ANNUAL REPORT OF PROGRAM ACTIVITIES NATIONAL EYE INSTITUTE

Fiscal Year 1975

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

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ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1974 - June 30, 1975

STATEMENT OF THE INSTITUTE DIRECTOR

The National Eye Institute recently completed its fifth year of operation and I am extremely gratified at the hard-won advances we have been able to achieve over these first few years. First among these, of course, has been the establishment of a strong core of intramural research activity as represented by the Laboratory of Vision Research, the Clinical Branch, and the Office of Biometry and Epidemiology. Second is the increase in extramural research support that we have been able to provide and the increase in scientific activity that has resulted from this support. Third, a direct result of the first two achievements has been the establishment of a research base from which we are now in the position to organize a comprehensive research program to clinically attack several of the leading causes of blindness, such as glaucoma, cataract, and macular degeneration. The recent publication of the National Advisory Eye Council of its Vision Research Program Planning report contains the analytical plan upon which this accelerated research effort should be based. The report identified a total of 24 specific areas of priority research within the five major NEI programs that should be greatly expanded and also delineated general areas of activity which span all of the NEI programs and should be emphasized. Examples of this latter category are clinical research, clinical trials, and support for basic and clinical disciplines such as genetics, immunology, pharmacology, pathology, experimental ophthalmology, epidemiology, clinical research, controlled clinical trials and the training of research manpower. Just as important as noting the areas of research that require increased emphasis is the identification of areas that are currently receiving adequate levels of funding. Thus, a balanced framework has been established within which the national vision research program can move forward more effectively and efficiently.

Research offers the only real hope of relieving the enormous personal and economic costs of eye disorders. Through studies in the laboratory and clinic, great strides have already been taken toward this end. Now, through carrying out the recommendations of this report with the active participation of the research community, there is even more hope for the future.

Highlights of the progress made against the leading causes of blindness and visual disability in the United States is documented in this report on research conducted and supported by the National Eye Institute in FY 1975.

Carl Kupfer, M.D.

INTRAMURAL RESEARCH

ANNUAL REPORT
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July 1, 1974 - June 30, 1975

REPORT OF THE DIRECTOR OF INTRAMURAL RESEARCH

Carl Kupfer, M.D.

The establishment of a strong core of intramural research activity is indeed one of the great achievements of the NEI in its first five years of operation. This productive intramural component of the new Institute insures that the concerns, viewpoints, and technical expertise of the vision research scientist will be a prime determinant in NEI's policy determinations. As the scientific conscience of the NEI, the intramural program plays a role far beyond that of research organization.

From a strictly research perspective, the intramural research program can play a key part in the national vision research effort as described as follows in one of the major recommendations of the Vision Research Program Planning report of the National Advisory Eye Council:

> "With an extraordinary accumulation of scientific talent and excellent physical facilities, the intramural program should play a leadership role in areas of research just emerging, areas offering unusual promise, and in areas of investigation that require close coordination with the other categorical NIH Institutes. In addition, the intramural program can provide research training to young investigators, as well as advanced training to established scientists who wish to enter new areas of research."

The reports of Dr. Kinoshita, Chief, Laboratory of Vision Research and Dr. Ballintine, Chief, Clinical Branch concerning the research activity of their respective organizations over the past year provide a multitude of examples of the direct contributions these groups have made to vision research. Not mentioned in these reports, but of equal importance, are the contributions the scientists in these groups have made to the scientific management of the NEI by participating in workshops, serving as technical consultants to the Program Planning Subcommittee of the National Advisory Eye Council, assisting in the development of responses to inquiries from the public concerning the NEI programs, and serving as representatives of the NEI on special committees and commissions.

However, the NEI intramural program still suffers from a chronic deficit of staff which must be rectified in order for the Institute to move forward in an orderly manner in its research program.

Clinical Branch

ANNUAL REPORT
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July 1, 1974 - June 30, 1975

REPORT OF THE CLINICAL DIRECTOR

Elmer J. Ballintine, M.D.

The programs of clinical research and related laboratory investigations were continued and expanded. The principles on which the Clinical Branch operates continued to be that each patient admitted must be referred by his or her ophthalmologist and must be appropriate for study under one of the research plans. These plans must meet the same standards of scientific validity that are applied to non-human experiments and at the same time be ethically acceptable and incorporate appropriate safeguards for the patient's rights and welfare. In pursuit of these objectives each research plan is reviewed by a protocol review committee composed of representatives from the Clinical Branch as well as members who are not employees of the Clinical Branch or of the NIH. This review must be completed before patients are admitted for study.

Establishment of the supporting laboratories for neuro-ophthalmology, histopathology, biochemistry, and the clinical facilities for special examination and testing has been mostly completed.

The professional staff includes five senior staff physicians, four physicians who are clinical associates and one who is a staff associate. One physician is a consultant and two senior staff members are not physicians.

Protocols developed during the year emphasize the commitment to Clinical Research. A detailed specific protocol for a controlled randomized clinical trial of urokinase and heparin in the treatment of central retinal vein occlusion was developed and implemented. Clinical protocols for the study of correlations of capillary fragility, basement membrane structure and platelet aggregation with diabetic retinopathy were developed and are in the process of being approved and implemented.

In collaboration with the Experimental Pathology Section of the NEI, Laboratory of Vision Research, 230 eyes from the autopsy service of the Clinical Center were processed and examined histopathologically. Approximately 950 inpatient and 525 outpatient consultations were furnished for other Institutes at the Clinical Center and there were 1600 outpatient visits during the year. There were 108 admissions to the inpatient division and 116 surgical operations were performed.

The Clinical Branch continued to cooperate with other Institutes in the pursuit of unique research opportunities. The study of diabetic retinopathy among the Pima Indians in the project administered by the Epidemiology and Fields Study Branch of the National Institute of Arthritis, Metabolism, and Digestive Diseases was continued as was the study of microangiopathy among patients with acromegaly. A protocol for the study of ocular metastasis in patients undergoing treatment of the breast was begun.

Project No. Z01 EY 00150-02 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Ocular Hypertension Study

Previous Serial Number: NEI-73 CB 150(c)

Principal Investigator: Elmer J. Ballintine, M.D.

Other Investigator: Douglas E. Gaasterland

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	0.4
Other:	0.6

Project Description:

Objectives: Prolonged observation of a series of patients with ocular hypertension, some of whom are treated with miotics, will help to determine which signs have value in predicting those who will eventually require treatment and in determining if early treatment of ocular hypertension has any value in preventing visual field loss or in slowing the rate of development of abnormalities of aqueous humor dynamics.

Methods Employed: A detailed plan for classifying patients with ocular hypertension, observing them by repeated examinations over a period of five or more years and the randomized assignment of patients to treatment with pilocarpine collyria to one or both eyes, or to no treatment, has been standardized for repeated measurement of visual fields, aqueous humor dynamics, and photogrammetry of the optic discs.

Major Findings: The protocols for conduct of the study have been completed and registration of patients in the study is continuing.

Significance to Biomedical Research and the Program of the Institute: Early, precise identification of patients who require treatment because they are in the early stages of the simple glaucoma remains an unsolved problem. The data being collected on this research plan will furnish a basis for establishing treatment for criteria more precisely than is now possible. There is at present no detailed knowledge of the progression of optic disc

changes in ocular hypertension and the data being collected in this study as well as the development of better instruments for the measurements on this study will supply needed information in this field.

Proposed Course: It is expected that the project will continue for at least five years and we expect to enroll 100 subjects.

Keyword Descriptors: glaucoma, ocular hypertension, pilocarpine, tonography visual fields, aqueous humor dynamics, optic disc, photogrammetry

NEI Research Program: Glaucoma

Experimental Subject: Human

Research Objective: Treatment, Diagnosis

Honors and Awards: None

Publications: None

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

Project Title: Ocular Aqueous Humor Formation in Monkey

Previous Serial Number: NEI-73 CB 151(c)

Principal Investigator: Elmer J. Ballintine, M.D.

Other Investigators: Frank J. Macri, Ph.D.
Stanley Cevario
Richard Weiblinger

Cooperating Units: None

Man Years:

Total:	0.7
Professional:	0.3
Other:	0.4

Project Description:

Objectives: To determine the rates at which sodium and chloride ions enter the aqueous humor under physiologic conditions. From these data it will be possible to make some deductions that will help to decide whether the substance primarily secreted is sodium, chloride, or both, and especially give some indications as to whether a chloride ion pump operates in primates.

Methods Employed: A tracer dose of sodium 24 and chloride 36 are given intravenously in rhesus monkeys at the beginning of the experiment. At a later time a tracer dose of sodium 24 is given. Samples of anterior and posterior chamber aqueous humor are then obtained still later in the experiment from one eye and then from the other. The concentration of these ions is determined by the appropriate methods for measuring the radioactivity. The properties of the tracer ions are such that the concentration of each eye can be determined independently of the others by these methods. This method of procedure allows a determination of the time course of the accumulation of the ion at several points in the same eye. In general, a concentration of one of the ions at steady states higher than the plasma concentration indicates that some substance has been secreted into the aqueous humor against the chemical gradient. If the substance enters at a rate greater than the bulk flow through the chamber it is an indication that the substance gets in by a diffusional exchange. If the substance enters

at the same rate as the bulk flow through the chamber, it is an indication that that substance is primarily transported into the chamber.

Major Findings: Fifteen monkeys have been tested using the isotopic tracer methods. Calculations of the rates of entrance of the various ions are underway.

Significance to Biomedical Research and the Program of the Institute: The question of whether a chloride pump is active in the formation of aqueous humor of primates and whether sodium undergoes significant diffusional exchange in primates are unsolved questions of importance in understanding the formation of aqueous humor. Answers to these questions can furnish a basis for predicting the performance of drugs that influence the rate of aqueous humor production and might be useful in the treatment of glaucoma. Similar triple-labelled experiments have not heretofore been performed.

Proposed Course: The project will continue according to the research planned.

Keyword Descriptors: aqueous humor, ion secretion, radioactive tracers, ion transfer

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: Monkey

Research Objective: Etiology

Honors and Awards: None

Publications: None

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

Project Title: Tissue Culture of Trabecular Meshwork

Previous Serial Number: None

Principal Investigators: Elmer J. Ballintine, M.D.
Sheldon Buzney, M.D.

Other Investigator: Richard Weiblinger

Cooperating Units: None

Man Years:

Total:	.04
Professional:	.02
Other:	.02

Project Description:

Objectives: A variety of evidence indicates that in simple glaucoma the site of obstruction to the drainage of aqueous humor is somewhere within the trabecular meshwork and the inner wall of the canal of Schlemm. The marked increase in resistance to aqueous drainage that occurs in eyes with simple glaucoma but not in eyes of normal subjects when they are subjected to topically corticosteroids indicates that the trabecular meshwork in glaucomatous subjects differs in some unknown metabolic way from the tissue of normal subjects. The amounts of tissue available are insufficient for most biochemical methods. Tissue culture and the use of radiolabelled metabolites with small segments of surviving tissue using histochemical and radioautographic methods are the methods likely to yield information about the pathogenesis of deranged aqueous humor dynamics. The objectives of this project are to determine how the cells in the trabecular meshwork and inner wall of Schlemm's canal differ in their metabolic activities from those from normal eyes. The immediate objective is to determine if the two kinds of meshwork differ in their ability to synthesize hyaluronic acid polymer from its precursors and to determine if this process can be influenced by glucocorticoids. Small specimens of trabecular tissue are obtained from monkey eyes, human autopsy eyes, and as surgical specimens from patients undergoing trabeculectomy for relief of intractable glaucoma.

Methods Employed: Specimens of trabecular meshwork are sectioned into

small fragments under a dissecting microscope and placed in tissue culture medium. The specimens are observed with phase microscopy for evidence of growth and at various stages of tissue proliferation the appropriate radiolabelled metabolites are added to the medium. Agents which may influence the rate of incorporation of metabolites into the cellular structure and cellular products are also added to the medium. The proliferating cells are characterized by their histologic and histochemical properties and by their ability to incorporate labelled precursors as revealed by radioautography.

Major Findings: Trabecular meshwork from monkey eyes has been grown consistently in tissue culture and some of the factors necessary for rapid and consistent growth have been determined. Surgical specimens from two human glaucomatous eyes are at present growing in tissue culture. Histologic study of the growth from the monkey trabecular meshwork is underway.

Significance to Biomedical Research and the Program of the Institute: The mechanism by which the glaucomatous eye develops its resistance to drainage of aqueous humor and by which this resistance is influenced by glucocorticoids is completely unknown. The intraocular pressure elevation in response to treatment with corticosteroids is probably inherited as a single gene trait. This project is at present the only feasible plan for direct investigation of this mechanism. It is expected that this work will reveal some indication of the fundamental cause of simple glaucoma.

Proposed Course: The work will be continued according to the research plan.

Keyword Descriptors: trabecular meshwork, tissue culture, simple glaucoma, glucocorticoid, intraocular pressure

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: Monkey/Human

Research Objective: Etiology

Honors and Awards: None

Publications: None

Project No. Z01 EY 00038-03 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Studies of Choroidal-Retinal Degenerative Diseases

Previous Serial Number: NEI-72 CB 038(c)

Principal Investigator: Donald R. Bergsma, M.D.

Other Investigators: Muriel Kaiser-Kupfer, M.D.

Cooperating Units: NEI 71 CB 006(c)
NEI 73 LVR 134
Walter Reed Army Institute of Research (Division of Surgery), Washington, D.C., A. R. Rosenthal, M.D., and D. Huxall, D.V.M., Study of Chloroquine Induced Damage to the Retina of the Rhesus Monkey
NEI 73 OBE 120
Z01 NS 02152-01, NINCDS

Man Years:

Total:	0.40
Professional:	0.35
Other:	0.05

Project Description:

Objectives: The objectives of this study are to properly classify, to further clinically define, and to elucidate the cause, prevention, or therapy of selected degenerative diseases of the retina and choroid. Examples are retinitis pigmentosa, familial macular degeneration, and the effects of drugs toxic to the retina.

Methods Employed: Clinical studies utilize specialized tests of visual function (dark adaptation, cone thresholds, visual fields), electroretinography (ERG), electro-oculography (EOG), fundus photography and fluorescein dye studies. Appropriate testing of relatives is undertaken to document genetic patterns and define variation of severity within disease entities. A prospective study to evaluate vitamin A therapy in selected diseases has been completed. Surgical and electrophysiological studies in animals are continuing. Possible side effects of drugs are being evaluated.

Major Findings: Approximately 150 patients were studied this year.

At present the overwhelming majority of patients afflicted with choroidal and retinal degenerative diseases are not curable. High dosage oral vitamin A therapy has been shown to have no effect on the vast majority of patients with pigmentary retinal degenerations. Nevertheless, most are helped by a combination of genetic counselling, discussing of prognosis, and advice regarding visual aids and rehabilitation.

