159 deaths during the first year of life. Thus, 260 of the 720 infants either died during the first year of life or were considered neurologically abnormal by examination at one year. There were 76 infants lost to follow-up and this represents 10% of the original 720 cases. Over 50% of the 159 deaths occurred during the first 24 hours, and over 90% occurred during the first 28 days.

The 101 infants neurologically abnormal at one year were classified in the following way: isolated motor retardation, diplegia, hemiplegia, quadriplegia, monoplegia, athetosis, motor retardation with neurological signs insufficient for other diagnosis, and other (infants with neurological signs, but not fitting a specific diagnostic category). Congenital malformations were also monitored for this group.

Outcome at one year (death, abnormal, normal, and lost) was then tabulated within each 100-gram birthweight interval. In general for the 720 infants,

as the birthweight increased, the percent of deaths decreased. For infants in jeopardy (dead or abnormal at one year), the same relationship held. The percent of lost cases within each 100-gram birthweight interval varied slightly.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/PN 1336
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Effects on the Child of Convulsive Disorder in the Mother.

Previous Serial Number: None

Principal Investigator: Karin B. Nelson, M.D., PRB, NINDB

Other Investigators: None

Cooperating Units: Perinatal Research Branch, NINDB

Man Years

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

Project Description: All cases of non-eclamptic convulsive disorder during or preceding pregnancy for whom a definite pediatric outcome--either a one-year neurological examination or a death in the first year--was known, were assembled and reviewed by hand. There were 540 cases in all, of which 97 were instances of convulsive disorder during the Study pregnancy.

It was found that convulsive disorder was a significant high-risk factor in this sample. If total reproductive wastage (including stillbirths, neonatal and infants deaths, and neurological abnormality) is compared for this series and for the Core Study, it is found that the figure for the Core Study is 5.5%, for the convulsive disorder series as a whole 10.2%, for cases of convulsions within the two years preceding delivery 15.6%, and for cases of seizures during pregnancy 17.1%. Thus, there is three times the risk of reproductive wastage through the first year of life among children who are the products of a pregnancy in which a seizure occurred. These differences are statistically significant at the 0.001 level.

The greatest increase in risk lay in liability to neurological abnormality of children of mothers with convulsive disorders, this risk being greatest when a maternal seizure occurred in the pregnancy. No clear relationship to trimester in pregnancy could be ascertained in this sample.

A number of factors were examined in an attempt to analyze this relationship between maternal convulsions and increased pediatric risk. Cause of

seizures, role of anticonvulsant medication, and several other factors were investigated, but it was found that size of the sample, or the lack of consistent careful workup of the seizure diathesis in the mother, made it impossible to answer the questions raised by the observation of increased wastage in children of mothers with convulsive disorder.

It is possible in this sample at least to identify seizures within the past two years as constituting one form of high risk pregnancy, the mechanism of which remains unclear. Because of the practical nature of the questions raised with regard to management and drug therapy, etc., it would seem desirable to pursue this observation further.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/PN 1337
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: An Investigation into Relationships Between History of Signs,
Symptoms and Behavior Early in Pregnancy and Pregnancy Outcomes.

Previous Serial Number: None

Principal Investigators: Lenore Bajda, M.D., PRB, NINDB and
Rudolph Vollman, M.D., PRB, NINDB

Other Investigator: Martin Mendelson, Ph.D., PRB, NINDB

Cooperating Units: Perinatal Research Branch, NINDB

Man Years

Total: 6/12
Professional: 4/12
Other: 2/12

Project Description: This study will look for meaningful associations between maternal histories as recorded on OB-3 through OB-8 and outcomes of pregnancy as noted on the newborn 8-months and 1-year Diagnostic Summaries. The outcomes will be considered also in the light of prior pregnancy losses.

Cohort to be used are first trimester registrants (14 weeks or less from LMP) from onset of study to January 1966, who were first study pregnancies with single birth outcomes.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/PN 1338
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Association of Mental Subnormality with Head Circumference, Congenital Malformations, and Other Conditions of the Newborn Term Infant.

Previous Serial Number: None

Principal Investigators: Lenore Bajda, M.D., PRB, NINDB and
Martin A. Mendelson, Ph.D., PRB, NINDB

Other Investigators: None

Cooperating Units: Perinatal Research Branch, NINDB

Man Years

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

Project Description: The objective of this study is to determine the relationship between head size and certain other physical features taken on the child shortly after birth and at 1-year and indications of possible mental retardation as manifested by performance on the 8-month Psychological Examination, specifically the scores on the mental scales, fine motor and gross motor scales. A study somewhat along these lines was made on the basis of retrospective data at the Mayo Foundation of the Mayo Clinic, Rochester, Minnesota by Drs. Edward J. O'Connell, Robert H. Felt and Gunner B. Stickler. Their study was reported in Pediatrics, 36:1, July, 1965. The objective of our study based on prospective data is similar to the objective formulated for the Mayo Study in that it will be designed to determine whether there is a significant incidence in mental subnormality in CORE Study children, primarily in terms of physical features--head circumference, body length, and weight--and the relationships of these to scores on the 8-Month Psychological Examination (controlling for gross diagnosis of outcome at the 1-year level).

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/PN 1339
1. Perinatal Research Branch, NINDB
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Sudden Unexpected Death.

Previous Serial Number: None

Principal Investigator: Joseph S. Drage, M.D., PRB, NINDB

Other Investigators: Toshio Fujikura, M.D., PRB, NINDB
Lon White, M.D., PRB, NINDB

Cooperating Units: Perinatal Research Branch, NINDB

Man Years

Total: 3/12
Professional: 2/12
Other: 1/12

Project Description: Sudden unexplained death in infancy is a problem challenging medical research. The Collaborative Project has data, prospectively collected that has potential for contributing to the solution of this problem.

Pediatric records of the Study will be reviewed on infants who have died suddenly without explanation. This review will seek evidence of abnormality earlier in life. Apgar scores, need for resuscitation, cyanotic episodes, feeding problems, etc., will be evaluated regarding their incidence in these cases. In addition the socio-economic index will be studied. Obstetrical records will be reviewed in an effort to determine if specific maternal complications are significantly related. Evidence of infectious disease will be evaluated by examination of serum specimens obtained during the mother's pregnancy and during early infancy. Pathological material will be reviewed for evidence of pathology. This would include review of autopsy material, as well as placenta, cord, and membrane material.

Honors and Awards: None

Publications: None

Serial No. NDB-(CF)-63 PR/BS 1166

1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Evaluation of the Gross and Fine Motor Functions of Children Eight-Months of Age in Relation to the Neurological Results of the Examination of the Children at One-Year of Age

Previous Serial Number: Same

Principal Investigator: Dr. Martin A. Mendelson

Other Investigators: Dr. Emmy E. Werner

Cooperating Units: Collaborative Institutions, COLR Project, NINDB
(Data from records)
Section on Pediatric-Neurology, PRB, NINDB
Section on Behavioral Sciences, PRB, NINDB

Man Years:

Total: .5 man years
Professional: .2 man years
Other: .3 man years

Project Description:

The objective of this study is to determine whether the gross and fine motor performance of children, assessed at 8 months of age, is related to the results of neurological examinations given to the same children at one-year of age. It is hypothesized that the degree of dysfunction in fine and gross motor performance will be indicated correspondingly in the degree or dysfunction, as well as the type found neurologically in children at one-year of age.

This study has not been processed on account of delays in acquiring computer facilities and time.

Honors and Awards: None

Publications: None

13-

14-

Serial No. NDB-(CF)-63PR/BS 1167

1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Explorative Study for the Use of A Speech and Language Screening Examination for 3-Year Old Children in the Home Situation

Previous Serial Number: Same

Principal Investigator: Dr. Miriam F. Fiedler

Other Investigators: Dr. Martin A. Mendelson

Cooperating Units: Children's Medical Center, Boston Massachusetts
Section on Behavioral Sciences, PRB, NINDB
Section on Pediatric-Neurology, PRB, NINDB

Man Years:

Total: .5 man years
Professional: .2 man years
Other: .3 man years

Project Description:

This is the first of two studies to try and determine the most efficient way of identifying those 3 year olds whose speech and language development is atypical and may be associated with neurological and sensory defects. At Boston, the Children's Medical Center has developed a home interview form designed to obtain information on the child's speech, language and hearing from the mother; a comparative study of children whose mothers have been interviewed with this form, and on whom (the children) speech, language and hearing examination results are available, is contemplated.

This study is in progress, with sorting and analysis of the data being accomplished with manual rather than computer methods.

Honors and Awards: None

Publications: None

Serial No. NDB-(CF)-63 PR/BS 1168
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Indexes of Socio-Economic Status of Study Mothers in Relation to Outcomes of Pregnancy

Previous Serial Number: Same

Principal Investigator: Dr. Martin A. Mendelson

Other Investigators: None

Cooperating Units: Section on Epidemiology and Genetics, PRB, NINDB
Section on Behavioral Sciences, PRB, NINDB
Section on Obstetrics, PRB, NINDB
Section on Pediatric-Neurology, PRB, NINDB

Man Years:

Total: .8 man years
Professional: .5 man years
Other: .3 man years

Project Description:

This study involves an attempt to determine the relationship between specific socio-economic indexes and specific outcomes of pregnancy. A socio-economic index suggested by V. Packard is to be applied to the socio-economic data on Study Mothers. It is hypothesized that prenatal morbidity and mortality will increase as the socio-economic index decreases.

This project was discontinued during fiscal year '66.

Honors and Awards: None

Publications: None

Serial No. NDB-(CF)-63 PR/BS 1169

1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Antecedents and Correlates of Retarded Motor Development

Previous Serial Number: Same

Principal Investigator: Dr. Audrey Shechtman

Other Investigators: Dr. Martin A. Mendelson

Cooperating Units: Collaborative Institutions, COLR Project, NINDB
(Data from records)
Section on Pediatric-Neurology, PRB, NINDB
Section on Behavioral Sciences, PRB, NINDB

Man Years:

Total: .8 man years
Professional: .4 man years
Other: .4 man years

Project Description:

The major objective of this study is to investigate the variables on which 8-month old infants judged to be normal, suspect, or abnormal in motor development, in terms of Bayley Motor Scale norms, can be differentiated neonatally at the 4 and 8 month level. Additional objectives are: (1) To determine the level of functioning of these groups on the Bayley Mental Scale; and (2) To explore relevant behavioral variables and their relationships to the above.

This project was discontinued during fiscal year '66.

Honors and Awards: None

Publications: None

Serial No. NDB-(CF)-63 PR/BS 1170

1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Socio-Economic Status Grouping and Their Relationship to Illnesses and Disorders as Reported on the OB-3, "History Since Last Menstrual Period," and OB-8, "Repeat Prenatal History"

Previous Serial Number: Same

Principal Investigator: Dr. Martin A. Mendelson

Other Investigators: Dr. Rudolph Vollman

Cooperating Units: Section on Epidemiology and Genetics, PRB, NINDB
Section on Behavioral Sciences, PRB, NINDB
Section on Obstetrics, PRB, NINDB
Collaborative Institutions, COLR Project, NINDB
(Data from records)

Man Years:

Total: .5 man years
Professional: .3 man years
Other: .2 man years

Project Description:

The hypothesis of this study assumes that the number of symptoms of disorders reported by Study gravidae is related to their socio-economic status in such a way that the greater the number of illnesses or discomforts reported, as well as the type of illnesses or discomforts reported, increase, as the socio-economic status of the gravidae decreases. Information from OB-3 and OB-8 (History since LMP and Repeat Prenatal History) are the major dependent variables.

These studies are in progress; they have not been processed sufficiently as yet on account of delays in acquiring computer facilities and time.

Honors and Awards: None

Publications: None

Serial No. NDB-(CF)-63 PR/BS 1171
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: An Evaluation of the Offspring of Unwed Mothers in Terms of the Complications of Birth and Delivery, Results on the 4-Month Examination, 8-Month Examination and One-Year Examination, as Compared to a Matched Group of Married Mothers and Their Offspring.

Previous Serial Number: Same

Principal Investigator: Dr. Martin A. Mendelson

Other Investigators: None

Cooperating Units: Section on Behavioral Sciences, PRB, NINDB
Section on Pediatric-Neurology, PRB, NINDB
Section on Obstetrics, PRB, NINDB
Collaborative Institutions, COLR Project, NINDB
(Data from records)

Man Years:

Total: .6 man years
Professional: .4 man years
Other: .2 man years

Project Description:

The purpose of this study is to determine whether there are significant differences between the offspring of a selected group of unwed mothers as compared to a matched group of married mothers. There is reason to believe, based on previous research, that the unwed mother is subject to various cultural and psychological stresses that can effect the course of her pregnancy, may show up in complications of birth and delivery, and may have measurable influences on the development of the offspring.

This project was discontinued during fiscal year '66

Honors and Awards: None

Publications: None

Serial No. NDB-(CF)-65 PR/BS 1273
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Hypothesized Performance on 8-Month Old Children, Considered Premature at Birth, on the COLR Form of the Bayley Scales of Mental and Motor Development

Previous Serial Number: Same

Principal Investigator: Dr. Martin A. Mendelson

Other Investigators: Dr. Heinz Berendes
Mr. William Weiss
Mr. Jerome Deutschberger
Dr. Zekin A. Shakhashiri
Miss Esther Jackson

Cooperating Units: None

Man Years:

Total: .5 man years
Professional: .2 man years
Other: .3 man years

Project Description:

The objective of this investigation is to follow-up a premise originating from the studies of Drs. Pasamanick and Knobloch regarding postnatal performance of premature children on objective scales of mental and motor performance. It has been indicated that it may not be fair to judge, at least during the first years of life, the premature infant's performance on psychological instruments that have been developed on the basis of age-level norms.

Computer time has been obtained for this project and tabulations of data were made. The study was completed and presentation of the results was made at the Scientific Symposium sponsored by NINDB and held on March 24-25, 1966.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-63 PR/EG 1172

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Pregnancy Outcome and Prior Pregnancy Record

Previous Serial Number: Same

Principal Investigators: Dr. V. Elving Anderson, Univ. of Minnesota
Dr. N. C. Myrianthopoulos, PRB, NINDB

Other Investigators: None

Cooperating Units: University of Minnesota

Man Years:

Total:	.25
Professional	.25
Other:	.0

Project Description:

This Study was conducted to examine the extent to which information about the course and outcome of prior pregnancies of the gravida may be used to predict the outcome of the current pregnancy, and to look for important biological variables affecting reproductive wastage.

Preliminary analysis showed that women with a prior loss tended to register earlier in the Study than other women. In order to take time of registration into account a modified life table procedure was employed to determine for each week of gestation the number of women subject to risk of pregnancy termination. This permits calculation of "week-specific" rates of fetal loss adjusted for the changing population at risk. The results of this methodological approach were presented at the Second Scientific Meeting of the Collaborative Study, in March, 1966.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-63 PR/EG 1173

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A Study of Socioeconomic, Medical and Genetic Factors in
Major Congenital Malformations

Previous Serial Number: Same

Principal Investigator: Dr. N. C. Myriantopoulos, PRB, NINDB

Other Investigators: Dr. L. Bajda, PRB, NINDB
Dr. C. Chung, NIDR

Cooperating Units: None

Man Years:

Total	.45
Professional	.25
Other:	.20

Project Description:

The purpose of this project is to study the relation of medical and genetic factors to major congenital malformations. The study is based on 593 cases of liveborn and fetal deaths with malformations and 1,186 controls drawn from a total of 35,941 births and fetal deaths. The malformations include anencephaly, polydactyly, cleft palate and/or harelip, spina bifida, and meningocele, congenital heart defects and hypospadias. The information collected prenatally and analyzed consists of race, histories of medications, X-ray examination, pregnancy complications and diseases, gestation time, parity, parental age, sibling data and socioeconomic status.

Data processing conducted in the Branch and multivariate analysis conducted at the University of Hawaii are complete. None of the medical or sociological variables has shown significant relationship with the presence of any malformation. However, siblings of the affected are at elevated risk. Mothers of children with cleft lip, palate, gum or uvula tend to have shorter gestation time than those of normal children. These results are scheduled to be reported at the Third International Congress of Human Genetics and a manuscript is being prepared for publication.

Honors and Awards: None

Publications: None

Serial No. NDB(CF)-63 PR/EG 1174

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Birthweight in Relation to Selected Socioeconomic Variables

Previous Serial Number: Same

Principal Investigator: Dr. N. C. Myrianthopoulos, PRB, NINDB

Other Investigators: Dr. A. F. Naylor, PRB, NINDB

Cooperating Units: None

Man Years:

Total:	.45
Professional	.30
Other:	.15

Project Description:

A Study has been conducted to relate birthweight to socioeconomic variables such as family income, parental education and occupation, housing density and other information gathered in Study interviews.

A partial sample study using a card sorter had suggested that some curvilinear relationships exist. A multivariate covariance analysis of the race variable has been completed. The results indicate that socioeconomic (SE) variation may not be able to account for racial differences in birthweight and that SE variables have different predictive values in the different ethnic groups in the Study.

The major results have been presented at the Second Scientific Meeting of the Collaborative Study, March, 1966. A manuscript is now being prepared for publication.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-63 PR/EG 1175

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Determination of the Zygosity of Twins Born to Mothers
in the Collaborative Study

Previous Serial Number: Same

Project Coordinator: Dr. N. C. Myrianthopoulos, PRB, NINDB

Cooperating Units: All Institutions participating in the Collaborative Study

Man Years:

Total:	.25
Professional	.25
Other:	

Project Description:

This is a continuation of a project to determine the zygosity of twins born to Study mothers. This determination is basic and vital to any subsequent studies using twins. Twin zygosity is determined by comparison of sex, placentation, blood groups, and finger and palm prints. This information is forwarded by all Institutions to the Section on Epidemiology and Genetics where it is classified and analyzed by special methods. The results are kept on file and are available to anyone wishing to engage in twin research.

In progress.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-63 PR/EG 1176

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A Genetic and Socioeconomic Study of Habitual Aborters

Previous Serial Number: Same

Principal Investigator: Dr. N. C. Myriantopoulos, PRB, NINDB

Other Investigators: Dr. A. F. Naylor, PRB, NINDB

Cooperating Units: None

Man Years:

Total:	.20
Professional:	.20
Other:	

Project Description:

This is a continuation of prospective Study of gravidae whose prior pregnancy history shows three or more early or late fetal deaths, excluding known induced abortions. The objective is to determine if genetic and/or socio-economic factors are related to habitual abortions. Study gravidae are matched with controls by same number of prior pregnancies but no more than one fetal death, Institution, race and age. Study variables include: outcome of first Study pregnancy, outcome of subsequent pregnancies where available, consanguinity on two levels, known cause of repeated abortions, reproductive history of sisters in Study where available, and socioeconomic factors.

Preliminary listings of data needed for the design and programming of the analysis have been received and work is in progress.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-63 PR/EG 1177

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Genetic and Socioeconomic Factors in Early and Late Fetal Death

Previous Serial Number: Same

Principal Investigator: Dr. N. C. Myrianthopoulos, PRB, NINDB

Other Investigators: Dr. A. F. Naylor, PRB, NINDB
Blanche Vincent, PRB, NINDB

Cooperating Units: None

Man Years:

Total:	.30
Professional	.20
Other:	.10

Project Description:

This is a continuation of a retrospective study of gravidae whose first Study pregnancy terminated in early or late fetal death. The objective is to determine what effect do genetic and socioeconomic factors have on pregnancy wastage and if a distinction can be made etiologically between early and late fetal death. Study gravidae are matched with controls by Institution, race and age. Study variables include prior pregnancy history, parity, socioeconomic factors, and subsequent pregnancy history, where available.

Preliminary listings of data needed for the design and programming of the analysis have been received and work is in progress.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-63 PR/EG 1178

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: A Socioeconomic Index for the Population of the Collaborative Project

Previous Serial Number: Same

Principal Investigator: Dr. N. C. Myriantopoulos, PRB, NINDB

Other Investigators: Dr. Kathrine French, University of Oregon

Cooperating Units: None

Man Years:

Total:	.40
Professional	.30
Other:	.10

Project Description:

The purpose of this project has been to develop an index by which to describe the socioeconomic status of Study childrens' families on the basis of information obtained about the family of the gravida before the Study child is born. The main components of the index are: education, occupation and income. The index is derived for the whole population and by individual Institutions, in two ways: first, by using available U.S. Census scores for education, occupation and income. This index enables us to compare socioeconomically the Study population with the United States population. Second, by deriving empirical scores for the socioeconomic variables from the distribution of these variables in the Study population. This enables us to obtain an objective socioeconomic picture of our population and observe similarities and differences among the various participating Institutions.

The developmental phase of the Project is completed. The indices for much of the Study population are now available on tape and the tape file will be updated from time to time as more cases become available for electronic data processing.

Some investigations based on the available sample indicate that when distributions from the Collaborative Study data are corrected for race and age effects the socioeconomic circumstances of the group appear to be

fairly close to that for the U.S. as a whole. These facts have been reported at the Second Scientific Meeting of the Collaborative Study, in March, 1966.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-63 PR/EG 1179

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Association of Blood Groups to Amyotrophic Lateral
Sclerosis

Previous Serial Number: Same

Principal Investigator: Dr. N. C. Myrianthopoulos, PRB, NINDB

Other Investigators: P. Schmidt, CC
W. Leyshon, NIDR

Cooperating Units: Blood Bank, CC, Genetics Branch, NIDR

Man Years:

Total:	.20
Professional:	.20
Other:	.0

Project Description:

A continuation of pilot study to determine if there exists any selection for a specific blood type among patients with motor neurone disease.

The study is now completed. Analysis of the material shows a very slight association with the P group but considerable association with the Secretor factor. The material is being prepared for publication.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-63 PR/EG 1180

1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A Survey of Schizophrenia Among the Relatives of Schizophrenic Patients

Previous Serial Number: Same

Principal Investigator: Dr. N. C. Myrianthopoulos, PRB, NINDB

Other Investigators: None

Cooperating Units: Spring Grove State Hospital
St. Elizabeth's Hospital

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description:

A Study to determine the occurrence of schizophrenia among the relatives of patients who have already been selected for another project (Parkinsonism - ataraxic drugs) and to determine the role of inheritance in schizophrenia. This project being a by-product of another project, has the advantage of overcoming some of the biases involved in selecting an adequate sample. The collection of data from Spring Grove State Hospital has been completed. These will be implemented with data from St. Elizabeth's Hospital concurrently with the project on ataraxic drugs conducted there before genetic analysis is attempted.

No further progress has been made.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-63 PR/EG 1183

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

FHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A Genetic Study of Parkinsonism and the Dystonias Produced
by Ataraxic Drugs

Previous Serial Number: Same

Principal Investigator: Dr. N. C. Myrianthopoulos, PRB, NINDB

Other Investigators: Francis Waldrop
Blanche Vincent

Cooperating Units: St. Elizabeth's Hospital

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description:

A Study to confirm earlier findings. If the impression is confirmed that there is an hereditary susceptibility to Parkinsonism produced by phenothiazine derivatives then the effects of phenothiazines will be studied in those siblings and close relatives of patients and controls who have mental illness and are institutionalized. The known degree of relationship of relative to patient will serve as a basis for prediction of the eventual outcome if susceptibility to ataraxic drugs is inherited along Mendelian lines.

This project is discontinued as of 1966.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-63 PR/EG 1184

1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Population Dynamics of Tay-Sachs' Disease

Previous Serial Number: Same

Principal Investigator: Dr. N. C. Myriantopoulos, PRB, NINDB

Other Investigators: Dr. Stanley Aronson, State University of New York

Cooperating Units: State University of New York

Man Years:

Total:	.40
Professional:	.30
Other:	.10

Project Description:

This study has been designed to determine whether differential fertility favoring the Jewish heterozygote can account for the 100-fold higher frequency of Tay-Sachs' disease and the gene responsible for it among the Jewish compared with non-Jewish population in the U. S. The completed fertility of presumed Jewish heterozygotes was compared to that of adequate controls and the results suggest that the Jewish heterozygote has a reproductive advantage of about six percent over that of the homozygous normal. Historical evidence appears to corroborate this hypothesis and to place the rise of the TSD gene among the Ashkenazi Jews in historical times, perhaps during the early centuries of the diaspora. The study is continuing on the demographic level, to identify the factor or factors which confer the selective advantage.