Related experiments in monkeys show that the subcellular damage to retinal cells produced by high doses of chloroquine occurs more than two years before ERG or fundus abnormalities are detectable.

Significance to Biomedical Research and the Program of the Institute: This project is directed at improving classification, prevention, and treatment of choroidal-retinal degenerative diseases via new diagnostic techniques, controlled therapeutic trials, long term follow-up and animal and laboratory experimentation.

Proposed Course: Since an adequate panel of patients has been established for longitudinal studies, special laboratory investigations, and therapeutic trials when appropriate, emphasis has been shifted to related laboratory studies via project NEI 73 LVR 134.

Keyword Descriptors: eye, retina, choroid, degeneration, retinitis pigmentosa, night blindness, genetic, toxicity, macula

NEI Research Program: Retinal and Choroidal Diseases--Development, Structure, Function, and Degeneration

Experimental Subject or Tissue Source: Human/Rhesus Monkey

Research Objective: Etiology, Treatment, Diagnosis

Honors and Awards: None

Publications:

Trobe, J. D., and Bergsma, D. R.: Atypical retinitis pigmentosa masquerading as a nerve fiber bundle lesion. Am. J. Ophthalmol. 79: 681, 1975.

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

Project Title: Studies of Ophthalmic Familial and Genetic Diseases

Previous Serial Number: NEI-72 CB 039(c)

Principal Investigator: Donald R. Bergsma, M.D.

Other Investigators: Muriel Kaiser-Kupfer, M.D.
Kenneth Foon, M.D.

Cooperating Units: NEI 73 LVR 134
NEI 73 CB 152(c)

Man Years:

Total:	0.30
Professional:	0.25
Other:	0.05

Project Description:

Objectives: The objective of this study is to properly classify, to clinically define, and to elucidate the cause, prevention and treatment of genetic and familial diseases affecting the eye. Please refer to the detailed description of a closely related project, NEI(I)-72 CB 038(c). This project involves a broader range of ophthalmic manifestation of genetic and familial disease. For example, the objective of one sub-study is to define the relationships between the inheritance of glaucoma, the inheritance of steroid responsiveness (increases in intraocular pressure following steroid eye drops) and the inheritance and mechanisms of steroid sensitivity at the cellular level.

Methods Employed: Clinical workups are tailored to each disease entity studied with emphasis on family studies, controlled therapeutic trials and genetic counselling. Cellular mechanisms are studied where possible. In the example above, lymphocytes from patients with primary open angle glaucoma and age matched controls are preincubated with various steroid dilutions. The lymphocytes are then stimulated to transform in tissue culture and the degree of transformation is quantitatively assayed by measuring the uptake of tritiated thymidine into DNA.

Major Findings: Approximately 100 patients with familial and genetic

diseases involving the eye (excluding the choroidal-retinal diseases of project NEI(I)-72 CB 038(c)) were seen on referral or recall.

Most of these patients do not have curable diseases with present medical knowledge, but do benefit from a combination of palliative therapy, visual aids, genetic counselling, and advice regarding prognosis and rehabilitation.

Evidence for extreme variation of some inherited diseases within families has been documented, indicating that minor genes and environment influence the major genes significantly.

Significance to Biomedical Research and the Program of the Institute: This broadly defined project is focused on the classification, etiology and treatment of diseases which interfere with vision. The common denominator of familial and genetic occurrence enables the marshalling of statistical analysis, biochemical tests, genetic counselling, etc., around the patient's problem. Discovery of biological markers and mechanisms at the cellular level could provide means of prevention or therapy which cannot be obtained with clinical examination alone.

Proposed Course: Clinical characterization of diseases and therapeutic trials will be continued as appropriate. However, since an adequate panel of patients has been established for longitudinal studies, special laboratory studies and therapeutic trials, emphasis has been shifted to related laboratory investigations via project NEI 73 LVR 134.

Keyword Descriptors: eye, familial, genetic, glaucoma, steroid, lymphocyte transformation, intraocular pressure

NEI Research Program: Glaucoma--Primary Glaucoma - Including Intraocular Pressure Regulation

Experimental Subject or Tissue Source: Human

Research Objective: Etiology, Treatment, Diagnosis

Honors and Awards: None

Publications:

Bergsma, D. R., and Brown, K. S.: Animal models of albinism. Birth defects, Original Article Series, The National Foundation, New York, (in press).

Project No. Z01 EY 00010-01 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: In Vitro Studies of Retinal Capillaries

Previous Serial Number: None

Principal Investigators: Sheldon M. Buzney, M.D.
Robert N. Frank, M.D.

Other Investigators: Kathleen Kuehl
W. Gerald Robison, Ph.D.

Cooperating Units: Laboratory of Vision Research, NEI

Man Years:

Total:	0.9
Professional:	0.8
Other:	0.1

Project Description:

Objectives: This project represents an initial attempt to study the properties of retinal capillary cells maintained under tissue culture conditions. We have attempted to determine which cell types proliferate and the morphological, ultrastructural, and histochemical characteristics of proliferating cells.

Methods Employed: Retinas are dissected from eyes shortly after death using sterile conditions. The retinas are lightly homogenized and sieved through sterile nylon mesh of appropriate size, and the retained capillaries are washed off the mesh into Petri dishes containing culture medium. At various times, the capillaries in the Petri dishes are prepared for light and electron microscopy using standard techniques. Alternatively, the original medium is removed and fresh medium containing 3H-thymidine is added to study the cells undergoing division by radioautography. Initial biochemical studies of the isolated capillaries have involved standard biochemical assays for basement membrane glucosyltransferase and galactosyltransferase activities, for aldose reductase and sorbitor dehydrogenase, and histochemical techniques for aryl esterase.

Major Findings: Preliminary work on this project has demonstrated that it is possible to isolate vertebrate retinal capillaries with a minimum of

contamination from larger vessels and from other cellular fragments, and that the cells from these isolated capillaries will over a few days' time begin to multiply in tissue culture. The cells ultimately undergo transformation, since they attain a large size and polygonal shape with a large nucleus and multiple nucleoli, i.e., they have become polyploid rather than retaining their original diploid chromosomal content. There are two types of cells in retinal capillaries, the endothelial cells and the mural cells (or intramural pericytes). Attempts are being made to determine which of these cell types undergoes transformation and division in culture. Preliminary radioautographic evidence suggests that both types of cells may be involved, but many more experiments will be required to establish a definite conclusion. Electron microscopy has thus far been limited to cells that have been in culture for over one month from the time of their isolation, and they bear little morphologic resemblance to the original capillary endothelial and mural cells. These cultured cells contain many mitochondria arranged close to the nucleus, and multiple closely packed smooth muscle fiber bundles. There are also many micropinocytotic vesicles and lysosomes densely packed glycogen granules, vesicles of rough and smooth endoplasmic reticulum, and scattered vesicles of the Golgi complexe. The rodlike structures which have been designated "Weibel-Palade bodies" and which are considered typical of of vascular endothelial cells, have not been observed. Biochemical and histochemical studies are still too preliminary to include their results here.

Significance to Biomedical Research and the Program of the Institute: Knowledge of the biochemistry and physiology of the retinal blood vessels, especially the capillaries, is of the utmost importance for the understanding and treatment of a variety of retinal diseases. Diabetic retinopathy, sickle cell retinopathy, and retinal vein occlusions are among those retinal vascular disorders which are serious causes of visual disability and which, perhaps, can be more rationally treated with further understanding of the biochemistry and function of the capillary cells of the retina. Preliminary work on this project has yielded the first indication that the retina capillaries from several vertebrate species, including man, can be studied in isolation, and that their cells can be induced to grow under controlled conditions in culture. This result suggests that tissue culture methods can be used to study a variety of factors that may alter this growth and give clues to the origins of many disorders of the retinal blood vessels.

Proposed Course: The anatomical, biochemical, and radioautographic studies noted in the Project Description and in the Methods section will all be continued.

Keyword Descriptors: retinal blood vessels, retinal capillaries, tissue culture, radioautography, vascular endothelium, capillary mural cells (intramural pericytes), basement membrane, electron microscopy

NEI Research Program: Retinal and Choroidal Diseases--Vascular and Circulatory Abnormalities--Including Disturbances in Blood Vessel Formation

Experimental Subject or Tissue Source: Rhesus Monkey/Cattle/Human

Research Objective: Etiology

Honors and Awards: None

Publications:

Buzney, S. M., and Frank, R. N.: In vitro proliferation of retinal capillaries, Paper presented at Wilmer Residents Association Meeting, The Johns Hopkins Hospital, Baltimore, Md., April 18, 1975.

Project No. Z01 EY 00020-01 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Abnormalities of the Human Oculomotor System

Previous Serial Number: None

Principal Investigator: David G. Cogan, M.D.

Other Investigators: Robert D. Yee, M.D.
David Zee, M.D.

Cooperating Units: BME, DCRT, NINCDS-Medical Branch

Man Years:

Total:	0.8
Professional:	0.6
Other:	0.2

Project Description:

Objectives: Recording and analysis of abnormalities of eye movements in patients with ophthalmologic and neurologic diseases to identify and describe specific defects in components of the human oculomotor system in order to aid diagnosis of these diseases and to elucidate functions of components in the oculomotor system.

Methods Employed: Eye movements are recorded by a videotape system and by electro-oculography. Electro-oculography records changes in an electrical field about the eyes produced by movements of the eyes and allows accurate measurement of movements of each eye in both horizontal and vertical directions.

Eye movements represented as electrical impulses are stored on FM magnetic tape and analyzed by a digital computer. The computer system allows deletion of artifacts from the recordings, accurate measurement of the speed and size of eye movements and statistical analysis of data.

Each of the major components of the oculomotor system, i.e. smooth pursuit system, voluntary saccades, optokinetic nystagmus, vestibular nystagmus and fixation system, are tested. A large aluminum drum which surrounds the patient allows rotation of the patient's entire visual field, and a motorized chair can rotate the patient up to 200 degrees/sec to

stimulate the semicircular canals of the ears.

An electromyography system is being developed which can record the electrical activity of individual eye muscles. This system will allow differentiation of diseases in which the eye muscles themselves are abnormal from diseases in which the nerves innervating the muscles are abnormal.

Major Findings: To date patients with abetalipoproteinemia, a disorder of fat metabolism in which retinal and neurologic degenerations occur, with hereditary cerebellar degeneration and with congenital ocular nystagmus have been studied. Characteristic eye movement abnormalities, nystagmus and paralysis of movements, have been observed in patients with abetalipoproteinemia which cannot be explained by known defects in one or more components of the oculomotor system. This suggests that there might be a combination of abnormalities in eye muscles and brain centers in this disease.

Patients with some forms of hereditary degeneration of the cerebellum have been observed to have characteristic types of nystagmus and abnormalities of the ocular tracking systems. Patients with congenital nystagmus have been found to have more complex types of nystagmus than the two types, jerk nystagmus and pendular nystagmus, usually appreciated. The causes of congenital nystagmus appear to be more complex than just disorders of the eyeball damaging vision or abnormalities in the motor coordination of fixation without damage to the eyeballs.

Significance to Biomedical Research and the Program of the Institute: Greater ability to record and analyze abnormalities of eye movements should lead to better understanding of specific defects in the oculomotor system which produced these abnormalities. Since components of the oculomotor system are represented throughout the central nervous system and are often affected by diseases of the brain, e.g. tumors, vascular anomalies, cerebrovascular accidents, and disorders of metabolism, a more precise diagnosis and localization of disease should be possible. Study of abnormalities of eye movements should elucidate the parts that components of the oculomotor system normally play in the control of eye movements.

Proposed Course: Patients with a broad spectrum of abnormalities of eye movements referred by other institutes of the NIH and by physicians from the outside medical community will be studied. Techniques for recording and analyzing eye movements will continue to be refined and new techniques will be developed.

Keyword Descriptors: motor disorders of vision-etiology and diagnosis, recording of eye movements, electro-oculography, electromyography

NEI Research Program: Sensory and Motor Disorders of Vision--Oculomotor Disorders (Oculomotor Control)

Experimental Subject or Tissue Source: Human

Research Objective: Diagnosis

Honors and Awards:

Dr. Cogan received the Gonin Award - University of Lausanne, May, 1975

Publications:

Yee, R. D., Cogan, D. G., and Zee, D. S.: Ophthalmoplegia and dissociated nystagmus in abetalipoproteinemia. Arch. Ophthalmol. (in press)

Cogan, D. G.: Ophthalmic Manifestations of Systemic Vascular Disease. W. B. Saunders, Co., 1974.

Project No. Z01 EY 00155-02 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Combined Clinical and Experimental Animal Study of the Pathogenesis of Abnormal Proliferations in the Vitreous Cavity

Previous Serial Number: NEI-73 CB 155

Principal Investigators: Daniel M. Eichenbaum, M.D.
Steven Charles, M.D.

Other Investigators: Ralph Helmsen, Ph.D.

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS
Television Engineering Section, ADM, CC

Man Years:

Total:	3.0
Professional:	2.0
Other:	1.0

Project Description:

Objectives: The primary purpose of the vitrectomy research protocol, NEI-9, is to perform trans pars plana vitrectomy for established clinical indications in order to obtain diseased human vitreous. This material is then assayed in an animal system for a vasoproliferative factor. In the performance of the clinical aspects of the protocol certain secondary projects have been generated. By documentation of preoperative, operative and postoperative findings it was possible to learn the operative and postoperative complication rate and the frequency of significant visual improvement. This information has been useful in the experimental design of the projected cooperative vitrectomy trial in diabetic retinopathy. By close cooperation with the BEIB and television engineering section, new instrumentation has been developed to improve preoperative patient examination and operative technique.

Methods Employed: Patients are admitted to the Clinical Center who are in need of a vitrectomy operation. The vitrectomy operation is performed and the vitreous that is removed is then analyzed for factors that cause vasoproliferation in a bioassay system.

Major Findings:

I. Vasoproliferative Factor

Chemical compounds which stimulate the growth of new blood vessels are the subject of much current research in many fields of science. It is well established that certain solid tumors manufacture and release these vasoproliferative factors that stimulate new vessel growth toward the tumor to provide it with nourishment for growth. The idea that a vasoproliferative factor is important in the development of proliferative diabetic retinopathy is not a new one. The search for this chemical has been attempted before and is currently the subject of investigation in several laboratories around the country.