Honors and Awards: None

Publications:

Myriantopoulos, N. C. and Aronson, S. M. : Population dynamics of Tay-Sachs' disease. I. Reproductive fitness and selection. Am. J. Hum. Genet. to be published

Myriantopoulos, N. C. and Aronson, S. M. : Reproductive fitness and selection in Tay-Sachs' disease. In, Cerebral Sphingolipidoses, Pergamon Press, to be published

Serial No. NDB (CF)-65 PR/EG 1274

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Genetic Bases of Neonatal Reflexes

Previous Serial Number: Same

Principal Investigator: Dr. A. F. Naylor, PRB, NINDB

Other Investigators: Dr. N. C. Myrianthopoulos, PRB, NINDB

Cooperating Units: None

Man Years:

Total:	.40
Professional:	.30
Other:	.10

Project Description:

The validity of regarding the suck, rooting and other neonatal reflexes as definite genetic entities is under study. Cases involving loss of single reflexes without evidence of hypoxia or other trauma may be genetically based. Such cases are being sought.

A computer output, initially obtained by the Section on Systems, Design and Procedures and retrieved in modified form by the principal investigator, is now available. This is a listing of approximately 435 Study baby cases for which computer processing alone cannot eliminate the possibility of the absence of one or more reflexes. These cases await critical hand review of the full case histories.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-65 PR/EG 1275

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Study of Family Size with Respect to Rh Blood Type and
Other Variables

Previous Serial Number: Same

Principal Investigator: Dr. A. F. Naylor, FRB, NINDB

Other Investigators: Dr. N. C. Myrianthopoulos, PRE, NINDB

Cooperating Units: None

Man Years:

Total:	.05
Professional:	.05
Other:	.00

Project Description:

The Project will re-examine the suggestion made by Glass in 1950 that fetal loss in Rh negative women may actually be over-compensated for in a population practicing birth control, which may be lifted when incompatibility loss occurs. Information on the Rh type of gravidae, previous incompatibility complications, family planning practices and race will be related to age-corrected family sizes of gravidae.

A standing request for appropriate data processing by the Section on Systems, Design and Procedures has been withdrawn because the principal investigator believes the desired output requires such detailed specification that he may best attend to the required computer programming himself.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-65 PR/EG 1276

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Sequential Aspects of Occurrence of Spontaneous Abortion
in Family Histories

Previous Serial Number: Same

Principal Investigator: Dr. A. F. Naylor, PRB, NINDB

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.35
Professional:	.25
Other:	.10

Project Description:

Collaborative Project data have been used to check whether abortion frequency has true age or parity relations and/or whether abortions tend to cluster in the reproductive histories of multiply-aborting women. The question of a "true" age-dependence is important especially since James (1963) suggested that an artifactual or spurious age correlation could arise if women who are innately more likely to abort, independently of age at conception, continue to attempt to satisfy family-size aspirations through later ages when non-aborters have restricted their reproductive activities.

The principal investigator has seen the analysis through computer processing, has prepared a manuscript suitable for prepublication circulation and has presented the results at the Second Scientific Meeting of the Collaborative Study, March, 1966. The results, briefly, are that a genuine age or gravidity effect but no clustering has been detected.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-65 PR/EG 1277

1. Perinatal Research Branch
2. Section on Epidemiology
and Genetics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: A Study of the Properties of B. Woolf's Statistic for
Combined Analysis of 2 x 2 Tables

Previous Serial Number: Same

Principal Investigator: Dr. A. F. Naylor, PRB, NINDB

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.10
Professional:	.09
Other:	.01

Project Description:

As an outgrowth of methodological discussions within the Section, attention has been directed to the problem of combining tests of pairs of samples from binomial populations. The problem arises widely in the treatment of epidemiological data. B. Woolf (1955) has suggested a statistic for the purpose of making a combined test and stating the strength of association in a two-fold classification of discrete data. The statistic is a fairly new one when its generality of application is considered and its mathematical properties have not been exhaustively considered.

Computer investigations programmed by the Principal Investigator have indicated that at least by one important criterion the old fashioned statistic (in its strictly correct form) which compares the difference of two proportions to the estimated standard error of the difference is superior to Woolf's statistic for hypothesis testing. The refinements which were mentioned in the last annual report are completed and a manuscript giving the results is in preparation.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-65 PR/P 1278

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Biologic Pattern Data Processing

Previous Serial Number: Same

Principal Investigators: Lewis E. Lipkin, M. D.
Russell A. Kirsch

Other Investigators: None

Cooperating Units: Artificial Intelligence Group,
National Bureau of Standards

Man Years

Total: 6.2
Professional: 3.3
Others: 2.9

Project Description:

The work that was done on the Collaborative Project between the National Bureau of Standards and the National Institutes of Health may for purposes of reporting be divided into four categories: the first concerned with language processing; the second with picture processing; the third with the use of elaborate direct access interrogations systems; and, the fourth a miscellaneous category.

The language processing investigations have been, as in previous reporting period, aimed toward the development of a language which can be used by neuropathologists for interrogating a system for processing photomicrographs and other microscope images. The notion of a habitable language, that is one which an investigator with comparatively little prior training can use and find himself able to stay within, has received further study during this period. Some general discussion of the desirable properties of such an interrogation language appear in NBS Technical Note 258, "A Prerequisite to the Utility of Microgrammars," by William C. Watt. A microgrammar such as is discussed in this report appears in NBS Technical Note 281, "Materials for Placebo V," by William C. Watt, and National Bureau of Standards Report 8934, "Concordance to the Rules of Placebo V," by William C. Watt. These two reports describe a large language whose grammar was made explicit in such a form as to lead directly to machine processing of interrogations within that language.

The language concerned here is a superset of a language previously reported on and which therefore has the property that computer programs written for the prior language can be absorbed into programs written for the larger language Placebo V. Some attention was given to this methodological matter of building large languages in such a way that each one properly includes the previous one so as to preserve all processing programs written for earlier versions when they are absorbed into later and larger versions of an interrogation language. In NBS Report 281, two such languages are exhibited side by side so as to demonstrate the manner in which a language may be expanded to provide greater adequacy and greater habitability for the biologist using it as his prime interrogation medium.

In order to analyze statements and commands and interrogations made to a system in such a language, a syntactic analyzer or parser is necessary which determines the syntactic analysis of such interrogations with respect to a grammar such as that of Placebo V. A syntactical analyzer or parser was completed early during the present reporting period which made use of FORTRAN for exhibiting the syntactic analysis of sentences and of all sub-sequences of sentences furnished to the machine. The logic of this syntactic analyzer was proved adequate but the use of FORTRAN and the degree of effort put into the development of the program did not yield an efficient parser. Several minutes of machine time were required to parse a single sentence. It is fairly easy to see, however, how improvements by perhaps two decimal orders of magnitude can be made in the running time of such programs. There presently exist several syntactic analyzers written for various computers by various investigators. None of them however are compatible with each other and it will inevitably be the case that for a productive system such as we envision, a tailor made syntactic analysis program will be necessary even though it exploits some of the insights developed here and elsewhere.

For a machine to interpret syntactically analyzed sentences furnished to it by an investigator, it is necessary to convert the syntactically analyzed sentences into a form used for internal representation in the machine. Although this form may bear close resemblance to the form used for exhibiting the syntactical analyses of the sentences, it is nevertheless the case that early versions of such systems as our interrogation system will probably use more arbitrary formal representation languages internally. To this end, an extensive study was made of the internal representation language into which we might translate the sentences of the earlier language Placebo IV. This translator which we call a formalizer was constructed for the Placebo IV grammar through the use of the MAC computer system which will be described below. For each of the over 1,000 rules in the Placebo IV grammar, corresponding rules were written which enable the machine to separate the semantically meaningful from the semantically not meaningful parts of a syntactically analyzed sentence of the grammar. This formalizer provides the critical input to a table driven syntax directed interpreter for the English sentences of Placebo IV.

The second major class of tasks during this period concerned the processing of pictorial images. A language was constructed based on the PAX language at the University of Illinois for operation on the 7094 computer at NBS. This language called STANDPAX enables pictures scanned with the rotating drum scanner at NBS to be processed in such a way that ten-inch square pictures may be handled in squares three-eighths of an inch on a side at a time. Each of these so-called windows is recorded sequentially on the magnetic tape input to STANDPAX and may be referred to in terms of its X-Y coordinates on the input photographs. STANDPAX primarily provides capabilities for transforming pictures into other pictures, hence, for doing such operations as Boolean algebraic, smoothing, filtering, enhancement, etc. It is necessary, however, to extract numerical data from such pictures and such transformed pictures.

To accomplish this, a language was constructed consisting of STANDPAX embedded in FORTRAN. This language SPIFII provides the capabilities for ordinary arithmetic processing of numerical quantities extracted from pictures by the STANDPAX part of SPIFII. One such program currently being completed jointly at NBS and NIH enables the 7094 computer to take a photograph containing about 1,000 cells and to select out some subset of these cells which are then subjected to an analysis to determine such properties as their average optical density and geometric moments of the density function which represents the cell image. Such geometric properties may be useful invariants to use in classification of cells for application like counting.

The STANDPAX language was modified to be compatible with the MAC computer (again to be mentioned below) and several programs were written on-line to process individual cell images. One set of such programs took spatial derivatives of the cell images and then assigned labels to the boundary components in terms of the predominating directions of the cell boundaries. It was intended to apply these programs to the problem of three-dimensional serial reconstruction of cells from optical serial sections but this work was not completed during the time we had access to the MAC computer.

Another picture processing investigation was concerned with the study of texture in images. Most of the study of images for pattern recognition has been concerned with black and white images, hence, images either of a synthetic nature or which could be so quantized as to be converted to binary form. For the images of biological interest, however, we must encounter multiple gray scales and hence the possibility that closed regions in such pictures will be characterized by statistically discriminable but otherwise non-constant density functions, hence texture. Thus far, this investigation has been successful in characterizing textures which are uniform but discriminable from each other in terms of certain statistical properties of them which can be measured.

Another study was made which can be considered both pictorial and linguistic in nature. This was the study of a particular class of images which have a codified system of natural language descriptions. W. C. Watt in NBS Report 9050, "Morphology of the Nevada Cattle Brands and Their Blazons," Part 1, studies the covariance between the pictorial objects in brands and their descriptions. He exhibits a grammar for the pictorial objects and shows how linguistic considerations determine the pictorial pattern processing that presumably might take place in a pattern recognition system.

Still in picture processing some work was done on methods for creating synthetic photographs of tissue sections by juxtaposing objects within such photographs. The work was abandoned because of the difficulty of dealing with the pictures mechanically. The work will be resumed, however, when the forthcoming use of a Stromberg-Carlson 4020 printer for creating high quality images is undertaken during the next reporting period.

Finally, in the category of picture processing, some initial investigation was undertaken in the use of the COMIT programming language for dealing with two-dimensional, i.e., pictorial objects, and some early investigation was made of possible correspondences between the linguistic phenomenon of depth and a potential counterpart in pattern recognition.

The third category of tasks performed during this period was concerned with the use of interrogation systems in which a computer is provided with immediate access facilities usually to many simultaneous users. In order to study the on-line interrogation aspects of a biological interrogations system such as we ultimately will design, some extensive investigation was made of the use of the Project MAC time-sharing system at MIT. As mentioned above, a large grammar was put into this system as was the facility of the STANDPAX language. If more time had been available, a more direct connection between these two disjoint programs would have been established. One important investigation was made regarding interrogating a system about a complex grammar. Programs were written in the COMIT language which enabled a grammar of the type of Placebo IV to exhibit tree structures corresponding to specified sentences within the grammar. The fact that such programs can be run on-line with immediate consequences displayed to the user suggested the important use for trying to teach a potential customer (i.e., a neuropathologist) to use what would otherwise be a grammar too large for him to bother learning. Consequently, Dr. Lipkin spent several hours at the teletype interrogation console exploring the structure of the Placebo IV grammar. He was able to determine that in such a comparatively short time he could learn the use of such a grammar even though its size was such as to make it unlikely that he could otherwise learn and memorize the rules necessary to use it if the on-line interrogation system were not available as a teaching device.

It thus appears that whatever advantages there are in using a natural language for interrogation, these advantages begin to show only with languages of some considerable size, primarily because with a direct access interrogation system, rather large arbitrary, i.e., artificial language, can be conveniently learned and used.

Subsequent to the experience with the MAC time-sharing system, an effort was undertaken, which is currently underway, to study comparable problems on the ANFS-Q-32 computer at the System Development Corporation in Santa Monica, California. This time-sharing system is the only other one of comparable size to the MAC system which can serve the purposes of our investigation. It is too early to have any results from the Q-32 computer experiments.

In the final category, there were two activities of a miscellaneous nature. The first was a conference sponsored by the Association for Computing Machinery on the Pragmatics of Programming Languages at which R. A. Kirsch organized a session on pictorial information processing. The results of this conference appear in the Communications of the Association for Computing Machinery, 9:3, March 1966. The paper by Lipkin, Watt and Kirsch, "Analysis, Synthesis and Description of Biological Images," appeared in the Annals of the New York Academy of Sciences, 128:3, January 31, 1966. This paper provides something of an overview of the work on the Collaborative Project as of about the middle of the present reporting period.

On fiscal matters, there are several observations worth noting. First, the level at which the project has been conducted has been somewhat less than anticipated. This is due largely to present government requirements for a lower staffing level than had been anticipated and also due in some small part to the move of the National Bureau of Standards to the new location in Gaithersburg, Maryland. The staffing level problem is partly offset by successful collaborative efforts that have progressed with other institutions where it has been possible to encourage work of a nature that will contribute to our project although over a somewhat longer period of time than if it were done within-house. Since indications are that the present low staffing level will continue, it is anticipated that we will recommend the extension of the collaborative project into fiscal 1968. The level that had been budgeted for the third year of the project should be achieved in this fourth recommended year. Expenditures through April 1, 1966 have been a total of \$60,000 out of a budgeted total of \$114,000.

Honors and Awards: None

Publications:

Author: Watt, W.C.: Materials for Placebo V. U. S. Dept. of Commerce, National Bureau of Standards Technical Note 281. Washington, D. C., U. S. Government Printing Office, 1966, 92 pp.

Author: Watt, W.C., and Hsu, R.W.: Concordance to the Rules of Placebo V. U. S. Dept. of Commerce, National Bureau of Standards Report 8934. Washington, D. C., 1965, 191 pp.

Author: Watt, W.C.: Morphology of the Nevada Cattle Brands and Their Blazons, Part 1. U. S. Dept. of Commerce, National Bureau of Standards Report 9050. Washington, D. C., 1966.

Kirsch, R.A.: Conference on a session on pictorial information processing. Communications of the Association for Computing Machinery, 9:3, March 1966.

Lipkin, L.E., Watt, W.C., and Kirsch, R.A.: Analysis, synthesis and description of biological images. Ann NY Acad Sci, 128:3, Jan. 1966.

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Influence of Race and Other Factors on Pulmonary Hyaline Membranes

Previous Serial Number: Same

Principal Investigators: Toshio Fujikura, M. D.
Luz A. Froehlich, M. D.

Other Investigators: None

Cooperating Units: All Collaborating Institutions

Man Years

Total: .37
Professional: .33
Others: .04

Project Description:

Objectives: To determine the incidence of pulmonary hyaline membranes in the study population; to determine the influence of race, birthweight, survival time, and associated pulmonary findings on the presence and degree of pulmonary hyaline membranes.

Methods Employed: The pulmonary sections of 434 neonatal deaths out of 27,407 single live births in consecutive deliveries were reviewed. Hyaline membranes were graded slight, moderate, or marked, depending upon the thickness and distribution of membranes.

Major Findings: The hyaline membrane rate in the Study population was 4.8 per 1,000 single live births. There were nearly twice as many Negro infants with birthweights of less than 2,501 grams as there were whites. However, the neonatal death rate was lower in the Negro than in the white for this birthweight group, but higher in the Negro than the white for birthweights over 2,500 grams. The hyaline membrane rate was consistently lower in the Negro for all birthweight groups. These suggest that the Negro is of more advanced maturity than the white at specific birthweights. In the hyaline membrane infants of very low birthweight (500-1,500 grams) death occurred within 24 hours in more than 80% of cases. The heavier babies with hyaline membranes survived longer. In the non-hyaline

membrane group of neonatal deaths, more than 50% of the cases died within 24 hours regardless of birthweight. In the hyaline membrane group with survival times of less than 24 hours slight membranes had a higher incidence than moderate or marked membranes in each birthweight group. The reverse was true with survival times of between 24-72 hours.

Infants of very low birthweight had a much higher incidence of slight than moderate and marked membranes.

In the white, the survival time pattern of the hyaline membrane group was similar to that of the non-hyaline membrane group. In the Negro, 71.2% of the hyaline membrane group were dead within 24 hours, as against 49.4% of the non-hyaline membrane group. Conversely, only 1.5% of the Negroes in the hyaline membrane group were still alive at 72 hours in contrast to 23.1% of the Negroes in the non-hyaline membrane group.

Pulmonary alveolar hemorrhage of moderate or marked degree was frequently seen in association with pulmonary hyaline membranes (46.6%). Pneumonia was noted in 25% of hyaline membrane cases and the lesion was frequently associated with moderate or marked membranes.

Significance to Bio-medical Research and the Program of the Institute: Numerous papers have appeared in the literature on the subject of so-called hyaline membrane disease and its relationship to factors such as prematurity, diabetes mellitus, and cesarean section have been frequently emphasized. This investigation purports to study the relationship of hyaline membranes to basic factors particularly race.

Proposed Course of Project: Additional studies revolve around the incidence of associated acute chorioamnionitis, and the results compared with the incidence of chorioamnionitis in (1) the general Study population, (2) neonatal deaths not showing hyaline membranes. It is also expected that a combined clinical and morphologic study will be made in collaboration with other members of the Hyaline Membrane Task Force, in an effort to shed some light into this intriguing perinatal problem.

Honors and Awards: None

Publications:

Fujikura, T., and Froehlich, L.A.: The influence of race and other factors on pulmonary hyaline membranes. Amer J Obstet Gynec, to be published.

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Umbilical Cord Inflammation

Previous Serial Number: Same

Principal Investigators: Muyesser Tuncer, M. D. (University of Buffalo)
_____ Ueda, M. D. (University of Buffalo)
Luz A. Froehlich, M. D.

Other Investigators: None

Cooperating Units: Child Development Program,
University of Buffalo

Man Years

Total: .40
Professional: .08
Others: .32

Project Description:

Objectives: To develop a rapid simple method of detecting neutrophilic infiltration in the umbilical cord and to determine the relationship between neutrophilic infiltration of the cord and perinatal infection.

Methods Employed: A total of 990 placentas of consecutive deliveries at the Buffalo Children's Hospital were utilized for this study. A simple method was devised consisting of making umbilical cord imprints, staining the slides with methylene blue, and scanning for neutrophils. The entire procedure lasted an average of 15 minutes for each case. To verify the efficacy of the method, sections of cord and placental membranes were obtained from each case, fixed, forwarded to this laboratory, processed and the permanent histologic sections read by Dr. Froehlich, guided by standardized quantitative criteria for evaluating the degree of inflammation.

Major Findings: There was a significant correlation between positive imprints (5 or more leukocytes per high power field) and prolonged rupture of membranes (more than 12 hours after delivery). Thus, there were 17.4% (10 out of 93) positive imprints with membrane rupture of more than 12 hours after delivery in contrast to 7.5% positive imprints (27 out of 839) cases when membranes ruptured 0 to 12 hours after delivery. The corresponding figures based on histology of the cord

were 15.2% (14 out of 92) and 3.0% (25 of 831) respectively. There was a step-wise increase in the incidence of positive cord imprints with increasing histologic evidence of cord inflammation; 6.6% of positive imprints were associated with histologically negative cords, whereas 35.9% of positive imprints were associated with histologic evidence of moderate or marked cord inflammation. None of the 13 stillbirths and the 11 neonatal deaths showed intrauterine pneumonia. Neither of the two cases that were clinically suspected by X-ray to have intrauterine pneumonia had histologic or imprint evidence of cord inflammation; both infants did well without benefit of antibiotic therapy. The lack of unequivocal cases of intrauterine pneumonia in this relatively small sample of 990 is unfortunate but is to be expected. However, the significant ($p < 0.05$) correlation between prolonged membrane rupture and positive imprints is enough evidence to show that this method could be utilized as a simple emergency diagnostic tool in perinatal infections.

This hopefully will be completed and published during F.Y. 67.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Significance of a Single Umbilical Artery

Previous Serial Number: Same

Principal Investigators: Luz A. Froehlich, M. D.
Toshio Fujikura, M. D.

Other Investigators: None

Cooperating Units: All Collaborating Institutions

Man Years

Total: 1.00
Professional: .50
Others: .50

Project Description:

Objectives: To determine the incidence of single umbilical artery based on a completely prospective study on a large number of gravid mothers and their resultant offspring enrolled in the different Collaborating Institutions; to evaluate the relationship between single umbilical artery and other important perinatal conditions, as well as the incidence of associated congenital malformations.

Methods Employed: The material studied was obtained from patients from 12 different institutions enrolled in the Collaborative Study. All single births from the beginning of the Study (1959) to April 30, 1963, were included in the present study. The incidence of single umbilical artery was determined from the pathologic reports on 26,539 placentas, the two investigators confirming the diagnosis by examination of duplicate microscopic slides of cords where there was a discrepancy between gross and microscopic reports on the number of umbilical vessels. A careful review of the neonatal, the 4-month, and the 12-month examinations of the infants with single umbilical arteries was done in order to obtain an accurate picture of the incidence of associated congenital malformations. Other perinatal factors such as maternal age, race, gravidity, associated maternal diseases, infant sex, birthweight, placental weight, etc., were analyzed.

Major Findings: The incidence of single umbilical artery in the white was 1.22%, a higher incidence than in the Negro, which was 0.44%. This racial difference was consistently present in the various institutes and in all maternal age and birthweight groups. Conversely, the incidence of associated congenital malformations was higher in the Negro (42.1%) than in the white (23.0%). Associated congenital malformations were classified into three types: A (lethal or major), B (non-lethal), and C (borderline) malformations. The overall incidence of associated malformations (Types A,B, plus C) was 28.6%. When the Type C malformations were excluded, the incidence was 16.7%. Type A malformations were found in 9 of the 20 stillbirths and in 4 of the 6 neonatal deaths, but only in 7 (4.0%) of the 174 survivors. Skeletal system anomalies showed the highest incidence in the associated malformations. The incidence of SUA was 1.98% in infants of low birthweight (2,500 grams and under). In infants weighing over 2,500 grams the incidence was 0.63%.

Maternal diabetes was 6.4%, an incidence five times higher than in the control group.

Significance to Bio-medical Research and the Program of the Institute: Previous reports on the incidence of associated malformations with single umbilical artery have varied widely. This could be explained by the fact that the number of SUA cases in prospective series was relatively small, and the rest of the studies were retrospective based largely on autopsied material. The Collaborative Study data is especially suitable for analyzing the problems of SUA in that they represent collected material from several institutions on a large number of mothers and their offspring.

Proposed Course of Project: A follow-up study of the surviving SUA cases is contemplated, with particular emphasis on the neurologic course of these infants. New cases of SUA will be added to the original group. More detailed analysis of possible associated placental and cord abnormalities is contemplated.

Honors and Awards: None

Publications:

Froehlich, L.A., and Fujikura, T.: Significance of a single umbilical artery. Amer J Obstet Gynec, 94: 274-279, Jan. 1966.

Serial No. NDB (CF)-66 PR/P 1340

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Intrauterine Pneumonia in Relation to Birthweight and Race

Previous Serial Number: None

Principal Investigators: Toshio Fujikura, M. D.
Luz A. Froehlich, M. D.

Other Investigators: None

Cooperating Units: All Collaborating Institutions

May Years

Total: .21
Professional: .17
Others: .04

Project Description:

Objectives: To determine the incidence of intrauterine pneumonia in the Study population; to determine the effect of race, birthweight, and associated placental infection on this incidence.