In the search for a vasoproliferative factor, certain early success has been achieved. The diseased vitreous from diabetic patients removed during trans pars plana vitrectomy is processed to eliminate excess fluid, salts, and unwanted high molecular weight compounds. This crude, but semi-purified solution is then injected into the vitreous of experimental animals. In most of these eyes into which human vitreous extract has been injected, new blood vessels have been seen to grow on the iris, simulating what occurs in association with proliferative diabetic retinopathy; that is, the growth of new vessels on the iris with resultant neovascular glaucoma. This is the first time that any material from the eyes of diabetics has been used successfully to produce new vessel growth in an experimental animal model. Further experiments are currently underway to make certain that these early and preliminary results are not artifactitious and to clarify the chemical nature of this vasoproliferative factor.

II. Clinical Studies

A. Results of Vitrectomy

Since the initiation of the vitrectomy research protocol in November, 1973, approximately 90 primary trans pars plana vitrectomies have been performed. The two major indications for surgery in this patient population were opacities of the vitreous cavity such as vitreous hemorrhage and certain types of retinal detachments related to vitreous traction. By far the single largest disease group consisted of patients with proliferative diabetic retinopathy and retinal vascular disease related to high blood pressure. Essentially all of the eyes operated upon had no useful vision before surgery. Initial examination of the data reveals that approximately 70% of those operated eyes regained useful vision.

A major effort is now underway to examine in greater detail the preoperative, operative and postoperative data on each of these individual eyes. In addition to the rate of success or failure of surgery, there will be determination of the complication rate during surgery, immediately following surgery and in the extended postoperative follow-up period.

Analysis of these data will be completed within the next 60 days.

B. Fluorescein Angiography of the Iris

As more experience with trans pars plana vitrectomy is gained across the country by a number of surgeons, it has become apparent that neovascular glaucoma is one of the major complications of this surgical procedure. If this complication does occur, new blood vessels are seen to grow on the surface of the iris. These blood vessels block the filtration angle, thereby preventing the outflow of aqueous humor from the eye.

In order to examine this problem more directly, fluorescein angiography of the iris was performed in a number of patients preoperatively as well as at various times during the postoperative course. The blood vessel patterns on the iris could, therefore, be studied in detail. The initial observation has been that in spite of the fact that no new vessels may be seen on the iris of many diabetic patients, abnormal new vessels can be demonstrated by iris angiography prior to surgery. By comparing the postoperative and the preoperative angiograms, it is possible to demonstrate the changes in new vessel pattern on the iris. It is hoped that the study of these new vessel patterns can perhaps help the early diagnosis of neovascular glaucoma and can give the physician an insight into the pathological process ongoing in the diabetic eye.

III. Instrumentation Development

A. Evaluation of Patients for Vitrectomy

1. B-scan ultrasonography

The commercially available B-scan ultrasound equipment was utilized in all cases preoperatively and in many patients that did not receive vitrectomy. This technique was found to be helpful in planning surgical approach and in some cases determining necessity for surgery. It was hoped that certain improvement in this equipment would make possible greater accuracy in diagnosis. A real time mechanical sector scanner has been developed in conjunction with Mr. Jim Griffith of BEIB. The ophthalmic sector scan is displayed on a gray scale variable persistance oscilloscope. This oscilloscope can be observed directly or converted to television format with a vidicon camera. With the gray scale, real time B-scan in television format it is possible to perform kinetic analysis by means of a video disc recorder. This, in turn, makes it possible to determine if vitreo-retinal structures are mobile or taut. The television format also enables the investigator to color-encode the gray scale to analyze the spatial distribution of echo amplitudes and to generate deflection modulation pictures for similar purpose. The gray scale, color-encoding and deflection modulation techniques have been used over the past several months to aid in our diagnostic capabilities.

2. Increased Intensity ERG's

Increased intensity ("bright flash") ERG's have been utilized in conjunction with two new techniques to better assess preoperative retinal function. A twin flash technique is being evaluated to assess cone function. A transcleral delivery system is being tested to permit assessment of retinal attachment in individual quadrants.

3. Measurement of Sub 20/200 Vision

A device has been designed, constructed and evaluated to rotate a 20/200 "E" and stop randomly in 90° separated positions. This motorized rotating "E" is housed in a hand-held self-illuminated enclosure with a tape measure to assess the distance to the patient. This device has made possible careful documentation of vision less than 20/200. This device is important because many patients that improve from 1/200 to 8/200 have achieved significant functional improvement via vitrectomy. With this method it is possible to accurately determine these patients' vision.

B. New Techniques for Trans Pars Plana Vitrectomy

1. Chin switch - a chin operated switch for motorized three axis microscope movement has been designed, constructed, and evaluated. A patent is pending on this device. This device decreases microscope positioning time, prevents spurious hand movements, and allows a second foot to operate other surgical modalities.

2. Syringe drive - a hand-held mechanical syringe drive has been designed, constructed and evaluated to provide the suction force at the time of vitrectomy. This device is held by the surgeon and provides greatly increased control over the suction compared to the assistant-held syringe technique. This device eliminates operator fatigue, is autoclavable, uses disposable syringes and provides precise mechanical control with feedback feel of the force utilized.

3. Coaxial cannula - a coaxial cannula has been designed, constructed and clinically evaluated to instill a single, large intraocular gas bubble. This is done through the vitrectomy site and without losing intraocular volume or producing hypotony. In addition, this device is blunt tipped unlike the needle technique presently in use. This prevents inadvertent perforation of the retina.

4. Bipolar, bimanual intraocular cautery - a technique has been developed and clinically evaluated to utilize the bipolar cautery technique within the vitreous cavity. This technique provides excellent control over the distribution of the coagulation effect. The vitrectomy machine is utilized as one pole and a 23 needle connected to a handle as is the other pole with this cautery technique.

5. Slot cutter - a slot cutter has been developed to fit the Roto-Extractor to permit cutting vitreous without utilizing suction force for imbrication of structures to be cut.

6. Intraoperative fluorescein funduscopy and television angiography - working in conjunction with Mr. Willard C. Whitehouse in Television Engineering a technique has been successfully utilized to permit the recording on videotape of a fluorescein angiogram while the vitreous infusion suction cutter is in the eye. A fluorescein blue exciter filter is placed in the fiber optic light source, the patient is given intravenous fluorescein and the fundus observed for leakage of dye and patency of vessels. An electron bombarded silicon television camera with a fluorescein barrier filter records the passage of dye through the eye and the leakage pattern for instant replay and analysis in the operating room. Utilizing this new technique it is possible for the surgeons to do photocoagulation, cryotherapy, or diathermy to specific vascular abnormalities in the eye at the time of vitrectomy.

7. Fiberoptic delivery of intraocular photocoagulation - a device has been designed, constructed and clinically evaluated which utilizes the Zeiss xenon photocoagulator as an energy source for intraocular photocoagulation. The energy is delivered through a fiberoptic bundle placed through the pars plana opposite the entry site of the vitreous infusion suction cutter. This makes possible photocoagulation of retinal areas while viewing illumination suction and infusion are provided by the vitreous cutter. It is hoped that by combining these new treatment modalities and intraoperative fluorescein angiography further improvement in the results of vitrectomy can be possible.

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy is one of the leading causes of blindness in the United States. The underlying pathology is the proliferation of endothelial cells in the blood vessels of the retina with resulting neovascularization vitreous hemorrhage, scarring, and retinal detachment. The same pathologic sequence is found in other neovascular diseases; namely,sickle cell retinopathy, central retinal vein occlusion, Eales disease, and others. A secondary complication of these retinal disorders is the production of neovascularization of the iris and subsequent glaucoma. We postulate that an intraocular vasoproliferative factor that is diffusable in the eye is responsible for the vasoproliferation seen in these disorders. It is obvious that the identification of such a factor would be of great importance in the treatment and prevention of Blindness from these disorders.

Proposed Course: Dr. Eichenbaum and Dr. Charles will complete their Clinical Associate service on 30 June 1975. Their work on the vitrectomy project will end at the Clinical Center. They expect to pursue some of the aspects of the work in their new location. The vitrectomy service at the Clinical Center will be continued by Dr. Carol Kollarits, Dr. Martin Fishman, and Dr. John Christianson. The main emphasis on the work will be:
1) Completion of research plan which will enable us to participate in the

diabetic vitrectomy trial; 2) the testing in a controlled trial of alternative features of the vitrectomy procedure, for example, the efficacy of lensectomy, and the value of improved irrigating media; and, 3) the continuation of the search for the vasoproliferative factor by developing improved assays and alternative methods of processing and characterizing the fractions obtained from vitreous washings.

Keyword Descriptors: proliferative diabetic retinopathy, vasoproliferative retinopathy, traction retinal detachment, trans pars plana vitrectomy, instrumentation, angiography, vasoproliferative factor, ultrasound

NEI Research Program: Retinal and Choroidal Diseases--Vascular and Circulatory Abnormalities / Retinal Detachment and Vitreous Abnormalities

Experimental Subject or Tissue Source: Human/Monkey

Research Objective: Etiology, Treatment, Diagnosis

Honors and Awards:

Dr. Charles, Mr. McCarthy (BEI), and Dr. Eichenbaum have submitted for patent the syringe drive and chin switch, but a patent number has not been assigned.

Publications:

Charles, S. T., McCarthy, C. and Eichenbaum, D. M.: A mechanical syringe drive for vitreous surgery. Am. J. Ophthalmol. (in press).

Charles, S. T., McCarthy, C., and Eichenbaum, D. M.: A chin-operated switch for motorized three-axis microscope movement. Am. J. Ophthalmol. (in press).

Project No. Z01 EY 00023-01 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Neovascular Glaucoma Trial

Previous Serial Number: None

Principal Investigator: Martin L. Fishman, M.D.

Other Investigators: Carol R. Kollarits, M.D.
Elmer J. Ballintine, M.D.
Douglas E. Gaasterland, M.D.
Carl Kupfer, M.D.

Cooperating Units: None

Man Years:

Total:	.3
Professional:	.3
Other:	0

Project Description:

Objectives: Neovascular glaucoma is a blinding, painful eye disease that occurs in patients with severe diabetic retinopathy, central retinal vein occlusion, and other less common diseases. A new surgical procedure, the aqueous-venous shunt, has been proposed for treatment of eyes with neovascular glaucoma not controlled with medications. To determine if this technique is useful in the treatment of neovascular glaucoma, the aqueous-venous shunt must be compared to other operations designed to control elevated intraocular pressure.

Methods Employed: There is no known effective treatment for neovascular glaucoma and many cases cannot be controlled with medicines. Such cases will be randomized into one of several surgical treatments. A new surgical treatment, aqueous-venous shunt, is now being evaluated in experimental animals. If this technique is found to be useful in animals, it will be compared with other surgical techniques of managing neovascular glaucoma in human patients.

Major Finding: Polyethylene tubing has been used for performance of an aqueous-venous shunt operation in the rabbit. The tubing appears to be too large and not quite flexible enough for this purpose. We are in the

process of obtaining a new collagen tubing to be used for performing aqueous-venous shunts.

Significance to Biomedical Research and the Program of the Institute: At this time there is no really effective treatment for neovascular glaucoma. If a surgical procedure such as the aqueous-venous shunt technique could be developed for the treatment of neovascular glaucoma, it may prevent blindness and pain in many eyes that would otherwise be lost to this disease.

Proposed Course: When the technique and materials for the aqueous-venous shunt procedure have been thoroughly investigated in animals, human patients with medically uncontrollable neovascular glaucoma will be treated with aqueous-venous shunts or a standard operation in an attempt to favorably alter the course of their disease. Until such time as this new technique is fully developed and ready for use on human patients, patients being referred to the Clinical Center with neovascular glaucoma will be randomized into one of several available surgical procedures to evaluate their relative effectiveness in the treatment of eyes with neovascular glaucoma.

Keyword Descriptors: neovascular glaucoma, aqueous-venous shunt, trabeculectomy with cautery, cyclocryotherapy

NEI Research Program: Glaucoma--Secondary Glaucoma

Experimental Subject or Tissue Source: Human/Rabbit/Monkey

Research Objective: Treatment

Honors and Awards: None

Publications: None

Project No. Z01 EY 00140-02 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Clinical Studies of Retinal and Choroidal Diseases (Previous Title: Argon Laser Photocoagulation of Retinal and Choroidal Diseases)

Previous Serial Number: NEI-73 CB 140(c)

Principal Investigator: Robert N. Frank, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: This project has three major, long-range objectives: (1) To elucidate factors involved in the pathogenesis of diseases of the retinal blood vessels and of diseases involving the macular region of the retina by clinical and laboratory studies of affected patients and of matched, normal controls; (2) to describe the natural history of certain of these diseases by long-term followup of affected patients with the aid of standard clinical examinations abetted by such techniques as stereoscopic fundus photography, fluorescein angiography, and psychophysical and electrophysiological testing; and (3) to determine if the natural history can be altered by appropriate treatment, in particular at the present time by the use of argon laser photocoagulation in randomized, controlled clinical trials.

Methods Employed: Patients are examined by standard clinical ophthalmologic techniques, with the addition of the specialized methods noted above. Two new protocols which have been approved by the NIH Medical Board and added to this project this year will involve studies of platelet aggregation by a densitometric method which depends on increased light transmission by serum samples as the platelets aggregate and settle out, and the measurement of conjunctival capillary fragility by application of a calibrated suction cup to the bulbar conjunctiva following topical anesthesia, with determination

of the pressure required to produce a minimum number of petechiae following an application of one minute.

Major Findings: The group of patients described in last year's report who have been treated with the argon laser for either proliferative or background diabetic retinopathy, for retinal vein occlusions, and for several macular diseases have been followed for another year, and the findings at this time do not differ from those reported previously. A study of visual field and electroretinographic changes following massive argon laster photocoagulation for proliferative diabetic retinopathy has suggested that the degree of treatment now being recommended in many centers may destroy an average of 40% of the functioning neural retina although, surprisingly, visual field changes have not been marked save in a very few cases. Since the clinical course of these diseases may be protracted, it is still too early to draw conclusions regarding the benefits, or lack of same, from laser treatment, and followup will continue.

Significance to Biomedical Research and the Program of the Institute: It is hoped that this study will provide both clinical and basic scientific information about several retinal and choroidal diseases which are common, and which cause severe visual disability in large numbers of people. In addition, it will continue to investigate one, and, perhaps in time, will add other, proposed methods of treatment for these diseases.

Proposed Course: Two new protocols have been approved by the Medical Board and should be initiated shortly. These include a study of platelet aggregation in long-term diabetics with and without retinopathy, who will be matched with a group of normal controls, and a study of conjunctival capillary fragility in the same group of patients and controls, in which it will be attempted to correlate capillary fragility with the presence of retinopathy, and with capillary basement membrance thickness as determined from electron microscopy of conjunctival biopsy specimens. Increased platelet aggregability, increased capillary fragility, and increased capillary basement membrane thickness have all been implicated as possible factors in diabetic small vessel disease in the retina and elsewhere. Formal protocols involving random allocations for laser treatment of background diabetic retinopathy and several varieties of macular disease (senile macular degeneration, the presumed ocular histoplasmosis syndrome, and angioid streaks) are now in preparation and will, it is hoped, be initiated within the year. Patients with proliferative diabetic retinopathy are no longer being recruited for this project, since photocoagulation treatment of this type of diabetic retinal disease is being studied in the Diabetic Retinopathy Study now being carried out with extramural support from the NEI.

Keyword Descriptors: Retina, choroid, macula, diabetic retinopathy, senile macular degeneration, presumed ocular histoplasmosis syndrome, angioid streaks, retinal photocoagulation (argon laser photocoagulation), fluorescein angiography, electroretinography, clinical trial, platelet aggregation, capillary fragility

NEI Research Program: Retinal and Choroidal Diseases--Vascular and Circulatory Abnormalities--Including Disturbances in Blood Vessel Formation/ Macular Diseases

Experimental Subject or Tissue Source: Human

Research Objective: Etiology, Treatment

Honors and Awards:

Lecturer in Ophthalmology, The Wilmer Ophthalmological Institute, The Johns Hopkins University School of Medicine.

Publications:

Frank, R.N.: Visual fields and electroretinography following massive argon laser photocoagulation for diabetic retinopathy. Arch. Ophthalmol. (in press).

Frank, R.N.: Diabetic Retinopathy, in Selected Topics on the Eye in Systemic Disease, Ryan, S.J., Jr. and Smith, R.E., eds. New York: Grune & Stratton, 1974, pp. 65-118.

Frank, R.N.: Argon laser photocoagulation and subretinal neovascularization. Ophthal. Surg. 5: 56-64, 1974.

Project No. Z01 EY 00141-02 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Biochemistry of Vertebrate Retinal Receptor Outer Segments

Previous Serial Number: NEI-73 CB 141(c)

Principal Investigators: Robert N. Frank, M.D.
Sheldon M. Buzney, M.D.

Other Investigators: William Robinson, Ph.D.
David Rodbard, M.D.

Cooperating Units: Laboratory of Physical Biology, NIAMDD
Reproduction Research Branch, NICHD
Atomic Pile Facility, National Bureau of Standards, Gaithersburg, Maryland

Man Years:

Total:	0.9
Professional:	0.9
Other:	0.0

Project Description:

Objectives: This project involves the study of the light-activated phosphorylation of visual pigment molecules in the retinal photoreceptors (rods and cones) involving adenosine triphosphate (ATP) and a particular enzyme system, or kinase, which catalyzes the reaction. Attempts are being made further to characterize the reaction, to determine if it occurs in living animals and, if so, to quantitate its extent, and to determine its role in the physiology of the visual process.

Methods Employed: Retinal rod and cone outer segments, containing the visual pigments, are isolated by standard differential centrifugation techniques. Measurements of various enzyme activities, including protein kinase and cyclic nucleotide phosphodiesterase, involve the addition of radioactively labelled substrates which undergo reaction. The reaction products are then separated from the unreacted material and are measured in a liquid scintillation counter. The visual pigment molecules can be separated from other components of the retinal outer segments by polyacrylamide gel electrophoresis in the presence of the detergent, sodium dodecyl sulfate and their specific radioactivity or other properties measured. Some

preliminary experiments have been performed to measure the visual pigment phosphorylation reaction in living animals (frogs) using neutron activation analysis, in which the inorganic phosphorus in the visual pigments isolated from the animal retinas is converted to the radioactive isotope, phosphorus-32, by bombardment with neutrons in an atomic pile. This avoids the need to administer large quantities of radioactive materials to the animals, and also has the theoretical possibility of allowing much better quantitation of the reaction in the intact animal.

Major Findings: A major focus of research this year has been an attempt to elucidate the mechanism of the light-activation of the phosphorylation reaction. Two possibilities have been examined: either the kinase enzyme itself is sensitive to light, or the enzyme has the same activity under all conditions of light and darkness, but the phosphorylation site on the visual pigment molecule is "hidden" in the dark, and is "exposed" in light. Previous experiments in this laboratory had suggested the second possibility, but were not felt to be conclusive. The experimental procedure utilized involved isolation of cattle retinal outer segments which had been prepared either under darkroom conditionsor in bright, room light ("bleached"), followed by extraction of most of the kinase activity by mild ultrasonic treatment, and centrifugation. The sedimented retinal material containing the visual pigments, could then be treated with various agents to destroy the remaining kinase without harming the visual pigments themselves. The extracted retinal kinase, or kinases from other tissues, could then be added back to the visual pigment preparations and incubations carried out in light or darkness to measure the extent of phosphorylation. Of a variety of kinases from several cattle tissues tested in this system, only that from the retina was capable of phosphorylating visual pigments. This specificity of the enzyme system is a strong argument that the phosphorylation has physiological significance. When the retinal kinase was added back to the visual pigment preparations, the phosphorylation reaction was stimulated by light even when the visual pigments, or the kinase, or both had been prepared under bleaching conditions. Preliminary measurements of the spectral sensitivity of this reaction suggest that it is stimulated maximally by light at 440 nm in the blue, and 600 nm at the red end of the spectrum and not at 500 nm, the wavelength of maximal absorption for rhodopsin, the visual pigment of the retinal rods. This indicates that, under the conditions of these experiments, retinal cone visual pigments may be responsible for the observed results.

Significance to Biomedical Research and the Program of the Institute: Recognition that visual pigments are phosphorylated in the light has stimulated considerable interest in this reaction as a possible clue to the mechanism of the visual process. While this study is primarily of importance in advancing our basic knowledge of the biochemistry and physicology of vision, it may also provide clues to the pathogenesis of clinical disorders of the retinal rods and cones, such as the various retinitis pigmentosa syndromes and the cone degenerations.

Proposed Course: Studies of visual pigment phosphorylation in isolated retinal receptor outer segments and in intact animals will be continued. During the past year also, initial experiments were carried out to determine if a relationship exists between retinal outer segment protein kinase and cyclic nucleotide phosphodiesterase activity. Since this offers a promising lead to the possible function of these enzymic reactions in retinal physiology, this work will be expanded.

Keyword Descriptors: visual pigments, rhodopsin, protein kinase, cyclic nucleotide phosphodiesterase, retinal rods and cones, liquid scintillation counting, polyacrylamide gel electrophoresis, differential ultracentrifugation, neutron activation analysis, sodium dodecyl sulfate

NEI Research Program: Retinal and Choroidal Diseases--Development, structure, Function, and Degeneration/Visual Pigments, Photoreceptors, and Visual Transduction Disorders

Experimental Subject or Tissue Source: Cattle/Frog (Rana pipiens)

Research Objective: Etiology

Honors and Awards: None

Publications:

Frank, R.N. and Buzney, S.M.: Mechanism and Specificity of Rhodopsin Phosphorylation, presented at Spring National Meeting, Association for Research in Vision and Ophthalmology, Sarasota, Florida, April 30, 1975.

Frank, R.N. and Rodbard, D.: Precision of sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the molecular weight estimation of a membrane glycoprotein: Studies on bovine rhodopsin. Arch. Biochem. Biophys. (in press).

Project No. Z01 EY 00143-02 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Study of the Use of Radioiodinated (I-125) Chloroquine Analog for the Differential Diagnosis and Detection of Intraocular Melanoma

Previous Serial Number: NEI-73 CB 143 (C)

Principal Investigators: Douglas E. Gaasterland, M.D.
Elmer J. Ballintine, M.D.
Carl Kupfer, M.D.

Other Investigators: None

Cooperating Units: Nuclear Medicine Department, CC
Radiopharmacy, CC

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To determine the value of using I-125 labelled chloroquine analog for the detection of ocular melanoma.

Methods Employed: These were outlined in project reports for the year 1974 and 1973 and have not been altered.

Major Findings: Since the previous report an additional ten patients have been enrolled in the study. Thus, 36 patients have been studied altogether. Some of the results were previously outlined. The current status in the test group is as follows: In eleven patients with choroidal melanoma which has been verified by enucleation and histopathologic examination, six tests have been positive and five have been negative. One patient with ocular melanosis and histopathologically verified malignant change has had a positive test. Three patients with histopathologically verified iris melanoma have had negative tests. Nine tests have been done in patients strongly suspected to have maglignant lesions on clinical grounds. Two of these tests have been positive and seven negative. One positive test occurred in an elderly person who refuges operative treatment for what is surely malignant melanoma. The second positive

test occurred in a patient whose lesion has been noted to be present for over ten years. This lesion has not changed during the period of observation and enucleation has not been advised in the case. The other seven tests have been negative; one of these patients has been advised to have enucleation for clinical reasons, but refuses. The other patients have remained under observation.

It was previously observed that two patients with monocular metastatic breast adenocarcinoma and one patient with a hemangioma as most likely diagnosis have had negative tests. Ten patients with various lesions which enter into the differential diagnosis of melignant melanoma have been tested in this study. During the past year the one patient who was added to this file was a patient with a phthisical eye. The patient was about to undergo enucleation. The test was negative. Subsequent histopathologic examination in his case did not disclose malignancy. Observation of the patients with suspicious lesions and some of the patients with benign lesions in the differential diagnosis continues.

An alternative protocol was prepared during the last part of the present year. In this protocol it was proposed that the relative effectiveness of the idochloroquine diagnostic test be assessed by performing both the iodochloroquine test and the P-32 test in the same patients. The equipment for performing beta radiation monitoring for the P-32 test was obtained. Radiation dosimetry calculations done for the modified protocol are of interest. The estimated total body dose is approximately 5.2 rads and the dose to the critical tissue, the bone marrow, liver and spleen, is approximately 37 rads. This protocol was submitted for consideration by the Radiation Safety Committee. An objection to pursuing the study was voiced. This is that this amount of radiation is in excess of permitted burdens for normal subjects. It was suggested that this burden be permitted only in cases wherein no other alternative diagnostic modality will fully identify the nature of the ocular lesion. It was pointed out that the dose of radioactive phosphorous (^{32}P) employed in the test (up to 700 microcuries) is approximately 1/3 of the dose used in the treatment of polycythemia, though polycythemia patients frequently require additional dose increments in the range of 2-3 millicuries. It was further pointed out that several studies indicate that acute myologenous leukemia occurs only in patients with polycythemia who have been treated with radioactive phosphorous.

The lesions of most patients enrolled in the present study are not undiagnosable. Under the guidelines suggested by the Radiation Committee only those patients in the highly suspicious group could be considered for the 32 phosphorous test. Therefore it is felt inappropriate to pursue the alternative protocol at this time.

Significance to Biomedical Research and the Program of the Institute: The conclusion regarding the I-125 chloroquine test last year is further substantiated by additional observations. The test has some use in differentiating patients with intraocular melanoma from other patients with benign lesions or metastatic disease. The test is not sufficiently sensitive to identify all patients with intraocular melanoma. There have still been no verified false positive tests. This test is attractive in that it employs a gamma radiation

emitter as the tracer isotope. It may be appropriate to devote some energy to refining this test or a modification of it.

Proposed Course: The enrollment period for the project as it has been described will terminate at the end of fiscal year of 1975. Appropriate patients will continue to be followed to define more clearly their course and the diagnosis of their lesions.

Keyword Descriptors: Ocular malignant melanoma, diagnosis, radioactive tracers, radioiodinated labelled chloroquine analog, ocular gamma radiation detection.

NEI Research Program: Retinal and Choroidal Diseases--Tumors

Experimental Subject or Tissue Source: Human

Research Objective: Diagnosis

Honors and Awards: None

Publications:

Gaasterland, D.E.: Evaluation of radiolabelled chloroquine in the diagnosis of ocular melanoma. Presented at the monthly ophthalmic seminar of Washington, D.C., 15 May 74.

Project No. Z01 EY 000154-02 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Experimental Glaucoma in the Rhesus Monkey

Previous Serial Number: NEI-73 CB 154(c)

Principal Investigators: Douglas E. Gaasterland, M.D.
Carl Kupfer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To study retinal and optic nerve function in the eyes of rhesus monkeys after glaucoma is caused by argon laser anterior chamber angle photocoagulation; to study effects of chronic elevation of intraocular pressure upon formation of aqueous humor; and to define in the same eyes the effect of argon laser photocoagulation upon outflow pathway structure and function.

Methods Employed: Circumferential argon laser photocoagulation applications are made confluently to the trabecular meshwork area of the eye through a modified Koeppe lens. In most animals one eye is treated, the other eye serves as an untreated control. Animals are examined externally with slit lamp, with applanation pressure measurements, and with fundoscopy at intervals, before treatment and after treatment. Observations of the anterior chamber angle and the fundus are routinely recorded by photography. The status of the outflow tract is determined physiologically by anterior chamber perfusion with a constant pressure method, a modification of the method used by Grant for enucleated eyes. Following perfusion in four animals the anterior chamber angles have been examined histopathologically throughout the circumference. Studies of aqueous production have not been initiated. Studies of retinal and optic nerve have been performed with histopathologic methods. Preparations to perform autoradiography of retinal ganglion cell function and to assess axoplasmic flow have been made and initial experiments utilizing

tritium labelled leucine, to assess the second and third component of fast axoplasmic flow have been started.

Major Findings: Both eyes from four monkeys have been studied extensively, histopathologically to assess the status of the anterior chamber angle and to assess the status of the retina and optic nerve. Repeated argon laser photocoagulation of the monkey trabecular meshwork causes scarring, with obliteration of the canal of Schlemm. In perfusion studies of the same eyes the outflow facility was greatly diminished in treated eyes; it was normal in untreated eyes and in one eye treated once. This observation in the latter eye agreed with the fact that no elevation of intraocular pressure had occurred in this eye. A review revealed that in 66% of sessions, when the anterior chamber was treated some bleeding occurred from the region of the trabecular meshwork immediately after application of photocoagulation energy. No correlation could be defined between the occurrence of bleeding and whether or not the intraocular pressure subsequently became elevated. In addition there was no relation between the occurrence of bleeding and previous experiences with bleeding in the same eye. Of 16 eyes 15 bled at least once, 8 bled twice, none bled three times (out of 6 that had either three or four treatments). In one eye that had only one treatment there was no bleeding. This was not generally the case with first treatments. In a total of 16 first treatments, there was a 50% incidence of bleeding. This observation, of bleeding, is important because it occurs only when the photocoagulation energy penetrates through the trabecular structures to the interior of the canal of Schlemm, allowing a pathway to be open for blood, from the interior of the canal of Schlemm to the interior of the eye.

The histopathologic changes within the retina and optic nerve after the onset of experimental glaucoma have been previously described and have been corroborated by additional studies during the past year.