Methods Employed: The pulmonary sections of 512 Study infants dying within the first 48 hours of life out of 36,212 single live births were reviewed. In the pneumonia group, incidence of associated chorioamnionitis was compared with that of a control group matched according to survival time, race, birthweight and institution.

Major Findings: Intrauterine pneumonia remains a common and often fatal infectious process despite the advent of antibiotics. Acute chorioamnionitis is generally accepted as its frequent precursor. In a histologic evaluation of the pulmonary sections of 512 neonatal deaths within the first 48 hours of life out of 36,212 single live births, 100 or 19.5% were found to have pneumonia. The proportion of neonatal deaths showing pneumonia was higher in the Negro (27.7%) than in the white (11.3%) and consistently higher in the Negro at each birthweight interval. The association of pneumonia and chorioamnionitis was more pronounced in the Negro (50.7%) than the white (28.0%). In the white the incidence of pneumonia was higher in the mature (birthweight over 2,500 grams) than in the premature infants; in the Negro it was not higher in prematures compared to mature infants. This is in contrast to chorioamnionitis, which is more common in premature births.

Significance to Bio-medical Research and the Program of the Institute: Although it is generally accepted that acute chorioamnionitis is the frequent precursor of intrauterine pneumonia, and it is well known that chorioamnionitis is more frequent in premature births, this study has revealed somewhat unexpected findings. When the diagnosis of pneumonia was based on morphologic evaluation of the pulmonary sections of diseased neonates in the first 48 hours of life, pneumonia was found more frequently among mature than premature whites; in the Negro the incidence of pneumonia was not higher among prematures compared to mature infants. It is postulated that this contrast in incidence between fetal and placental (maternal) inflammatory reaction could be explained by the parallelism between fetal maturity and ability for neutrophilic response.

Honors and Awards: None

Publications:

Fujikura, T., and Froehlich, L.A.: Intrauterine pneumonia in relation to birthweight and race. Amer J Obstet Gynec, to be published.

Serial No. NDB (CF)-66 PR/P 1341

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Placental Shape According to Race, Birthweight,
Gestational Age and Placental Weight

Previous Serial Number: None

Principal Investigators: Toshio Fujikura, M. D.
Luz A. Froehlich, M. D.
Lewis E. Lipkin, M. D.

Other Investigators: None

Cooperating Units: All Collaborating Institutions

Man Years

Total: .04
Professional: .04
Others: 0

Project Description:

Objectives: To retest the hypothesis that the definitive shape of the placenta is established early in pregnancy and changes little in the last five months of gestation.

Methods Employed: All coded PATH-I's on single livebirths of whites and Negroes were used. Placental shape was estimated by obtaining the ratio of the small diameter to the large diameter. Placental shape was defined as follows: round - ratio of 1.0 to 0.9; oval - ratio of 1.0 to 0.9; and, elongated - ratio of 1.0 to 0.9. Placental shape was analyzed according to race, birthweight and gestational age.

Major Findings: Preliminary analysis showed that placentas tended to be rounder with increasing birthweight and placental weight. The association with round shaped placentas appeared to be stronger with placental weight than with birthweight. There was no significant influence of gestational age on placental shape.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/P 1342

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: The Length of the Umbilical Cord

Previous Serial Number: None

Principal Investigators: Toshio Fujikura, M. D.
Luz A. Froehlich, M. D.
Lewis E. Lipkin, M. D.

Other Investigators: None

Cooperating Units: All Collaborating Institutions

Man Years

Total: .04
Professional: .04
Others: 0

Project Description:

Objectives: To determine if there is a relative correspondence between cord length and the two main indices of embryonic growth, namely birthweight and gestational age.

Methods Employed: The cohorts in the Study were single birth core cases where the total length of the umbilical cord was available in the PED-I form (Examination of the Neonate). Only infants with birthweights of 1,001 grams and over and gestational ages of 28 to 43 weeks were included. For birthweight-cord length associations, a total of 8,696 whites and 9,290 Negroes were available for analysis and for gestational age-cord length relationships, 4,869 whites and 6,434 Negroes were analyzed.

Major Findings: Mean cord lengths showed a progressive increase in both white and Negro with increments of gestational age and birthweight. Percentage distribution of cord lengths also showed a gradual shift from the shorter to the longer cord with increasing gestation and birthweight.

The mean cord length in the Negro was usually less than its white counterpart, the difference being constant at about 1.4 cm at each gestational interval.

Significance to Bio-medical Research and the Program of the Institute: There is a paucity of comprehensive studies on the length of the umbilical cord. In two separate studies, it has been claimed that there is very little correspondence between cord length and fetal weights and there is no evidence of growth in cord length after the 28th week of gestation. These hypotheses were not supported by the results of the present study where cord lengths on a much larger study group were analyzed.

Proposed Course of Project: Because of the availability of total cord lengths in a vast number of cases, the study of the length of the umbilical cord and its association with a multiplicity of factors using Collaborative Study data lends itself to wide exploitation. It is contemplated that this study be enlarged to include analysis of its association with other indices of growth such as crown-rump length, crown-heel length, placental weight, as well as other placental findings such as type of cord insertion, placental shape, etc. In addition, possible effects on cord length of selected conditions such as single umbilical artery, true knots of cord, cord entanglement, cardiovascular and other malformations, anemia in pregnancy, diabetes mellitus, dysmaturity, etc., will be investigated.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A Simplified Anatomic Classification of Abortions

Previous Serial Number: None

Principal Investigators: Toshio Fujikura, M. D.
Luz A. Froehlich, M. D.
Shirley G. Driscoll, M. D.

Other Investigators: None

Cooperating Units: The Boston Lying-in Hospital

Man Years

Total: .08
Professional: .08
Others: 0

Project Description:

Objectives: To devise a simplified anatomic classification of abortions which would result in more uniformity of reporting and widespread use of what should be an integral part of the morphologic description of abortions.

Methods Employed: The pathologic reports on abortion material from patients of the Boston Lying-in Hospital enrolled in the Collaborative Study were reviewed. A modified classification was applied to the abortion data.

Major Findings: Between January 1, 1959 to April 30, 1963, there were 7,818 total deliveries in which 353 abortions (4.5%) were reported. The pathologic reports of 327 abortions where specimens were available for morphologic examination were reviewed and the specimens were grouped according to the following anatomic classification:

- Group I - Incomplete specimen
- Group II - Ruptured empty sac
 - A. With cord stump
 - B. Without cord stump
- Group III - Intact empty sac

- Group IV - Embryo or fetus present
- A. Normal embryo or fetus
 - B. Deformed embryo - deformity of the embryonic mass as a whole
 - C. Embryo or fetus with anomalies - localized deformities of organs or organ systems
 - D. Unable to determine normality

In this classification, primary emphasis is placed on the identification of a fetus or embryo. The presence of maceration should be indicated. Stunted but otherwise morphologically normal embryos or fetuses should be classified as normal (Group IV-A). Of 147 embryos and fetuses, 6 or 4.1% had localized anomalies. The intact empty sac suggests early death of the embryo or fetus, and in this series there were 18 specimens (5.5%) belonging to this group. The general maceration rate was 89 of 147 abortuses or 60.5%, specific maceration rates showing inverse relationships with C-R length. Using the Streeter curve as the standard it was noted that in most abortuses, particularly the macerated, the calculated gestational age based on LMP was usually greater and sometimes considerably so than what was expected for a given C-R length. Because of variable differences in measuring technique and type of material used the use of Streeter's curve should be made with certain reservations. It is apparent that in at least a few of our Study abortuses where the C-R length-gestational age disparity was marked, the mechanism of intrauterine growth retardation plays a prominent role. Other factors may be erroneous LMP's or intrauterine retention of a dead embryo or fetus.

Significance to Bio-medical Research and the Program of the Institute: Pathologic reports on abortion material are often grossly inadequate and frequently verify only that the specimen is placental tissue. Except for a few institutions interested in the study of abortions, little or no attempt is made to identify the chorionic vesicle and to classify the material anatomically. The system of classifying and reporting abortions described above is based on the morphology of products recovered and requires neither a special interest in embryology nor an undue expenditure of time. Uniform recording of such data would provide a basis for studies of mechanisms of abortions and their pathophysiology. Patterns of special interest, such as abnormal fetal growth, might also be recognized. The survey also revealed that with diligence and mutual cooperation between patient, obstetrician and pathologist most reported abortions can be histologically verified (93% for the Boston Lying-in Hospital) and properly classified. In a few Study institutions the number of pathologic reports received on abortions was practically nil.

Honors and Awards: None

Publications:

Fujikura, T., Froehlich, L.A., and Driscoll, S.G.: A simplified anatomic classification of abortions. Amer J Obstet Gynec, to be published.

Serial No. NDB (CF)-66 PR/P 1344

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Predictors of Organ Weight at Autopsy - A Multivariate Analysis, I. Heart Weight

Previous Serial Number: None

Principal Investigator: Lewis E. Lipkin, M. D.

Other Investigators: Luz A. Froehlich, M. D.
Esther Jackson

Cooperating Units: Section on Systems Design and Procedures, NINDB
Section on Statistical Analysis, NINDB

Man Years

Total: 0.1
Professional: 0.1
Others: 0

Project Description:

In order to assess normality or abnormality in the fetal and neonatal period, the pathologist must have a convenient (preferably linear) comparison scale based on an easily measured or obtained parameter. The object of this study was to construct such a referent from the accumulated quantitative data available in the autopsy records of the Collaborative Project.

The initial phase of the study concerned itself with determining the best predictor(s) of heart weight. Among the independent variables subjected to a multivariate analysis were crown-rump and crown-heel length, gestational age, and body weight. The same analysis attempted to assess factors such as duration between death and autopsy and postnatal age as modifiers of the observed organ weight.

This phase concluded with the determination that the best estimator of heart weight (among those independent variables studied) was body weight and that moreover a simple linear empirical relationship exists between the logarithm of heart weight and the logarithm of body weight at autopsy. The equation found was present along with envelopes of

percentile bands, so that in effect the probability of a given value occurrence as a member of the postulated "normal autopsy room" population is directly ascertainable. It was further shown that the heart-body weight data obtained in the Collaborative Project is very similar to that found in two other independent studies (Gruenwald - Minh and Potter - Yerushalmy).

It is planned to analyze the data relating other parenchymatous organs in a similar manner during the course of the next year.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Influencing Factors in Sudden Unexpected Death

Previous Serial Number: None

Principal Investigators: Toshio Fujikura, M. D.
Joseph Drage, M. D.

Other Investigators: Luz A. Froehlich, M. D.

Cooperating Units: All Collaborating Institutions

Man Years

Total: .08
Professional: .08
Others: 0

Project Description:

Sudden unexpected death in infancy presents a serious unsolved problem. It accounts for over 10% of infant mortality in the United States and 20% in England. In the majority of cases, the pathologic findings are minimal. Previous exhaustive studies have concentrated on the neonatal and postnatal conditions. The purpose of this study is to investigate all possible influencing factors on sudden death, especially prenatal and maternal problems which have not been explored in previous studies. Two hundred and seven cases of sudden unexpected deaths in the Collaborative Study material were identified. The possible influence of the following factors will be investigated: sex, race, birthweight, gestation, placental weight, placental infection, autopsy findings, maternal diseases, type of feeding, Apgar score and socio-economical factors.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: An Epidemiological Assessment of Primary Endocardial Fibroelastosis

Previous Serial Number: None

Principal Investigators: Shiela C. Mitchell, M. D.
Luz A. Froehlich, M. D.
John S. Banas, Jr., M. D.
Mary Ruth Gilkeson

Other Investigators: None

Cooperating Units: All Collaborating Institutions
Section on Infectious Diseases, NINDB

Man Years

Total: .50
Professional: .42
Others: .08

Project Description:

Objectives: (1) To identify well documented cases of primary endocardial fibroelastosis in the Collaborative Study population following strict clinical and pathological criteria, and, in combination with cases reported in the literature satisfying the same criteria, attempt to assess the disease entity epidemiologically; and, (2) to test existing theories on the etiology of this condition with particular emphasis on the possible role of viruses.

Methods Employed: A review of the literature of autopsied cases of primary endocardial fibroelastosis was undertaken. From this review 54 reports were identified which detailed 199 cases meeting the following criteria: (1) a complete autopsy report accompanied each; (2) there was no associated intracardiac abnormality apart from the involvement of the aortic or mitral valve with the fibroelastotic process; (3) the left ventricle was markedly dilated and hypertrophied and involved with diffuse pearly white thickening of the endocardium; and, (4) an elastic tissue stain demonstrated microscopically a marked increase in the elastic tissue of the endocardium. Among the living cases, a typical clinical course and specialized cardiac investigation were mandatory. The same criteria were applied to the cases uncovered in the Collaborative Study population (6 autopsied and 1 living).

Major Findings: Of 40,400 live infants born to the Study between January 1, 1959 to October 1, 1965, 7 cases of primary endocardial fibroelastosis were identified, based on the aforementioned criteria. The incidence of primary EFE in this population is therefore 1 in every 5,000 to 6,000 total births. The 7 cases were analyzed along with 2 controls for each case matched for maternal residence, age, parity, and date of delivery for maternal viral antibody titer during pregnancy. A total of 28 different viruses were tested using the complement fixation technique. While the maternal sera of the 7 cases contained some antibody to all but 4 of these viruses, a fourfold rise or fall was only to be found for 8 of the 28 viruses in 4 of the cases. It is our belief that titer changes less than fourfold are of doubtful significance. Because of the current interest in the association between maternal viral infection and primary EFE in the offspring, the actual data for mumps, coxsackie B₄, B₅ and Echo 11 are presented. These data fail to demonstrate any consistent association between maternal viral infection as measured by repeated complement fixation antibody tests and primary EFE in the offspring.

Significance to Bio-medical Research and the Program of the Institute: It has been shown that primary EFE is a relatively rare lesion. While clinical cases are associated with a positive skin reaction to mumps, there is at present no consistent proof that pre or postnatal maternal infection is the underlying causative agent.

Proposed Course of Project: The paper was presented at the Second Scientific Session of the Collaborative Study held in Washington, D.C., March 24-25, 1966. It has had NIH approval and has been submitted for publication.

Grants and Awards: None

Publications: None

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: A Case of Marchiafava Bignami Disease with Clinical Recovery

Previous Serial Number: None

Principal Investigators: J. Richard Baringer, M. D.
Carl M. Leventhal, M. D.

Other Investigators: Barry G. Arnason, M. D.
C. Miller Fisher, M. D.

Cooperating Units: Department of Neurology,
Massachusetts General Hospital

Laboratory of Neuropathology,
Harvard Medical School

Man Years

Total: 0.3
Professional: 0.2
Others: 0.1

Project Description:

A case of cystic degeneration of the corpus callosum, a rare complication of chronic alcoholism, is reported in which the diagnosis was made on clinical grounds three years prior to death and ultimately confirmed pathologically. The clinical syndrome is described in greater detail than has been previously possible. The illness was shown to be reversible. The pertinent literature is reviewed and neuropathological and neuroanatomical features are studied. A report was presented to the American Neurological Association on June 15, 1965 and is being amplified for publication.

Honors and Awards: None

Publications:

Leventhal, C.M., Baringer, J.R., Arnason, B.G., and Fisher, C.M.:
A case of Marchiafava Bignami disease with clinical recovery.
Trans Amer Neurol Ass, 90: 87-91, 1965.

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Embryologic Analysis of Multiple Congenital Anomalies

Previous Serial Number: None

Principal Investigator: Carl M. Leventhal, M. D.

Other Investigators: Lewis E. Lipkin, M. D.
Barbara Burke, M. D.

Cooperating Units: Child Development Study,
University of Minnesota

Man Years

Total: 0.4
Professional: 0.3
Others: 0.1

Project Description:

A case of multiple congenital anomalies, submitted in the Collaborative Study, is presented in clinical detail and studied pathologically. The separate anomalies of diverse organ systems are subjected to intensive analysis based on embryologic organogenesis as a model for testing the hypothesis that the constellation of anomalies may have arisen as the result of an insult at a definable stage of early embryonic development. A report was presented to the Scientific Meeting of the Collaborative Study on March 24, 1966 and will be expanded for publication.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/F 1349

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Alzheimer's Disease: A Family Study

Previous Serial Number: None

Principal Investigators: Leonard L. Heston, M. D.
Dale L. W. Lowther, M. D.
Carl M. Leventhal, M. D.

Other Investigators: None

Cooperating Units: Medical Neurology, NINDB
Veterans Administration Hospital, Portland, Oregon

Man Years

Total: 0.6
Professional: 0.4
Others: 0.2

Project Description:

A detailed clinical, genetic and pathological study of a large kinship with presenile dementia is undertaken. Neuropathologic emphasis has been placed on the use of fluorochrome staining methods for the demonstration of amyloid plaques by fluorescence microscopy. A report is being prepared for publication.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Birthweight, Gestational Age and Type of Delivery
in Rhesus Monkeys

Previous Serial Number: None

Principal Investigators: Toshio Fujikura, M. D.
Wendell H. Niemann, M. D.

Other Investigators: None

Cooperating Units: Perinatal Physiology Laboratory, NINDB,
San Juan, Puerto Rico

Man Years

Total: .08
Professional: .08
Others: 0

Project Description:

Objectives: Since there is the recognized uncertainty as to length of gestation in humans based on LMP, birthweight has been widely accepted as the most desirable single index in investigations of intrauterine development. Various opinions have been reported on the controversial subject regarding birthweight and its influencing variables. The influence of environmental factors during gestation could be minimized by a planned breeding program with a caged colony of Rhesus monkeys. Because of timed matings in the breeding colonies, length of gestation is supposed to be accurate as an index of fetal maturity. Furthermore, artificial termination of normal pregnancy by cesarean section for experimental purposes could provide the desired data on birthweight studies. It is interesting to evaluate the birthweights of monkey infants which include vaginal deliveries and cesarean sections.

Methods Employed: The newborn monkeys (*Macaca mulatta*) used in this study were obtained from a caged breeding colony at the Laboratory of Perinatal Physiology in San Juan, Puerto Rico. All newborn monkeys were of known gestational age and born by cesarean section or vaginal delivery. Conceptions occurred throughout the year. Single live births of which 179 were delivered vaginally and 292 by cesarean section were included in this study. Their lengths of gestation ranged from 135 to 171 days. The extreme premature and postmature infants were excluded. Timed mating, technique of surgery, prenatal and postnatal cares have been described in previous papers. With the advent of a natural birth, the infant was separated from its mother the morning after the delivery and birthweights were recorded. The cesarean section group was also separated from their mothers at birth, and birthweights recorded immediately.

Major Findings: The newborn monkeys (*Macaca mulatta*) used in this study were obtained from a caged breeding colony. Gestational ages were presumed to be accurate because of timed matings. Mean birthweights in males were consistently higher at each gestational interval than in females. This trend was true in both the vaginal delivery group and the cesarean section group.

The variability in birthweights, as indicated by the coefficient of variation, was wide and roughly constant regardless of gestational age, sex and type of delivery. Such a variability in birthweights could be a biological phenomenon and cannot be interpreted as due to errors in determining gestational age. The newborn monkeys delivered prematurely by cesarean section tended to be heavier than those born vaginally at corresponding gestational intervals and sex.

Paper is being prepared for publication.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/P 1351

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Fetal Vessel Ligation in Rhesus Monkey Placentas

Previous Serial Number: None

Principal Investigators: Ronald Myers, M. D.
Wendell H. Niemann, M. D.
Toshio Fujikura, M. D.

Other Investigators: None

Cooperating Units: Perinatal Physiology Laboratory, NINDB
San Juan, Puerto Rico

Man Years

Total: .04*
Professional: .03*
Others: .01*

Project Description:

The purpose of this study is to produce offsprings which are abnormal in neurological and/or morphological aspects. The vessel ligation was done in the interlobar area of the Rhesus monkey placenta at around 120 days gestation. Dr. Myers has generously made available the ligated placentas which Dr. Fujikura examined microscopically for pathologic lesions. Relevant findings in the offspring will be reported by Dr. Myers and in the placenta by Dr. Fujikura.

Honors and Awards: None

Publications: None

*Indicates man hours used only by workers in Bethesda and does not include hours used by workers in Puerto Rico.

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Neuropathological Study of a Series of Selected
Monkey Brains from Animals in the Perinatal Period

Previous Serial Number: None

Principal Investigator: Jack H. Carleton, M. D.

Other Investigators: Lewis E. Lipkin, M. D.

Cooperating Units: Bionetics Research Laboratory, Inc.

Man Years

Total: .5
Professional: .3
Others: .2

Project Description:

Objectives: This study was initiated to evaluate as a comparison to the Perinatal Research Project material the brains of the primate perinatal period according to the protocol established for the processing of whole brains coming from the Collaborative Study. Data on the prenatal period, delivery, neonatal care, developmental aspects, and circumstances of death in these cases has been collected and will be considered after the neuropathological findings have been described.

Methods Employed: The specimens for study have been processed according to Project methods including whole and sectioned brain photography, and project staining procedures. A microscopic examination protocol is being devised so that all brains can be compared in tabular form.

Major Findings: Intake of cases to the series ended in January 1965 and 98 of the 103 brains included have been processed and classified as to age, species and quality of histological preparation. Ages range from stillbirth to 2½ years for the three major species which will be evaluated. Except for one case, which has been exhaustively studied and reviewed, major findings await microscopic evaluation.

Significance to Bio-medical Research and the Program of the Institute:
It is anticipated that this study will enhance our understanding of perinatal neuropathology and serve as a laboratory control for evaluation of technical procedures used in the Project's protocol.

Proposed Course of Project: Continue to completion as outlined.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/P 1353

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: A Sequential Study of Ultrastructural Changes in an Experimentally Produced, Traumatic, Brain Lesion

Previous Serial Number: None

Principal Investigator: Jack H. Carleton, M. D.

Other Investigators: John L. Priester

Cooperating Units: None

Man Years

Total: .8
Professional: .4
Others: .4

Project Description:

Objectives: To describe in detail the sequential ultrastructural changes occurring as a response to a reproducible wound in the mouse brain.

Methods Employed: The project has demanded specific and extensive modifications of existing techniques in the area of fixation, embedding and tissue orientation for electron microscopy.

Major Findings: The project has been divided into seven phases each dependent upon the satisfactory completion of the previous one to achieve the projected goal of the project.

Phase I, A Preliminary Study of Glial Scar Formation in the Mouse, has established the feasibility of the study and determine the most appropriate method for producing the wound.

Phase II, The Preliminary Electron Microscopic Study of a CNS Wound, enabled us to ascertain reproducible methods of fixation, tissue processing and localization of the CNS lesion.

Phase III, The Light Microscopic Study of the CNS Lesion in Timed Sequence, has allowed us to describe the pathogenesis of the traumatic lesion using the light microscope. This is an essential feature to proceeding with the description of ultrastructural changes.

Phase IV, The Electron Microscopic Study of a Traumatic CNS Lesion, has produced a pictorial atlas of the sequence of structural changes occurring in one day and eight days after the lesion was made in 30 animals. This phase has shown the necessity to establish an improved way of comparing electron microscopic and light microscopic findings. Therefore, the technical phases have been initiated and are nearing completion. They are:

Phase V, Thick Serial Sections for Computer Analysis of CNS Traumatic Lesion.

Phase VI, The Comparison of Various Embedding Media.

Phase VII, Large Epoxy Tissue Sections of Mouse Brain.

Significance to Bio-medical Research and the Program of the Institute: This investigation has been undertaken to apply improved electron microscopic techniques in an effort to enhance and clarify existing knowledge pertaining to the response of the brain to trauma.

Proposed Course of Project: The seventh phase of this investigation is nearing completion and will be followed by a final phase in which the technical improvements and knowledge gained from the preceding seven will be applied to an expanded sequential series on lesions. It is anticipated that this final series will be completed during the 1966-1967 fiscal year and that in the future this investigation can be expanded to include the relationships of host age and other factors to the response of the central nervous system to trauma.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Automated Microspectrophotometry Employing the LINC
Computer

Previous Serial Number: None

Principal Investigator: Lewis E. Lipkin, M. D.