Several studies have been performed in both normal and glaucoma eyes during the past year to serve as the basis for the studies of axoplasmic flow. A series of injections have been done to determine the ocular rigidity of the monkey eye. When injections are done to the anterior chamber, the average rigidity is 0.020 with a range from 0.016 up to 0.024. This has been determined by injections of 10 microliter volumes to four eyes. In two eyes, with normal intraocular pressure and outflow facility the pressure rise induced by an intravitreal injection was assessed. A 25 microliter volume to the vitreous cavity in either eye elevated the pressure from the mid to high teens up to the high fifties. Ocular rigidity was approximately 0.020. A 50 microliter injection to the vitreous cavity in one eye raised intraocular pressure from the mid teens to the level of 90. The interval before the intraocular returned to the pre-injection level varied from 3 1/2 to 15 minutes in these experiments.

These observations define the necessity to concentrate commercially available solutions of tritiated amino acids before they can be used for autoradiography. Tritiated leucine is available as 5 millicuries in

5 milliliters. In conjunction with Dr. Shichi and Dr. O'Brien of the NEI, Laboratory of Vision Research, it was possible to dehydrate one specimen to dryness by lyophilization and then to resuspend the amino acid in a 0.5 ml. volume of sterile injectable saline. Analysis of this sample revealed that the activity was now 8.2 microcuries per microliter. The pH was determined by the use of pHydrion paper and was determined to be in the range of 6.8 to 7 pH units. Since autoradiography is done by installing approximately 100 microcuries of tritiated amino acid into the eye, using the solution as prepared will allow two injections each of a six microliter volume for each eye.

In one monkey with binocular glaucoma, before ^{3}H-leucine injection, the intraocular pressure of the right eye was 38, left eye was 36. This animal had had glaucoma in both eyes for 450 days. The injections were made in both eyes and the animal returned to his cage. Twenty-four hours later the intraocular pressure in both eyes was 14 mmHg. The animal was sacrificed and the eyes obtained for autoradiographic study. The second animal had had high elevation of intraocular pressure in the right eye only, for approximately 90 days. In the left eye the pressure had been intermittently elevated to the low 20's for the same period of time. On the day of injection the right eye pressure was 44, the left eye pressure was 14 before injection. Twenty-four hours after injection the right eye pressure was 21 and the left eye pressure was 13 mmHg. The animal was then sacrificed and the eyes obtained.

In the four eyes obtained for autoradiography, an assessment of fixative radioactivity has been made. The total radioactivity in the bottle of 10% formalin used as fixative is approximately 1/100 of that injected into the eye 24 hours before the eye was enucleated. The radioactivity in the fixative bottle did not increase after the eye had been opened and returned to the same bottle of fixative. When the specimens were changed to 60% ethanol an additional elution of one microcurie of radioactive tritium occurred. The conclusion with regard to this study is that radioactive elution from the specimens occurs; this will necessitate handling of specimens as if they are radioactive and will necessitate use of separate facilities from those used for routine histopathologic examination.

Significance to Biomedical Research and the Program of the Institute:
All parts of this work have clinical applicability.

1. This method of creating experimental glaucoma provides a mechanism whereby retinal and optic nerve function can be studied in eyes with chronic elevation of intraocular pressure. This will allow additional insight into the mechanism whereby visual function is lost in the patient with glaucoma.

2. The production of aqueous humor in the eye with elevated intraocular pressure is probably similar in magnitude and mechanism to that in the eye with normal intraocular pressure. That this happens and how it happens has never been studied. This model offers an opportunity to pursue this.

3. Practicing clinical ophthalmologists have been urged to consider

treating glaucoma by argon laser photocoagulation of the structures in the anterior chamber angle. The observation that in monkeys this treatment causes scarring and obliteration of the outflow pathways is important, and indicates that such treatment should be undertaken only cautiously and only with appropriate controls to determine the efficacy.

Proposed Course: Additional studies using this model will be continued. A study of the effects of cyclocryothermy in the normal and in the eye with experimental glaucoma of rhesus monkeys has been planned. Axoplasmic flow studies will continue. An assessment of the magnitude of intraocular pressure parameters related to aqueous formation by ultrafiltration is planned.

Keyword Descriptors: glaucoma, experimental model, trabecular meshwork, argon laser photocoagulation, autoradiography, axoplasmic flow, aqueous humor production, aqueous humor outflow, anterior chamber perfusion, histopathology, visual function, ocular vessel perfusion

NEI Research Program: Glaucoma--Primary Glaucoma (Open-Angle Glaucoma)/ Secondary Glaucoma

Experimental Subject or Tissue Source: Rhesus Monkey (Macacca Mulatta)

Research Objective: Etiology, Treatment

Honors and Awards: None

Publications:

Gaasterland, D., and Kupfer, C.: Experimental glaucoma in the rhesus monkey. Invest. Ophthalmol. 13: 455, 1974.

Gaasterland, D., and Kupfer, C.: The effects of low power, long duration argon laser photocoagulation on the anterior chamber angle of the rhesus monkey. Presented at the ARVO Atlantic Section Meeting, November 2, 1974.

Project No. Z01 EY 00030-04 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Studies of Parameters of Intraocular Pressure

Previous Serial Number: NEI-71-CB 030(c)

Principal Investigators: Douglas E. Gaasterland, M.D.
Carl Kupfer, M.D.

Other Investigators: Karyn Ross
Lessie McCain
Phyllis Aaron
Roy Milton, Ph.D.

Cooperating Units: Normal Volunteer Officer, CC, NIH
Pharmaceutical Development Service, NIH
Biomedical and Engineering Instrumentation Branch, DRS, NIH
Electronic Technician, NEI
Office of Biometry and Epidemiology, NEI

Man Years:

Total:	2.8
Professional:	0.5
Other:	2.3

Project Description:

Objectives: This is a continuing study of the parameters of intraocular pressure in both young and older normal subjects as well as patients with ocular hypertension and glaucoma. The areas of interest are first, the actual values of the parameters and second, the effect upon the parameters of both acute anc chronic administration of medications which might be used in the treatment of glaucoma.

Methods Employed: Eight parameters--intraocular pressure, episcleral venous pressure, total facility, true facility of outflow, pseudofacility, aqueous humor flow, P_k of Goldmann, and the ocular rigidity, are determined before and after medication is given topically or systemically. This allows assessment of acute effects of medication. Replicate measurements on sophisticated subjects are made. Chronic studies of the effects of topical

dexamethasone 0.1% given four times a day to one eye with the other eye being an untreated control have been done. Similar chronic studies, in patients with ocular hypertension, have been initiated on the effects of pilocarpine. Dose response studies with catecholamines and acute effects of medication combinations have been emphasized during the past year.

Major Findings: In the eyes of older subjects 2.0% epinephrine appears, in preliminary analysis, to have an effect upon the outflow facility. This was also the case in the observations on younger subjects. Studies to more clearly define the dose response characteristics in both younger and older subjects are continuing.

In both younger and older subjects the effects of combinations of medications of parameters have been initiated. The combination of acetazolamide (given systemically) with topical isoproterenol has been studied in nine young subjects. The addition of isoproterenol to one eye when both eyes receive acetazolamide results in a significant additional lowering of intraocular pressure in the eyes which receive both medications. In most subjects this seems to be caused by an additional reduction of the aqueous production, whereas in some subjects the combination appears to have an effect of lowering the resistance to aqueous outflow. This latter observation is unexpected and difficult to explain. Other combinations under study include pilocarpine with epinephrine, pilocarpine with isoproterenol, pilocarpine with norepinephrine, acetazolamide with norepinephrine, acetazolamide with pilocarpine and acetazolamide with epinephrine. Some attention is being given to the effects of neosynephrine.

In cooperation with the Office of Biometry and Epidemiology considerable effort was directed to assessing whether by plotting the determined value for pseudofacility versus a combined parameter made up of multiples of the intraocular and episcleral venous pressure against total facility and true facility of outflow (respectively), it would be possible to separate the rate of aqueous production due to secretion from that due to ultrafiltration. Should the separation be possible, the effects of medications such as acetazolamide and the effects of aging upon aqueous production might be analyzable. This effort met with frustration due primarily to the large number of variables under analysis, each with large standard deviations. No significant interpretation was possible.

Modifications of a fluorophotometer as designed by Maurice were completed during the year. Assessment of the status of the instrument indicates that it gives meaningful numbers which represent the fluorescein concentration within the anterior portion of the eye. With this instrument it will be possible to evaluate aqueous turnover in the anterior chamber in vivo, non-invasively. Initial studies in patients have indicated a lack of agreement between the fluorescein turnover and the turnover calculated from the Goldmann equation. In order to assess the accuracy of the turnover evaluated from fluorescein readings a series of experiments have been undertaken in isolated, enucleated eyes which are connected to an exogenous flow source at a known rate.

Finally, during the past year, renovations have been completed to the laboratory facilities where patients and normal volunteers are seen. This extensive work essentially shut down the operations for a three months period of time. During the last half of the year an additional person has joined the technical staff to work on the project. She is learning to perform measurements of parameters of intraocular pressure. During the past year there have been approximately 325 visits by patients or normal volunteers to the facilities. The majority of these visits have been for three hour experiment sessions.

Significance to Biomedical Research and the Program of the Institute: Study of the patterns of alteration of parameters of intraocular pressure allows a clearer interpretation of the nature of the mechamisms of the action of pharmacologic agents used to treat glaucoma. This allows identification of desirable and undesirable properties of various pharmacologic agents. The additive effects of combinations of agents can be studied. This will hopefully allow development of agents or combinations having only desirable properties.

Proposed Course: This project will continue with an extension of studies in the areas outlined. There will be continued emphasis on incorporating into the studies patients with glaucoma or with ocular hypertension as well as additional studies in normal eyes.

Keyword Descriptors: glaucoma, pharmacology, anti-glaucoma medications, aqueous humor dynamics, parameters of intraocular pressure.

NEI Research Program: Glaucoma--Primary Glaucoma/Secondary Glaucoma

Experimental Subject or Tissue Source: Human

Research Objective: Etiology, Treatment, Diagnosis

Honors and Awards: None

Publications:

Gaasterland, D.: Effects of cycloplegia upon responses to pilocarpine. Presented at Fifth Biannual Walter Reed Post-Graduate Course and Alumni meeting. April 8, 1974

Gaasterland, D., Kupfer, C., and Ross, K.: Additive affects of isoproterenol combined with acetazolamide on parameters of intraocular pressure. Presented at the Meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Florida, April 30, 1975.

Project No. Z01 EY 00006-04 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Design and Construction of Ophthalmic Instruments; Research in Methods of Evaluating Visual Processes

Previous Serial Number: NEI-71 CB 006(c)

Principal Investigator: Ralph D. Gunkel, O.D.

Other Investigators: Donald R. Bergsma, M.D.
Mary E. Hendricks

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	1.1
Other:	0.1

Project Description:

Objectives: Broad objectives remain as before, being the continued improvement of psychophysical tests for visual functions. Obviously the ultimate goal would be the replacement of all subjective tests with objective methods for threshold measurement. Since this goal does not yet appear to be readily attainable, we attempt to reduce the subjective element by simplification or further confirmation.

A further objective is to adapt and contribute any devices, skills, ideas or materials to other projects and investigators in the Clinical Branch, at their invitation.

Methods Employed: In collaboration with Clinical Associates, appropriate patients are seen, often serially, for the measurement of visibility and chromatic thresholds under different conditions. Electroretinograms and electro-oculograms are obtained where indicated, but while being objective, they are of limited usefulness.

Problems are discussed with either clinical or laboratory investigators and when potential solutions appear, help may be given in their implementation.

Major Findings: Psychophysical tests have been done on 390 patients, most

of which were being studied for the various types of toxic or degenerative retinopathies. This includes dark adaptation measurements in four quadrants in 45 candidates for the vitrectomy project of Drs. Charles and Eichenbaum and color vision test on 15 patients in Dr. Cogan's vincristine protocol. About 20 electro-oculograms have been performed.

A paper entitled, "A Ganzfeld Stimulator for Electroretinography" was presented at the last meeting of the Association for Research in Vision and Ophthalmology and a related article has been accepted for publication in the AMA Archives of Ophthalmology.

A modified chromaticity diagram has been proposed for clinical delineation of color defects, with an appropriate instrument for quickly and easily measuring and plotting them on triangular coordinates. A few optical and/or electrical problems remain to be solved, but they do not seem formidable, and preliminary demonstrations of the present instrument suggest that it may provide a much-needed means for determining and describing all types of color defects. This has not been reported before.

Several components have been designed and constructed for the pupillograph of Dr. Kollarits.

A light-funnel and fiber-optics conductor has been made for transilluminating the iris in photography, but will probably be redesigned.

The electroretinography apparatus was redesigned to include a completely separate transistorized unit for optional use.

Several suction cups of different size and form were made for Dr. Frank in testing conjunctival capillary fragility.

Numerous other small parts or components were made or suggested for other investigators.

Significance to Biomedical Research and the Program of the Institute: Functional testing of patients is frequently a major factor in medical management and genetic counselling, both of which are vital to the clinical program.

The new system for measuring and plotting defects in color vision shows some promise of becoming a major contribution in that field.

Clinical and laboratory investigators appear to be grateful for any benefits resulting from consultations.

Proposed Course: It is proposed that this project be continued in its present flexible form.

Keyword Descriptors: psychophysical, subjective, objective, visibility

thresholds, chromaticity, dark adaptation, retinopathy, Ganzfeld, electro-oculogram, pupillograph, vitrectomy, electroretinography

NEI Research Program: Retinal and Choroidal Diseases--Development, Structure, Function, and Degeneration/Macular Diseases; Sensory and Motor Disorders of Vision--Congenital, Developmental, and Degenerative Abnormalities/Visual Sensory and Perceptual Disorders/Sensory and Motor Disorders Related to Specific Disease Processes

Experimental Subject or Tissue Source: Human

Research Objective: Etiology, Treatment, Diagnosis

Honors and Awards: None

Publications: None

Project No. Z01 EY 00018-01 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Ophthalmologic Screening for Metastatic Lesions to the Eye

Previous Serial Number: None

Principal Investigator: Muriel Kaiser-Kupfer, M.D.

Other Investigators: Joan Bull, M.D.

Cooperating Units: MBS, MO, OCT, NCI

Woman Years:

Total:	.1
Professional:	.1
Other:	0

Project Description:

Objectives: To determine the incidence of metastatic eye disease in patients with metastatic breast carcinoma, to evaluate the effects of irradiation on ocular tumors which threaten central vision and monitor the effects of irradiation on the eye, and to evaluate effectiveness of the hormonal manipulations and chemotherapy on ocular metastasis in relation to systemic effect on tumor.

Methods Employed: All NEI/MBS patients having metastatic breast carcinoma are examined ophthalmoscopically. Those patients having metastatic disease to the eye are then followed frequently as indicated. The course of the ocular metastatic disease is followed with serial color and infrared fundus photography, Goldmann perimetry and fluorescein fundus photos when indicated.

Major Findings: To date approximately 45 patients have been seen and of those approximately ten patients have had evidence of ocular metastasis.

Significance to Biomedical Research and the Program of the Institute: The response of choroidal metastatic lesions to cancer chemotherapy could serve as an indication of response of metastatic disease elsewhere in the body.

Proposed Course: To continue for one additional year.

Keyword Descriptors: breast carcinoma, metastatic disease, choroidal metastasis

NEI Research Program: Retinal and Choroidal Diseases--Tumors

Experimental Subject or Tissue Source: Human

Research Objective: Treatment, Diagnosis

Honors and Awards: None

Publications: None

Project No. Z01 EY 00011-01 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Pigmentary Dispersion With and Without Glaucoma

Previous Serial Number: None

Principal Investigator: Muriel Kaiser-Kupfer, M.D.

Other Investigators: Carl Kupfer, M.D.
Kenneth Foon, M.D.
Carol Kollarits, M.D.

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objectives: To compare patients having pigmentary dispersion with and without glaucoma by documenting and following the clinical features and course of their disease, and evaluating the patient's performance on a variety of diagnostic tests. To determine the presence of abnormal aqueous humor dynamics using provocative testing in those patients having pigmentary dispersion without glaucoma. To compare pigmentary dispersion with and without glaucoma with respect to possible genetic markers (i.e. lymphocyte transformation, PTC taste testing and family history of open-angle glaucoma). To determine whether pupillary dynamics to light stimulation are abnormal in cases having iris transillumination.

Methods Employed: At the first visit, the following examinations are performed:

Complete family history with detailed pedigree
Best corrected visual acuity with manifest refraction
Slit lamp examination
Visual field examination (Goldmann I_{2_e} and I_{4_e})
PTC Taste-testing
Applanation Goldmann tension (app)

Photography of iris transillumination
Goniophotography

At the next visit, the following examinations are performed:

Lymphocyte transformation test (LTT)
Base-line tonography and water-drinking tonography one hour later

At the third visit, the following examinations are performed:

Slit lamp photography of Krukenberg spindle
Dilated ophthalmoscopic examination (10% phenylephrine and 1% cyclogel)
Stereophotographs of the optic nervehead

At the fourth visit, pupillography is performed.

Major Findings: Patients may have pigment dispersion syndrome for as long as 15 years with developing glaucoma.

There may be a hereditary predisposition in some cases as seen in mother and daughters and two brothers.

The steroid testing and PTC taste testing do not appear to show any particular categorization of these patients.

It may be noted with or without filtering procedures pigment may be lost from the trabecular meshwork in time.

Significance to Biomedical Research and the Program of the Institute: These data may enable a determination to be made of the risk of patients having pigmentary dispersion to develop glaucoma. In addition, it may be possible to identify which features of these determinations may have predictive value in forcasting those patients having pigmentary dispersion who may develop a field defect. In addition, the relationship of "pigmentary" glaucoma to the known characteristics of open-angle glaucoma can be investigated.

Proposed Course: Four more years.

Keyword Descriptors: pigment dispersion syndrome, pigmentary glaucoma

NEI Research Program: Glaucoma--Developmental Glaucoma/Secondary Glaucoma

Experimental Subject or Tissue Source: Human

Research Objective: Treatment, Diagnosis

Honors and Awards: None

Publications: None

Project No. Z01 EY 00022-01 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: The Urokinase Central Retinal Vein Occlusion Trial

Previous Serial Number: None

Principal Investigator: Carol R. Kollarits, M.D.

Other Investigators: David C. Allen, O.D., M.A.
Frederick L. Ferris, M.D.
William Bell, M.D.
Harvey R. Gralnick, M.D.
Joseph C. Frantantoni, M.D.
Elmer J. Ballintine, M.D.
Martin L. Fishman, M.D.

Cooperating Units: Office of Biometry and Epidemiology, NEI
Division of Blood Diseases and Resources, NHLI
Department of Medicine, Johns Hopkins University Hospital
Clinical Pathology Department, NIH Clinical Center

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: Urokinase is a new drug for dissolving blood clots which has been shown to be effective in treating pulmonary emboli and will probably become available to American physicians within the next several years. Urokinase may be useful in the treatment of an eye disease known as central retinal vein occlusion. Patients with this disease usually have only one eye affected, but more than 90% of such eyes will end up with sufficient visual loss, and as many as 30% of these eyes may develop neovascular glaucoma and eventually require surgical removal for pain. Because treatment with urokinase exposes the patient to some risk of bleeding complications, it would be desirable to conduct a controlled randomized clinical trial to determine if the potential benefits to be gained from treatment of central retinal vein occlusion with urokinase justify the risk of this therapy.

Methods Employed: Patients with central retinal vein occlusion will be referred by their personal ophthalmologists to the Eye Clinic at the Clinical Center. Blood tests will be made and each patient will be examined to determine if he or she has any contraindications for use of urokinase or anticoagulant drugs. If the patient is a candidate for the study and gives informed consent to participate, then he or she will be randomized into one of three possible treatment groups: 1) Unmedicated intravenous solution for 14 days, 2) Intravenous heparin for 14 days, or 3) Urokinase for 24 hours followed by intravenous heparin for 13 days. Except for the content of the intravenous solutions, all patients will receive identical care while hospitalized in the Clinical Center Eye Ward. Visual acuity and visual field examinations will be made by a masked technician and the patient will be followed at intervals by an ophthalmologist.

Major Findings: This study is scheduled to start on May 5, 1975.

Significance to Biomedical Research and the Program of the Institute: There is no known effective treatment for central retinal vein occlusion. It is hoped that this study will answer the question whether or not urokinase therapy can improve the visual outcome in eyes with central retinal vein occlusion, or reduce the incidence of neovascular glaucoma.

Proposed Course: It is anticipated that enough patients to complete the study will be recruited in three or four years.

Keyword Descriptors: central retinal vein occlusion, urokinase, anticoagulation, heparin, thrombolysis

NEI Research Program: Retinal and Choroidal Diseases--Vascular and Circulatory Abnormalities-Including Disturbances in Blood Vessel Formation

Experimental Study or Tissue Source: Human

Research Objective: Etiology, Treatment, Diagnosis

Honors and Awards: None

Publications: None

Project No. Z01 EY 00021-01 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Videotape Pupillometry

Previous Serial Number: None

Principal Investigator: Carol R. Kollarits, M.D.

Other Investigators: Frank J. Kollarits, Ph.D.
Muriel I. Kaiser-Kupfer, M.D.
William Schutte
William Whitehouse

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS and Television Engineering Section, ADM, CC

Man Years:

Total:	.01
Professional:	.01
Other:	.00

Project Description:

Objectives: To develop a video tape system for recording the pupil movements of both eyes of patients. In the past few years infrared television and integrated circuit techniques have become available which makes possible the constructions of pupillometer which will record the size of a patient's pupils on video tape. The tape is then analyzed automatically and a record of the time course of pupillary changes is produced.

Methods Employed: In cooperation with the Biomedical Engineering and Instrumentation Branch, DRS and the Television Engineering Section, CC an infrared video tape pupillometer is now being developed. The instrument is not yet perfected, but is already useful for recording clinical findings in patients with pupil abnormalities.

Major Findings: Pupil dynamics in a series of patients with pigmentary dispersion syndrome have been investigated with the videotape pupillometer. The pupillometric findings have correlated well with the known defect in the dilator muscle of the iris that has been described in patients with pigmentary dispersion syndrome.

Significance to Biomedical Research and the Program of the Institute: Videotape recordings made of abnormal pupil dynamics are useful for teaching purposes. In addition, the recordings obtained from this device will be useful in detecting and analyzing the abnormalities of the components of pupillary responses that accompany several neurologic and intrinsic eye diseases. Usually pupil activity is too rapid for detailed analysis by simple inspection. This pupillometer will make detailed analysis a straight forward clinical procedure.

Proposed Course: Development of the videotape pupillometer will continue in an effort to make this a clinically useful research tool. The automatic measurement and graphing of the pupil changes and their differentiation by non-optical, electrical techniques has been demonstrated. The videotape pupillometer will be used to evaluate as many patients as possible with pupillary abnormalities.

Keyword Descriptors: videotape, pupillogram, pupil abnormalities, pigmentary dispersion syndrome

NEI Research Program: Sensory and Motor Disorders of Vision--Optical and Pupillary Disorders

Experimental Subject or Tissue Source: Human

Research Objective: Etiology, Diagnosis

Honors and Awards: None

Publication: None

Project No. Z01 EY 00014-01 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Applications of Surgical Vitrectomy

Previous Serial Number: None

Principal Investigator: Carol R. Kollarits, M.D.

Other Investigators: Martin Fishman, M.D.
Ronald G. Michels, M.D.

Cooperating Units: The Wilmer Eye Institute
Johns Hopkins Hospital
Baltimore, Maryland

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: Vitrectomy is a new operation, developed within the last several years, for removing the vitreous gel within the eye. This surgical technique may be employed in diabetics with vitreous hemorrhage, patients with intraocular infections, patients with severe eye injuries, and patients with uveitis involving the vitreous. We will define the conditions in which vitrectomy will be useful by performing careful clinical trials in human patients with diseased vitreous. Technical problems are being investigated in animals before the experience gained will be applied to human patients.

Methods Employed: Adequate facilities for vitrectomy are available in the human operating room and in the animal operating room at the National Eye Institute. These instruments are equipped with a special suction and cutting mechanism that has been miniaturized so that it can be inserted into the eye through an opening no larger than 4 mm. These instruments can be used to cut off and suck out diseased vitreous from inside the eye.

Major Findings: Our experience in rabbit vitrectomies indicates that removal of the lens at the same time that the vitrectomy is done predisposes some eyes to a higher risk of complications.

Significance to Biomedical Research and the Program of the Institute: Technical advances in miniaturization have made vitrectomy instruments possible. It is not yet clear, however, in just which cases vitrectomy will be a useful operation for restoring vision. Its application to eyes threatened with blindness from diabetes offers hope for favorably influencing the visually disastrous course of this disease.

Proposed Course: Humans with diseased vitreous (e.g. diabetics with visual loss due to longstanding vitreous hemorrhage) will be admitted to the Clinical Center for vitrectomy. Investigations of the material obtained at vitrectomy will be performed. Animal experiments are now being undertaken to answer the question of whether or not cataract should be removed at the same operation. Other animal experiments are being planned to investigate the place of vitrectomy in treatment of endophthalmitis and ocular trauma.

Keyword Descriptors: vitrectomy, diabetic vitreous hemorrhage, endophthalmitis, ocular trauma, traction retinal detachment

NEI Research Program: Retinal and Choroidal Diseases--Retinal Detachment and Vitreal Abnormalities

Experimental Subject or Tissue Source: Human/Rabbit/Monkey

Research Objective: Etiology, Treatment, Diagnosis

Honors and Awards: None

Publications: None

Project No. Z01 EY 00019-01 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Vitrectomy Perfusion Solutions

Previous Serial Number: None

Principal Investigator: Carol R. Kollarits, M.D.

Other Investigators: Martin Fishman, M.D.
Jin Kinoshita, Ph.D.
Henry Fukui, Ph.D.
Ikuo Mikuni, M.D.

Cooperating Units: Laboratory of Vision Research, NEI

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: During vitrectomy, it is necessary to flush the inside of the eye with a constant infusion of a solution to prevent the eye from collapsing and to wash away any blood. We have determined that the most commonly used solution for vitrectomy operations, (Ringer's injection) causes cataracts in monkey lenses. We are now developing a perfusion solution to be used during vitrectomy operations that will maintain the clarity of the lens.

Methods Employed: Monkey lenses are obtained from adult rhesus monkeys sacrificed for polio virus vaccination studies. The lenses are incubated in different solutions and observed for 48 hours to determine whether the lens becomes cataractous or remains clear in each solution.

Major Findings: One hundred percent of monkey lenses incubated in Ringer's injection become cataractous within several hours. A complicated solution containing glucose, bicarbonate, glutathione and adenosine maintained the clarity of the lens for several days, but this solution is difficult to make up for routine use and is not yet available for practicing ophthalmologists to use. We are now trying to determine if a simpler solution may give equally good results.

Significance to Biomedical Research and the Program of the Institute: Although the biochemistry of many lower animal lenses has been investigated extensively, very little work has been done on the conditions necessary to preserve the clarity of either the monkey or the human lens. If a perfusion solution can be found that will not damage the lens during vitrectomy, use of this solution may improve the results of vitrectomy surgery.

Proposed Course: Continued laboratory investigation will determine the best solution for lenses cultured in an incubator. This solution then will be used in vitrectomy surgery on living rhesus monkeys. If the new solution proves superior to solutions now in use for vitrectomy, the new solution may be used on human patients during vitrectomy surgery.

Keyword Descriptors: perfusion solutions, intraocular surgery, lens toxicity, lens clarity, biochemistry, monkey lens, human lens

NEI Research Program: Cataract--Lens Structure, Function, and Metabolic, Toxic and Traumatic Cataract

Experimental Subject or Tissue Source: Monkey

Research Objective: Etiology, Treatment, Diagnosis

Honors and Awards: None

Publications: None

Project No. Z01 EY 00013-04 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Study on the Pharmacodynamics of Various Agents Affecting the Intraocular Pressure

Previous Serial Number: NEI-71 CB 013(c)

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: To determine the pharmacodynamics of agents able to alter the intraocular pressure with a view toward finding more effective compounds and possibly to furthering the understanding of mechanisms which maintain the intraocular pressure.

Methods Employed: Studies are made on the enucleated, arterially perfused cat eye. Perfusate is channeled through the ophthalmic artery to nourish the entire eye or a ligature is placed around the optic nerve at its insertion, so that only the anterior segment of the eye is perfused. Drugs and other test substances are added to individual bottles of perfusate fluid which can then be introduced into the system by stopcock control. Temperature and rate of arterial flow are easily regulated. The rate of aqueous humor formation was estimated by determining the rate of decay of intracamerally injected I^{125} tagged serum albumin. Additional, intact anesthetized monkeys were also studied for their ocular responses to administered drugs.

Major Findings: We have reported that acetazolamide and ouabain lowers aqueous humor formation in the enucleated arterially perfused cat eye by a vascular mechanism, affecting ultrafiltration. To determine if this mechanism applied to primates as well, the anesthetized monkey was studied. After a number of attempts, a drug was found (phencyclidine) which inhibited the

aqueous humor actions of acetazolamide without any notable action on the systemic circulation. Studies currently in progress indicate rather strongly that phencyclidine inhibits the action of acetazolamide in the primate, by a mechanism identical to that reported for the cat eye.