Other Investigators: None

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS

Man Years

Total: 0.8
Professional: 0.3
Others: 0.5

Project Description:

Microspectrophotometry of neural tissue sections is extremely difficult, time consuming, subject to serious instrumental errors and requires extensive computations (e.g., as in two wave length methods) to obtain meaningful results. In each aspect of the foregoing, the use of a computer as a control and analytic device is of very great assistance indeed.

The objective of this study is twofold:

1. To demonstrate the use of a small general purpose computer in morphology (analogous to its already demonstrated value in neurophysiology).
2. To investigate the dynamics, quantifiability and reproducibility of Gallocyenin staining of CNS tissues.

A precision prism monochromator has been fitted with stepping motors to render the wave length and slit size control to be subject to direct digital control by the computer. A stepping motor driven stage is now under construction. A combined differential photo-multiplier and power supply has been built which allows automatic subtraction of light source variation from the analog signal delivered to the LINC.

The LINC is employed as a control subsystem as follows:

1. The microscopist selects a series of fields. Selection will be accomplished by activating each of the two digital input terminals, generating the x and y coordinates of the field of interest.

Thereafter, the microscopist will not be required and the system will conduct all operations and analysis opted by the experimenter and stop and signal on the completion of a run. (A run would consist of up to 50 or 100 selected fields on a single slide.)

LINC will cause the stage to progress from each selected field to the next in a programmed order. It should be possible to construct a set of subroutines to permit different kinds of orders of presentation all but one of which being independent of the order of selection by the microscopist. These orderings of scan might be merely to minimize run time. On the other hand, the chosen sequence might bear a biologically interesting relationship to a given referent in the section.

2. LINC would control the grating monochromator.
3. LINC would control the exit slit size of the monochromator. This is of considerable value in studies requiring absorption measurements under constant energy conditions.

Of even greater importance is the fact that computer control of grating and slit make possible the unique opportunity for efficiency of variable precision and resolution. If, for example, the first and second derivatives of absorption with respect to wave length are also factors programmed to participate in control of the grating and slit, it becomes possible for the computer to "choose" to increase spectral resolution in precisely the most interesting segments of the absorption curve and to scan other segments with less detail. Moreover, this type of control option would allow the computer to correct over-shoot of an absorption peak with suitable programming.

4. The detector would be directly monitored by LINC via one of its analog inputs.
5. The computer pulsed digital output lines will be employed to control a 70 mm electric drive camera placed to photograph one of the display scopes. LINC will be programmed to display successively in appropriate order the following types of information:

- a. Housekeeping information such as date, run number, slide designation (entered alphanumerically), coordinates of field, etc.
 - b. The absorption curve, with numeric presentation of maxima and minima.
 - c. As an option at one or more maxima, a digitized (binary) image of the field at a selected series of absorption thresholds.
6. At the time of initiating the run the experimenter will, at his option, replace the observation tube by a Leitz-type 35 mm automatic camera. This will be activated by another digital output to take a series of color photomicrographs in sequences as programmed. These could be used for merely identification purposes, or matching the chosen with actual measured fields. Perhaps of more significance and interest would be a series of "natural" images, matched with the corresponding digitized images, thus contributing greatly to the direct evaluation of spectral and densitometric factors in cell recognition problems. This also is in effect a "stain simulator."

The LINC is employed as an analytic subsystem as follows:

1. Comparison of absorption curves among various fields in a given run. It is to be noted that multiple tissue specimens may be simultaneously embedded or alternatively, different and treated sections may be mounted on the same slide. The latter option permits inter-stain companions. The former would allow tissue or organism treatment comparisons, and a reduction in "statistical" section thickness variations (but not local). In either case, the opportunity for on-line analysis is intriguing and promising.
2. The ability of the computer to accept a multiplicity of simultaneous analog inputs, will allow us to avoid the confounding of light source intensity variations with local section thickness variation and thus allow an estimate of absolute quantity of absorbing material. The determination of stoichiometric factors thus becomes available, particularly if the scanning option is included.
3. The more ordinary computer application of Fourier analysis and application of the spectral sample theory to the taped records of individual absorption curves would most likely be performed off-line.

Honors and Awards: None

Publications: None

1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Factors Influencing Quantitative DNA Staining

Previous Serial Number: None

Principal Investigator: Jack H. Carleton, M. D.

Other Investigators: Charles Spencer, M. D.
Lewis E. Lipkin, M. D.

Cooperating Units: Mortimer Mendelson, M. D.
Department of Radiology,
University of Pennsylvania

Man Years

Total: .4
Professional: .3
Others: .1

Project Description:

Objectives: To devise ways of testing and to determine the degree of influence that various factors have on the microspectrophotometric measurements of DNA staining. Factors which are under investigation include stain fading, species and embryological development differences in absorption curve characteristics, and staining progressivity.

Methods Employed: Instrumentation and procedures used in this project have been developed under the direction of the investigators and include a specially constructed two wave length cytophotometer and a microscopic slide cell chamber.

Major Findings: A statistical analysis of the data on the stain fading aspect of this project has been completed by Mr. Weiss and Miss Jackson and the paper is to be published, showing that no significant fading of Feulgen stained cells occurs when Eukitt or Cargille's Immersion Oil is used as the mounting medium in contrast to marked fading with Permout. An initial investigation of Feulgen-DNA and Gallocyanin absorption curve inconsistencies in cells derived from different embryological germ layers and animal species has been completed and showed instrumentation variations to be too great to make statistically valid conclusions. It is anticipated

that this study will be reconducted using the unique microspectrophotometer under construction by Dr. Lipkin. A preliminary evaluation of progressiveness of Feulgen DNA staining using the developed cell chamber suggests the possibility of a real difference between the currently theorized staining of Feulgen DNA and its actual progressivity.

Significance to Bio-medical Research and the Program of the Institute: Greater understanding and documentation of the factors influencing quantitative DNA staining is mandatory if these methods are to become more useful tools in the microscopist's search to automate and quantitate the evaluation of images. It is to this aim that this project is directed both for use within the section's investigations of automated biological image processing and for this field of research in general.

Proposed Course of Project: Continuance of project to evaluate the above findings as methods and instrumentation develops.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/SA 1356
1. Perinatal Research Branch
2. Section on Statistical Analysis
3. Bethesda, Maryland

FHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Association of Maternal Age and Parity to Pregnancy Outcome and to Certain Obstetric Complications.

Previous Serial Number: None

Principal Investigator: Stewart H. Clifford, M.D.,
Boston Lying-In Hospital, Boston, Mass.

Other Investigators: None

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years: Total: 0.4
Professional: 0.2
Other: 0.2

Project Description:

This report is concerned with the influence of age and parity by race on pregnancy outcome and on the incidence of certain obstetric complications. Data pertaining to the following subjects have been analyzed:

Stillbirths, neonatal mortality; low birthweight infants; 8 month motor scores; 1-year neurologic abnormalities; education of the gravida; dystocia; toxemia; chronic hypertension; bleeding; hydramnios; anemia and prolonged labor.

In general, in both races, the most favorable age for pregnancy was from age 20-27. The second and third pregnancy appeared to be the one with the most favorable outcome. Most unfavorable results were associated with the very young and elderly gravida.

Para 0 and Para 5 and over for the Negro, as well as Para 3 and over for the White, carried increased complications.

Interesting evidence was found as to the unfavorable effect of too rapid succession of pregnancies in ages of 22 years and under. The influence produced unfavorable results in neonatal mortality, prematurity, mental scores, neurological abnormalities and anemia.

The findings associated with the education of the gravida were most provocative. The incidence of patients with schooling below the 8th grade

was high in the White as well as the Negro. The high proportion of these patients and their contribution to unfavorable factors was evident in both the early and advanced parities

Many other expected and unexpected associations were encountered - too numerous to be included.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/SA 1357
1. Perinatal Research Branch
2. Section on Statistical Analysis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Prior Pregnancy Loss and Present Infant Outcome

Previous Serial Number: None

Principal Investigator: W. S. Jones, M.D.,
Brown University, Providence, R.I.

Other Investigators: R.H. Holden, Brown University, Providence, R. I.

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.4
Professional	0.2
Others:	0.2

Project Description:

That a poor reproductive past performance, by any woman for whatever reason, predisposes to an even smaller yield in future pregnancies is an accepted obstetrical concept. Many components interact and/or combine to correlate prior loss with fetal death in the current pregnancy. This study attempts to isolate and evaluate the factors involved.

The perinatal loss of some 25,000 women, approximately half the Collaborative Study, who produced a single fetus in the current pregnancy, is related to loss in previous pregnancies. 43.4 percent of the women were white, 47.5 percent negro, and about 9 percent Puerto Rican. Roughly one-third were primiparous, one-third had one or two previous pregnancies, and the remaining third had three or more.

One-third of the women with at least one prior pregnancy had one or more prior fetal losses. The combined fetal and neonatal mortality rate of 6.9 percent in women with at least one prior loss is more than twice the rate of 3.2 percent for those without prior loss.

The combined abortion, stillbirth, and neonatal death rates in the current pregnancy show a significant association with the incidence of past failures. Peak mortality rates are concentrated in regions of heavy losses in prior pregnancies. Furthermore, the greatest relative frequency of each type of

death in the study occurs for the same type of death in the last prior pregnancy.

Frequency of prior loss closely associates with shortened gestation and attendant low birth weight, which obviously escalates neonatal mortality. Low Apgar scores, respiratory distress, neonatal infections, and early neurological abnormalities all show a trend of increased hazard in rough proportion to previous infant loss, with the findings consistent by race.

Both the "high risk" age groups, teenagers and women 35 and older, demonstrate a deteriorating fetal salvage closely parallel to prior pregnancy loss.

Medical and obstetrical complications are a veritable jungle. Maternal factors include small stature, uterine abnormalities, leiomyomata, and incompetent cervix. Diabetes, syphilis, toxemia, and psychosis are significant medical conditions. Obstetrical variants comprise hydramnios, premature rupture of membranes, prolonged labor, dystocia, breech presentation, abruptio, placenta previa, and ceserean section. Since seldom less than two and often five or more of these factors influence most complicated cases, the computers provide less meaningful information in any specific area; nevertheless, there is definite orientation of the poorest results in the current study to the greatest perinatal loss in prior pregnancies.

Summarizing: Analysis of 25,000 women indicates that gestational background has prognostic value of statistical significance. Probability of a successful outcome in the current pregnancy, among women with prior reproductive failures, diminishes in very close relationship to the number of previous losses.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/SA 1358

1. Perinatal Research Branch
2. Section on Statistical Analysis
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Dilatation Curves

Previous Serial Number: None

Principal Investigator: Kenneth R. Niswander, M.D.,
University of Buffalo, Buffalo, N. Y.

Other Investigators: E. Friedman, M.D.
Michael Reese Hospital, Chicago, Illinois

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.6
Professional:	0.5
Other:	0.1

Project Description:

This study, whereas currently in progress, entails the production of dilatation curves from the case records of some 1,000 women whose labors were complicated either by maternal dystocia, fetal dystocia, or uterine dysfunction.

Honors and Awards: None

Publications: None



- Serial No. NDB (CF)-66 PR/SA 1359
1. Perinatal Research Branch
 2. Section on Statistical Analysis
 3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Neonatal Pneumonia in Liveborn Infants

Previous Serial Number: None

Principal Investigator: Sheldon B. Korones, M.D.
University of Tennessee Medical College,
Memphis, Tennessee

Other Investigators: H. Abramson, M.D., New York Medical College,
New York, N. Y.
T. Fujikura, M.D., PRB, NINDB
A. Kantor, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.7
Professional:	0.6
Other:	0.1

Project Description:

Pneumonia has been cited frequently as the significant cause of neonatal mortality. The Collaborative Study's experience with neonatal pneumonia in liveborn infants was therefore analyzed in regard to significant obstetric factors and selected aspects of the clinical course of affected infants.