The studies relating the intra-ocular neurogenic pathway for the formation of aqueous humor have been completed. The proposed pathway proceeds from a synapse in the ciliary ganglion to a second synapse (intra-ocular) which has the properties of an E-2 (Nicotinic) receptor of a sympathetic ganglion. From the latter synapse, the nerve fiber continued to the posterior portion of the ciliary processes where norepinephrine is released from the nerve terminals to produce vasoconstriction.

Studies are continuing to clarify the neurogenic connection (if any) to the CNS of another type (E-1) of receptor. In the cat eye, the stimulation of this receptor has been reported to decrease the rate of aqueous humor formation.

Significance to Biomedical Research and the Program of the Institute: All drugs studied to date, which have been determined to decrease the rate of aqueous humor formation have been found to act via a vascular mechanism. These data introduce serious questions as to the validity of current concepts that aqueous humor is formed by secretion or that secretory processes are inhibited by certain pharmacologic agents to cause a reduction of aqueous humor formation.

The neurogenic pathway, described for the production of aqueous humor, emanates from the brain and is thus strong evidence of a central control of this function.

Proposed Course: Work will proceed to determine if intraocular E-1 (muscarinic) type receptors (which lower aqueous humor formation rate) communicate with the brain or whether they may represent an arc of an axonal reflex.

Studies will continue of drug effects on intra-ocular ganglion-like receptors in order to understand better these receptors and perhaps to uncover agents with greater efficacy for the treatment of glaucoma.

Keyword Descriptors: ouabain, acetazolamide, ganglion-like receptors, aqueous humor, inflow, ultrafiltration, eye

NEI Research Program: Glaucoma--Primary Glaucoma

Experimental Subject or Tissue Source: Cat/Monkey

Research Objective: Etiology

Honors and Awards: None

Publications:

Macri, F.J. and Cevario, S.J.: Ciliary ganglion stimulation. I Effects on aqueous humor inflow and outflow, Invest. Ophthalmol. 14: 28-33, 1975.

Macri, F.J. and Cevario, S.J.: A possible vascular mechanism for the inhibition of aqueous humor formation by ouabain and acetazolamide. Exp. Eye Res. (in press).

Macri, F.J. and Cevario, S.J.: Ciliary ganglion stimulation. II Neurogenic, intra-ocular pathway for excitatory effects on aqueous humor production and outflow. Invest. Ophthalmol. (in press).

Project No. Z01 EY 000153-02 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

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

Project Title: Ciliary Body Blood Flow and Aqueous Humor Formation in the Rhesus Monkey

Previous Serial Number: NEI-73 CB 153(c)

Principal Investigator: Karyn Ross

Other Investigators: Frank Macri, Ph.D.
Helen MacLellan

Cooperating Units: Bureau of Biologics, Division of Pathology, FDA

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: During this year the objective of this project has been modified to emphasize the relationship of ascorbic acid concentration in the posterior chamber as compared to that in the anterior chamber.

Methods Employed: The concentrations of ascorbic acid in the posterior and anterior chambers and in the serum are determined by titration with 2,6-dichlorophenol indophenol dye.

Major Findings: In monkeys sedated with phencyclidine hydrochloride, the ascorbic acid concentration in the posterior chamber is higher than that in the anterior chamber. Monkeys anesthetized with pentobarbital sodium also have a higher ascorbic acid concentration in the posterior chamber than the anterior chamber. After pentobarbital the posterior chamber concentration exceeds that in those animals sedated with phencyclidine hydrochloride. The mean concentration of ascorbic acid in the anterior chamber of animals, sedated with sernylan is equal to that of animals sedated with pentobarbital.

The diffusion coefficient calculated taking into account the effect of anesthesia on the formation rate of aqueous humor, is the same for each group of animals. In the rhesus monkey, the diffusion coefficient for ascorbic acid is considerably less than that reported for the rabbit by Becker.

Significance to Biomedical Research and the Program of the Institute: The definition of this anterior chamber-posterior chamber ascorbate ratio allows a more accurate determination of plasma flow through the ciliary body. The relation of this flow to the rate of aqueous humor formation, can be more accurately studied. Understanding of aqueous formation is important in the management of glaucoma patients.

Proposed Course: This project will continue through next year.

Keyword Descriptors: ascorbic acid, anterior chamber, posterior chamber, diffusion coefficient, aqueous humor, serum

NEI Research Program: Glaucoma--Primary Glaucoma (Open-Angle Glaucoma)--Secondary Glaucoma

Experimental Subject or Tissue Source: Rhesus Monkey (Macaca Mulatta)

Research Objective: Etiology, Treatment, Diagnosis

Honors and Awards: None

Publications: None

Laboratory of Vision Research

ANNUAL REPORT
LABORATORY OF VISION RESEARCH
July 1, 1974 - June 30, 1975

REPORT OF THE CHIEF, LABORATORY OF VISION RESEARCH

Jin H. Kinoshita, Ph.D.

The rich research environment that prevails at NIH has been responsible for initiating a number of projects pertinent to clinical problems. These projects result from the interaction on the investigators of the Laboratory of Vision Research and those of other Institutes. In addition, the unique facilities at NIH greatly enhance the research projects in several ways. A good example of such a project is the immunological study involving Dr. Helmsen of the LVR. The NIH scientists have access to the Poolesville farm where large animals such as horses, sheep and goats are available for preparation of antisera to specific antigens. This facility has proved extremely helpful to scientists involved in immunological studies. Not many research centers exist that have comparable facilities.

Another advantage at NIH is that information about new research findings developed in one Institute is rapidly disseminated to other Institutes. In addition, scientists are unusually cooperative and eager to extend their findings to other research areas. Thus the further development of the hybrid antibody technique by Dr. Henson of NCI as a more refined method of localizing antigens was capitalized on by Dr. Helmsen in applying it to the herpes simplex problem involving the cornea. Drs. Helmsen and Henson, culturing corneal cells infected with herpes simplex, demonstrated by the hybrid antibody technique that the viral antigen is found on the surface membrane of the corneal cells. Further refinement of the technique is in progress so that it may become possible to demonstrate the intracellular localization of the antigen. This research project also illustrates how the external eye research community can gain from advances made in the NEI/Intramural program. Dr. Herbert Kaufman of the University of Florida, known for his outstanding work on the herpes simplex problem, recognizing the significance of Dr. Helmsen's study has sent an associate to acquire knowledge of this new technique which may aid in further insights in the infective nature of this virus.

Another NIH facility that has been helpful to scientists of the Laboratory of Vision Research is the Veterinary Resources Branch of the Division of Research Resources. Dr. Hansen, the geneticist of the small animal section of this group, has extensively bred and studied the SH strain of rat, developed from the Wistar strain at Kyoto University, Japan. These rats, besides having chronic hypertension, also develop a retinal dystrophy that histologically resembles human retinitis pigmentosa. Dr. Hess of the LVR is particularly interested in the SH strain because the retinal dystrophy in these rats requires a longer time to develop than that observed in other animals models, such as the RCS rat. Since the destruction of the photoreceptors is of late onset in these animals, Dr. Hess believes this disorder may more closely resemble human retinitis pigmentosa. Drs. Hess and Hansen are planning to carry out cross breeding studies with this and other animal models to establish first that this is truly a gene-linked retinal dystrophy.

Other projects currently under investigation could be cited as examples of the advantages available to the scientists in the NEI/Intramural program. Taking advantage of the unique research opportunities at NIH substantially enhances the scope and the depth of the research conducted by the LVR scientists. I believe this fact becomes apparent in reviewing the individual reports in this year's Annual Report.

Project No.Z01 EY 00136-03 LVR
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

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

Project Title: Chemistry and Metabolism of the Lens

Previous Serial Number: NEI-73 LVR 136

Principal Investigators: Jin H. Kinoshita, Ph.D.
Izumi Kabasawa, M.D. (Visiting Scientist)
Ikuo Mikuni, M.D. (Visiting Scientist)

Other Investigators: Yasuhiko Tsunematsu (Guest Worker)

Cooperating Units: None

Man Years:

Total:	3.0
Professional:	2.5
Other:	0.5

Project Description:

Objectives: The many aspects of the chemistry and metabolism of the ocular lens will be studied. The changes in carbohydrate metabolism, chemistry and metabolism of lens proteins, and various lens enzymes will be studied during aging and as a result of organ or tissue culture.

Methods Employed: Aging effects on the lens are studied by examining the changes that occur in the enzymes, metabolism and chemistry of the important constituents of the lens. Organ and tissue culture procedures are used.

Major Findings: Our studies on the carbohydrate associated with lens proteins indicated that the protein with the highest level of sugar was found in the γ crystallin fraction. The amount of sugar associated with the γ crystallin fraction appeared to increase with age. Closer scrutiny revealed that the bulk of the carbohydrate was not associated with any of the major crystallins, but with a minor protein component. This carbohydrate rich protein eluted before the γ peaks on a SE-Sephadex column and did not appear to belong to γ crystallins.

In our quest to find the source of the glycoprotein we turned our attention to the lens membranes. For this study, cattle lenses, stripped of capsule and epithelium, were used. The plasma membranes were isolated in a particular fraction by sucrose-gradient centrifugation. The plasma membranes were

extracted by detergents and the proteins analyzed by polyacrylamide gel electrophoresis in SDS. The gel revealed a complex pattern of protein bands most of them unrelated to the crystallins. There were 4 main protein bands that stained strongly with PAS. The carbohydrate of the membrane glycoproteins was composed of fucose, xylose, mannose, galactose, glucose, hexosamine and sialic acid.

Proteolysis is one of the mechanisms that has been thought to be a factor in the formation of certain cataract. However, that proteolysis can occur in the intact lens has been difficult to demonstrate. We have been successful in demonstrating that proteolysis can occur under certain experimental conditions. Rat lenses were cultured in balanced salt solution without amino acids. When glucose was present, slow loss of free amino acids to the medium occurred, but sodium and potassium levels remained normal up to at least 48 hours of incubation. Glutathione and ATP slowly disappeared.

When lenses were incubated without glucose, loss of ATP and glutathione and leakage of amino acids were faster and some exchange of cations occurred. Starting abruptly after 24 hours without glucose, rapid net proteolysis was observed which produced linear increases in concentrations of proteogenic free amino acids. Accumulation to several times normal levels was accompanied by marked influx of sodium and water.

Studies to establish the optimal conditions to culture epithelial cells of lenses from normal and cataractous mice are being continued. Tissue culture has led to lentoid bodies which appear very much like lens fiber under electron microscopic examinations. Purification of γ crystallin from mouse lens has been accomplished and antiserum to it is now being prepared. γ-crystallin is a protein synthesized in the lens fiber but not in the epithelium. Use will be made of the flourescent antibody to γ crystallin to determine whether or not γ crystallin is present in these lentoid body. By these means we hope to show that the tissue cultured cells retain the differentiative characteristics of lens cells.

Significance to Biomedical Research and the Program of the Institute: An understanding of the basic chemistry and physiology of the lens is important to provide a more complete understanding of the cataractous process. The age-related change in the γ crystallins is one of the first examples of the effect of aging on the lens proteins.

Development of a tissue culture procedure of lens epithelial cells may become useful in studying human cataracts. The human congenital cataracts may be studied by this technique provided that epithelial cells from a cataract can be obtained. We are already looking into possibility of culturing congenital mouse cataracts.

Proposed Course: The studies described are being continued.

Keyword Descriptors: eye, lens, proteins; eye, lens, gamma crystallin; eye, lens, glycoprotein; eye, lens proteolysis; eye, lens, tissue culture; eye lens, organ culture.

NEI Research Program: Cataract

Experimental Subject or Tissue Source: Bovine/Rat/Mouse

Research Objective: Etiology

Honors and Awards: None

Publications:

Kabasawa, I., Lou, M.F., Merola, L.O. and Kinoshita, J.H.: Inositol metabolism in the lens. Ophthalmic Res. 6: 155-165, 1974.

Kabasawa, I., Barber, G.W. and Kinoshita, J.H.: Aging effects on the bovine lens γ-crystallins. Exp. Eye Res. 18: 457-466, 1974

Obazawa, H., Merola, L.O. and Kinoshita, J.H.: The effects of xylose on the isolated lens. Invest. Ophthalmol. 13: 204-209, 1974

Jedziniak, J.A., Kinoshita, J.H., Yates, E.M. and Benedek, G.B.: The concentration and localization of heavy molecular weight aggregates in aging normal and cataractous human lenses. Exp. Eye Res. (in press) 1974.

Gabby, K.H. and Kinoshita, J.H.: Aldose reductase from mammalian tissues. In Wood, W.A. (Ed.): Enzymes of Carbohydrate Metabolism. New York, Academic Press (in press) 1974.

Project No. Z01 EY 00003-03 LVR
1. Laboratory of Vision Research
2. Section of Biochemistry
3. Bethesda, Maryland

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

Project Title: Cataracts

Previous Serial Number: None

Principal Investigators: Jin H. Kinoshita, Ph.D.
Shambhu Varma, Ph.D. (Visiting Scientist)
Henry N. Fukui, Ph.D. (Staff Scientist)

Other Investigators: Lorenzo O. Merola

Cooperating Units: None

Man Years:

Total:	3.5
Professional:	2.5
Other:	1.0

Project Description:

Objectives: To study the mechanism of formation of cataracts in experimental animals and to explore possible means by which these cataracts can be prevented.

Methods Employed: Sugar cataracts can be induced in experimental animals by making them diabetic with appropriate chemical agents, or by making them galactosemic or xylosemic with a diet enriched with galactose or xylose. Another approach to study cataracts is to employ animal models. We have developed a colony of Nakano mouse strain with hereditary cataracts.

Major Findings: Further studies in developing means of delaying the onset of sugar cataracts have led to a new series of compounds known as flavonoids. These compounds, ubiquitously distributed in plants, were found to be effective inhibitors of lens aldose reductase, the enzyme which initiates the cataractous process in diabetes and galactosemia. The inhibitory activities of the flavonoids, quercetin, quercitrin and myricitrin, even surpassed those of AY-22,284 and TMG, the previously known inhibitors. The degree of inhibition was observed to differ with variation in the structure of the flavonoid moiety. Kinetic studies indicated that the inhibition of the enzyme by flavonoids is of the noncompetitive type. The flavonoids were also effective in blocking accumulation of polyol in rat lenses incubated in high sugar medium. At 10^{-4} M,

the polyol accumulation was decreased by 80% with quercitrin and by 40% with AY-22,284 as compared to contralateral controls. At 10^{-5} and 10^{-6}M, quercitrin reduced the polyol synthesis by about 30% and 20% respectively, while AY-22,284 was ineffective at these concentrations.

These results indicate that flavonoids are the most potent aldose reductase inhibitors available so far. Since there are so many possible forms of flavonoids, further studies of these compounds may reveal a potentially useful agent for delaying or preventing the onset of sugar cataracts.