Records of 42,205 liveborn infants were screened for the diagnosis of pneumonia during the nursery course. Individual consideration of 191 records with this diagnosis yielded charts of 95 infants for whom the clinical impression of pneumonia was supported by radiologic evidence, by microscopic evidence at necropsy, or both. Infants were excluded from the Study group when, in addition to pneumonia, they were noted to have major congenital anomalies, multiple sites of infection with or without septicemia, or antecedent surgical procedures. Available data precluded consideration of etiologic agents.

Incidence of pertinent obstetric factors associated with birth of infants with pneumonia was compared to that of the Collaborative Study as a whole. Sixteen percent of the infants with pneumonia were delivered by Cesarean section and 6.4% by breech extraction. Maternal complications and evidence

of intrauterine fetal distress were common findings. Though 46% of the infants weighed more than 2500 grams at birth, a distinct association between low birth weight and incidence of pneumonia was noted. Prolonged interval between rupture of membranes and delivery was considerably increased in frequency among infants with pneumonia. Most infants manifested respiratory difficulty in the first 20 minutes of life. Fatality in the group studied was 46.3%.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/SA 1360

1. Perinatal Research Branch
2. Section on Statistical Analysis
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1965 through June 30, 1966

Project Title: Neonatal Serum Bilirubin Levels and Subsequent Neurologic Deficits

Previous Serial Number: None

Principal Investigator: Thomas R. Boggs, M. D.,
Children's Hospital, Philadelphia, Pa.

Other Investigators: J. Hardy, M. D., Johns Hopkins University,
Baltimore, Maryland
T. M. Frazier, District of Columbia Health Department,
Washington, D. C.

Cooperating Units: Section on Statistical Analysis, PRE, NINDB

Man Years:

Total:	0.6
Professional:	0.4
Other:	0.2

Project Description:

Approximately 23,000 infants resulting from single livebirths among women registered in the Collaborative Project between January 1, 1959, and June 30, 1964, have had one or more neonatal total serum bilirubin determinations performed and have been examined at 8 and 12 months of age. Correlations between the maximum observed neonatal total serum bilirubin level and subsequent neurologic deficit have been made. These correlations were made both before and after the exclusion of major congenital malformations including those of the brain, the spinal cord, the skeletal system, the eye, the kidney, the upper respiratory tract, the heart and the gastro-intestinal tract and syndromes such as mongolism, cretinism, gonadal dysgenesis, Piere Robin's syndrome etc. The exclusion of such congenital malformations did not alter essentially the relationships observed before their exclusion and therefore infants with such defects are included in the survey. The study cases included both Coombs positive and Coombs negative infants.

The most striking finds were those encountered on evaluating the Motor and Mental Scores (modifications of the Bayley Scale) of these infants at their age of 8 months (\pm 2 weeks). There was a highly significant linear component to the regression lines of the associations between increasingly high total serum bilirubin levels and the incidence of infants performing in the lowest

decile of their group as concerns motor and mental scores. Although these observations are most striking for the lower birth-weight groups, they held for all birth-weight groups and continued to hold after the birth-weight groups were further controlled for the 5 minute Apgar score. Furthermore, the bilirubin-low mental and low motor score relationships were noted not to begin abruptly at the 20 mgm percent level but were observed to rise progressively and to become substantial at the 16 - 19 mg. percent concentration.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/SA 1361
1. Perinatal Research Branch
2. Section on Statistical Analysis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Marginal Sinus Rupture

Previous Serial Number: None

Principal Investigator: Kenneth R. Miswander, M.D.
University of Buffalo, Buffalo, New York

Other Investigators: Milton Westphal, M.D.
University of Buffalo, Buffalo, New York

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years

Total:	0.3
Professional:	0.2
Others:	0.1

Project Description:

Objectives: To determine the effects of marginal sinus rupture on the fetus, and the characteristics of the pregnant woman that predisposes her to the complication.

Methods: An abstract sheet, covering all variables chosen for study has been filled out for the Collaborative Project cases on which the definitive diagnosis of marginal sinus rupture can be made. Original listings were drawn from a screening form, OB-60, on which tentative diagnoses are made. The control group for the marginal sinus rupture group is the total study population.

Major Findings: Results are not yet reported, since tabulations are still incomplete.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/SA 1362
1. Perinatal Research Branch
2. Section on Statistical Analysis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Effect of Birth Injuries

Previous Serial Number: None

Principal Investigator: Myron Gordon, M.D.,
New York Medical College, New York, New York

Other Investigators: S. B. Korones, M.D.,
University of Tennessee, Memphis, Tennessee

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years (estimated)

Total:	0.3
Professional:	0.2
Others:	0.1

Project Description:

This study is still in progress. It represents an attempt to determine if birth injuries are a serious hazard of permanent impairment to the child, and what the major causes of these injuries may be.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/SA 1363
1. Perinatal Research Branch
2. Section on Statistical Analysis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Early Signs of Neurologic Disorders

Previous Serial Number: None

Principal Investigator: Marguerite J. Gates, M.D.
Columbia University, New York, New York

Other Investigators: Pediatric Staff, Babies Hospital, New York, New York

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years

Total:	-
Professional:	-
Others:	-

Project Description:

To attempt to determine if neurologic disorders of infancy and childhood can be predicted on the basis of early observation. Cases for this study are those observed in Babies Hospital, New York, New York. Several hundreds of variables are under study.

This study is continuing.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/SA 1364

1. Perinatal Research Branch
2. Section on Statistical Analysis
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Retinal Hemorrhages in the Newborn

Previous Serial Number: None

Principal Investigator: Myron Gordon, M.D.
New York Medical College, New York

Other Investigators: None

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years

Total:	0.2
Professional:	0.1
Others:	0.1

Project Description:

This study is in progress and represents an attempt to determine whether retinal hemorrhages in the newborn have a lasting significance or whether they are merely transient symptoms.

Honors and Awards: None

Publications: None



- Serial No. NDB (CF)-66 PR/SA 1365
1. Perinatal Research Branch
 2. Section on Statistical Analysis
 3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Effects of Uterine Dysfunction and Mechanical Dystocia

Previous Serial Number: None

Principal Investigator: Gilbert J. Vosburgh, M.D.
Columbia University, New York, N. Y.

Other Investigators: M. J. Gates, M.D., Columbia University
J. Mackenzie, M.D., Columbia University
J. Deutschberger, PRB, NINDB
H. Berendes, M.D., PRB, NINDB
W. Weiss, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.8
Professional:	0.6
Other:	0.2

Project Description:

The Collaborative Research Project records of 16,455 single pregnancies producing infants weighing over 400 grams were analysed in an effort to relate the complications of uterine dysfunction and/or mechanical dystocia to pediatric outcome.

Approximately 4.8% of gravidae studied had uterine dysfunction alone, 2.4% had mechanical dystocia alone, and 0.9% had both conditions, whereas 92% experienced neither condition.

Overall perinatal mortality for the population was 3.1%. For pregnancies uncomplicated by either condition perinatal mortality was 3.0%; after uterine dysfunction alone, 3.7%; after mechanical dystocia alone, 6.1%; after both conditions, 5.6%.

For all races the heaviest babies were born to mothers experiencing both conditions, with dystocia alone contributing larger infants than uterine dysfunction alone. Low birthweight infants occurred least frequently following pregnancies with both dysfunction and dystocia.

Within the delivery room, Apgar scores were measured at one and at five

minutes. Uterine dysfunction alone tended to lower the one-minute Apgar score, but the depressing effect of mechanical dystocia, either with or without uterine dysfunction, was much more marked. At five minutes the Apgar scores were affected in a similar manner, although the percentage of low scores was, as expected, markedly decreased.

At the time of discharge from the nursery, each infant's course was reviewed and a judgment made as to the presence of a central nervous system (brain) abnormality, either persistent or transient, during the nursery stay. Only following pregnancy complicated by both dysfunction and dystocia was there an apparent increase in incidence of brain abnormality during the nursery stay. However, this conclusion is based on only 4 cases in which the abnormality occurred among infants resulting from 116 pregnancies complicated by both conditions.

Similarly, at each successive re-evaluation during the first year of life the number of cases showing abnormalities was so small that definitive conclusions could not be drawn from the evidence available.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/SA 1366
1. Perinatal Research Branch
2. Section on Statistical Analysis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Incompetent Cervix

Previous Serial Number: None

Principal Investigator: Luke Gillespie, M.D.,
Boston Lying-In Hospital, Boston, Mass.

Other Investigators: S. L. Israel, M.D., Pennsylvania Hospital,
Philadelphia, Pennsylvania
J. Deutschberger, PRB, NINDB
W. Weiss, PRB, NINDB
R. Benson, M.D., University of Oregon, Portland, Ore.
H. Berendes, M.D., PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.9
Professional:	0.8
Other:	0.1

Project Description:

The surgical treatment of the incompetent cervical os is a relatively recent development in the practice of obstetrics. The diagnosis of the syndrome is not easily made, and more often than not is probably presumptive; indeed, there are obstetricians who doubt the existence of this entity.

Data, However, have been accumulating in the literature indicating the beneficial effects of operations (of one type or another) when the diagnosis is made. This report is intended to add to the detail by presenting observations drawn from the large-scale Collaborative Project.

A thorough review of some 25,000 antenatal histories produced 72 women for whom a verified diagnosis of incompetent cervix was made during the course of their first pregnancy in the Collaborative Project. Of these women, 26 never had operations, 6 were treated surgically only in some prior pregnancy, and 40 were subjected to operations during the course of the pregnancy under study.

The 72 women involved, who averaged some 5 pregnancies prior to that studied, had a history notable for loss, with about 70% of their pregnancies terminating in failure.

In the pregnancies studied here, the women who had no operations continued to have a high rate of loss, despite the special care they obtained from Project physicians. Moreover, the children of these pregnancies who survived the neonatal period gave strong evidence of neurological damage.

The loss rate among women on whom operations were performed during the study was 22%. This is high by any standard, but, in the light of their past histories, represents a substantial gain. In addition, damage to survivors did not appear to be extensive.

There are no overt differences in the gravidas that would lead to the conclusion that the effects noted derive from factors other than treatment. The groups do not appear to be overly different in terms of age, gravidity, work history, gynecological background, etc.

No distinction is made here among types of surgical procedures. While a variety of methods were used, there was no maternal mortality or alarming morbidity associated with any.

The suggestion is offered that surgical correction of the cervix may be a non-specific treatment for women with a history of chronic pregnancy loss, and that a therapeutic trial might be undertaken without breach of ethical considerations.

Honors and Awards: None

Publications: None

Serial No. NDB (CF)-66 PR/SA 136.7
1. Perinatal Research Branch
2. Section on Statistical Analysis
3. Bethesda, Maryland

PHS-NIH
Individual Project Reports
July 1, 1965 through June 30, 1966

Project Title: The Relationship of Certain Demographic Variables to the Conditions and complications of pregnancy.

Previous Serial Number: None

Principal Investigator: Sam P. Patterson, M.D.,
University of Tennessee Medical College,
Memphis, Tennessee

Other Investigators: S. B. Korones, M.D., University of Tennessee
J. Deutschberger, PRB, NINDB
W. Weiss, PRB, NINDB

Cooperating Units: Section on Statistical Analysis, PRB, NINDB

Man Years:

Total:	0.5
Professional:	0.4
Others:	0.1

Project Description:

The measurement of a relationship between an event or condition of pregnancy and pregnancy outcome is always made complex by the intrusion of associated factors, as for example, the variable parity complicates the assessment of the relationship between duration of labor and the size of the fetus.

This communication reports the measurement of the relationships among certain demographic variables and some 35 selected conditions and complications of pregnancy. Its purpose is to offer a base for determining which of these variables should be taken into account in studies of the effects of the conditions on the outcomes of pregnancy.

The variables under study are race of gravida, age, parity, and the measures of socio-economic state, family income and housing density. In addition, individual tabulations are derived for each of 6 of the hospitals of the Collaborative Project, in which the patient population is not mainly comprised of single racial group.

Many conditions and complications of pregnancy -- as, for example, toxemia, anemia, leiomyomas, KUB infections, viral infections, uterine dysfunction

and maternal dystocia -- appear to bear strong relationships to one or more of the study variables. The detailing of these relationships provides a preliminary for the study in depth within the Collaborative Project of the effects of the conditions on the outcomes of pregnancy.

Honors and Awards: None:

Publications: None



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