Another type of lens opacity currently being studied is a hereditary type of cataract observed in a certain mouse strain. In the lenses of the Nakano mouse strain an apparent decrease in the Na-K ATPase activity appeared to initiate the cataractous process. Further studies revealed that a non-dialyzable, heat sensitive inhibitor of Na-K ATPase was present in the cataract. Attempts were made to follow the cataract factor using alternate methods. For this reason the assay for the phosphatase activity of the Na-K ATPase was developed using p-nitrophenyl phosphate (PNPP) as the substrate and either calf lens or retina as the source of the enzyme. This reaction requires K ions, and is inhibited by ouabain was well as by high concentrations of Na ions. The mouse cataract factor was found to inhibit PNPPase. As shown with ATPase, the inhibitory activity was markedly enhanced by initially pre-incubating the factor and the enzyme before the enzyme assay was conducted.

Since the mouse cataract inhibitor appeared to be a positively charged peptide, a comparison was made between the cataract factor and protamine, a naturally occurring basic polypeptide, that is known to inhibit Na-K ATPase. Protamine was also found to inhibit PNPPase and required preincubation for maximum effect. However, a difference was observed in that high concentrations of K ions during the preincubation period abolished the protamine effect, but did not alter the inhibitory activity of the mouse cataract factor.

Significance to Biomedical Research and the Program of the Institute: Cataract is one of the major causes of blindness throughout the world. Even though vision can be corrected by appropriate surgery, loss of vision because of cataracts presents a problem. It is hoped that this type of study on sugar cataracts may serve as a model by which other mechanisms of cataract development can be uncovered, and also provide alternate means of preventing cataracts. The terminal stages of these sugar cataracts may have features common to other forms of cataracts. Even though the initial phase of cataract development may be different in the other forms of cataract, it appears that the terminal stages are quite similar.

Proposed Course: This project will be continued.

Keyword Descriptors: eye, cataracts, sugar; eye, cataracts, hereditary; eye, cataracts, Nakano mouse strain; eye, lens, enzyme, aldose reductase; eye, lens, enzyme, aldose reductase inhibitors.

NEI Research Program: Cataract

Experimental Subject or Tissue Source: Bovine/Rat/Mouse

Research Objective: Etiology

Honors and Awards: None

Publications:

Kinoshita, J.H.: Mechanisms initiating cataract formation. (Proctor Lecture) Invest. Ophthalmol. 13: 713-724, 1974

Kinoshita, J.H.: Cataractogenic effects of lactose and galactose. In Sipple, H.L. and McNutt, K.W. (Eds.): Sugars in Nutrition, New York, Academic Press 1974, pp. 375-386.

Varma, S.D. and Kinoshita, J.H.: The absence of cataracts in mice with congenital hyperglycemia. Exp. Eye Res. 19: 577-582, 1974

Varma, S.D. and Kinoshita, J.H.: Sorbitol pathway in diabetic and galactosemic rat lens. Biochim. Biophys. Acta 338: 632-640, 1974.

Varma, S.D., Mikuni, I., and Kinoshita, J.H.: Flavonoids as inhibitors of lens aldose reductase. Science (in press).

Hamai, Y., Fukui, H.N. and Kuwabara, T.: Morphology of hereditary mouse cataract. Exp. Eye Res. 18: 537-546, 1974.

Project No. Z01 EY 00134-02 LVR
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

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

Project Title: Biochemical Development and Function of Retina and Pigment Epithelium

Previous Serial Number: NEI-73 LVR 134

Principal Investigator: Gerald J. Chader, Ph.D.
Donald R. Bersgma, M.D.

Other Investigators: R. Theodore Fletcher
Barbara O. Wiggert, Ph.D. (Guest Worker)

Cooperating Units: None

Man Years:

Total:	1.7
Professional:	1.2
Other:	0.5

Project Description

Objectives: The aim of this project is to study factors which determine the development and function of retina and pigment epithelium in relation to retinitis pigmentosa and other retinal degenerative conditions.

Methods Employed: Biochemical techniques were used to study the uptake, binding and subcellular distribution of vitamin A in pigment epithelium and retina. Sucrose density gradient ultracentrifugation, gel chromatography, and other techniques were used to partially characterize vitamin A receptor proteins. In other experiments standard techniques of tissue culture were used to investigate retina and pigment epithelium cells growing separately and together under culture conditions.

Major Findings: 1) A high affinity receptor for vitamin A has been found in pigment epithelium and retina. It is a protein in nature, of low molecular weight and is specific for retinol, the alcohol form of vitamin A. It appears to be different from the retinol-binding protein of serum. 2) Under tissue culture conditions, pigment epithelial cells influence the morphology of retinal cells. The retinal cells become more neuronal in character with increased arborization and greater dendritic appearance.

Significance to Biomedical Research of the Program of the Institute: Factors involved in retinal dystrophy and degeneration are poorly understood. We are attempting to pinpoint, with biochemical techniques and tissue culture, the early signals that are critical to the normal development of retina and pigment epithelium and contrast them with those found in the diseased state. It is hoped that such studies will uncover defects in intracellular metabolism (eg. in vitamin A receptors) or intercellular communication (eg. effects of epithelial cells on the differentiation of retina) which will uncover the etiology of the degenerative condition(s).

Proposed Course: 1) Study the role of the vitamin A receptor in the metabolism of the retina and pigment epithelium and determine its presence or absence in tissues of various animals with inherited retinal degeneration. 2) Continue to examine the influence of various effectors (hormones, cyclic nucleotides, etc.) and of pigment epithelial cells on the early development of retina.

Keyword Descriptors: retina, pigment epithelium, retinitis pigmentosa, vitamin A, receptor, tissue culture

NEI Research Program: Retinal and Choroidal Diseases-Development, Structure, Function and Degeneration (Choroid, Pigment Epithelium and Related Disorders)

Experimental Subject or Tissue Source: Cow/Chick Embryo

Research Objective: Etiology

Honors and Awards: None

Invited Lecture: Symposium on "Developmental Ocular Abnormalities, Phil. Pa., Oct. 24-26, 1974.

Publications:

Gouras, P. and Chader, G.: Retinitis pigmentosa and retinol-binding protein. Invest. Ophthalmol. 13: 239-242, 1974.

Lippman, M., Wiggert, B., Chader, G. and Thompson, E.: Glucocorticoid receptors: characteristics, specificity and ontogenesis in the embryonic chick neural retina. J. Biol. Chem. 249: 5916-5917, 1974.

Wiggert, B. and Chader, G.: Studies on a specific glucocorticoid-hormone receptor in the developing chick retina. Exp. Eye Res. 18: 477-484, 1974.

Newsome, D., Fletcher, R., Robison, W., Kenyon, K. and Chader, G.: Effects of cyclic AMP and sephadex fractions of chick embryo extract on cloned retinal pigmented epithelium in tissue culture. J. Cell Biol. 61: 369-382, 1974.

Wiggert, B. and Chader, G.: A glucocorticoid and progestrone receptor in the chick optic tectum. J. Neurochem. 24: 585-587, 1975.

Chader, G., Newsome, D., Bensinger, R. and Fletcher, R.: Studies on the differentiation of retinal pigmented epithelial cells in culture. Invest. Ophthalmol. 14: 108-113, 1975.

Wiggert, B. and Chader, G.: A receptor for retinol in the developing retina and pigment epithelium. Exptl. Eye Res. (in press).

Project No.Z01 EY 00148-02 LVR
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

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

Project Title: Cyclic Nucleotides and Vision

Previous Serial Number: NEI-73 LVR 148

Principal Investigator: Gerald J. Chader, Ph.D.

Other Investigator: R.T. Fletcher

Cooperating Units: Gopal Krishna, Ph.D. Laboratory of Chemical Pharmacology, NHLI

Man Years:

Total:	1.2
Professional:	0.7
Other:	0.5

Project Description:

Objectives: The impingement of light on the retina causes changes in the photoreceptor protein, rhodopsin, which ultimately results in a visual response. It is probable that small intracellular "messengers" mediate this process. Likely candidates for such messengers are the cyclic nucleotides (cyclic AMP and/or cyclic GMP). This project was designed to study the enzymes of cyclic nucleotide metabolism and to determine the specific role of cyclic nucleotides in the photoreceptor unit.

Methods Employed: Retinal photoreceptors from retinas were isolated by sucrose gradient centrifugation and the activities of several enzymes of cyclic nucleotide metabolism were determined biochemically. Cyclic nucleotides were determined by immunochemical and high pressure liquid chromatographic techniques. Protein kinase activity was determined by a filter paper binding method.

Major Findings: Retinal photoreceptors are unique in containing a 100-fold higher concentration of cyclic GMP than cyclic AMP, a ratio that is reversed in all other tissues of the body yet studied. We have also found a specific GTP-dependent kinase activity which appears to phosphorylate rhodopsin. Its activity is increased 50-fold by light exposure. This effect is specific since other activators, neurotransmitters, cyclic nucleotides, etc. do not affect the phosphorylation.

Significance to Biomedical Research and the Program of the Institute: It is presently not known how the initial photic simulation of the retinal photoreceptor is converted into a neural response. The present findings indicate that cyclic GMP and GTP may play a central role in the visual proces or in normal photoreceptor functioning.

Proposed Course: We will continue to study the role of cyclic nucleotides in photoreceptor functioning and their possible role in mediating the response of the retina to light.

Keyword Descriptors: photoreceptor, cyclic nucleotides, cyclic GMP, cyclic AMP, phosphorylation

NEI Research Program: Retinal and Choroidal Diseases-Development, Structure, Function and Degeneration (Visual Pigments, Photoreceptors and Visual Transduction Disorders)

Experimental Subject or Tissue Source: Cow

Research Objective: Etiology

Honors and Awards: None

Publications:

Bensinger, R., Fletcher, R. and Chader, G.: "Piggyback" Chromatography: Assay for Guanylate Cyclase in Retina and Other Neural Tissue. J. Neurochem. 22: 1131-1134, 1974.

Chader, G., Fletcher, R., Johnson, M. and Bensinger, R.: Rod Outer Segment Phosphodiesterase: Factors Affecting the Hydrolysis of Cyclic AMP and Cyclic GMP. Exp. Eye Res. 18: 509-515, 1974.

Chader, G., Herz, L. and Fletcher, R.: Light Activation of Phosphodiesterase Activity in Retinal Rod Outer Segments. Biochem. Biophys. Acta 347: 491-493, 1974.

Chader, G., Herz, L. and Fletcher, R.: Cyclic Nucleotide Hydrolysis: Some Possible Natural Regulators of Phosphodiesterase Activity in Retina and Rod Outer Segments. J. Neurochem. 23: 873-875, 1974.

Chader, G., Fletcher, R. and Krishna, G.: Light-Induced Phosphorylation of Rod Outer Segments by Guanosine Triphosphate. Biochem. Biophys. Res. Comm. (in press).

Project No. Z01 EY 00027-04 LVR
1. Laboratory of Vision Research
2. Section of Biochemistry
3. Bethesda, Maryland

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

Project Title: Induction of Buphthalmos in Chicks by Feeding a High Level of Glycine

Previous Serial Number: NEI-71 LVR-027

Principal Investigator: Ralph J. Helmsen, Ph.D.

Other Investigators: Max Rubin, Ph.D. (University of Maryland)

Cooperating Units: Department of Poultry Science, University of Maryland

Man Years:

Total	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To study the chemical and physical factors which control the size and shape of the vitreous during development of the eye as at maturity.

Methods Employed: Weight determinations were made on the total eye and various ocular tissues. Colorimetry was employed to measure the quantity of each of the major macromolecules in dialyzed chicken vitreous.

Major Findings: No major findings were obtained in this fiscal year.

Significance to Biomedical Research and the Program of the Institute: Chicks grown on a high-glycine diet represent the first nutritional model for the study of buphthalmos in experimental animals. Because chickens possess a deficient blood-brain barrier during the first month post-hatching, buphthalmic animals prove not only useful for studying biochemical changes which take place in developing vitreous but in maturing nervous system as well.

* Work on amino acid profiles in vitreous and blood will continue when time is available on the automated amino analyzer for such analyses. Plans have been made to obtain new data in FY 1976.

Proposed Course: Amino acid profiles will be performed on dialysates of control and experimental vitreous from chicks as soon as time is available on the automated amino acid analyzer at the University of Maryland for processing a large number of samples. These studies will seek to determine if glycine or one of its metabolites is elevated in the connective tissue. This data will be correlated with the free amino acid levels in the serum from these animals.

Keyword Descriptors: eye, vitreous body; eye, intraocular fluid dynamics; amino acids, glycine; birds, chickens.

NEI Research Program: Retinal and Choroidal Diseases -- Retinal Detachment and Vitreous Abnormalities

Experimental Subject or Tissue Source: Chicken

Research Objective: Etiology

Honors and Awards: None

Publications: None

Project No. Z01 EY 00111-03 LVR
1. Laboratory of Vision Research
2. Section of Biochemistry
3. Bethesda, Maryland

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

Project Title: Chemistry of the Cornea

Previous Serial Number: NEI-72 LVR 111

Principal Investigator: Ralph Helmsen, Ph.D.

Other Investigators: Donald Henson, M.D. (NCI)
Harriet Kuzmiak
Rachel Levinson
Duran Harris

Cooperating Units: Laboratory of Pathology, (NCI)

Man Years:

Total:	2.4
Professional:	0.9
Other:	1.5

Project Description:

Objectives: To isolate tissue-specific soluble and membrane proteins from the epithelium and the stroma and to characterize these macromolecules by physical, chemical and immunological techniques.

Methods Employed: Distinct proteins are isolated and fractionated from fresh pooled corneal cell layers or tissue culture cells by chromatography on columns of glass beads coupled with salt fractionation and/or preparative gel electrophoresis. Purity of individual fractions are determined by the number of bands obtained by staining following polyacrylamide gel electrophoresis and isoelectric focusing.

Major Findings: An immunogenic polypeptide or small protein has been isolated from pooled calf corneal epithelia through the combined use of chromatography on small columns of 75 Å controlled-pore glass beads coupled with ammonium sulfate and sodium sulfate salt fractionation. The purification procedure was monitored at each step by demonstrating that the appropriate protein fraction could produce a precipitin line by the Ouchterlony technique when tested with a sheep antiserum prepared against a dialyzed extract of calf epithelial proteins.

The antigenic material thus prepared failed to enter a 6.5% polyacrylamide gel upon electrophoresis and was tentatively concluded to exist in the form of a calcium aggregate based on analysis by atomic absorption spectrophotometry. Addition of 0.001M EDTA to the immunogenic fraction prior to gel electrophoresis resulted in the appearance of a new protein band on Coomassie blue stained gels with an approximate R_f value of 0.70.

The proteinaceous material derived from the EDTA treatment was determined to be antigenic on unstained gels by use of immunodisc electrophoresis. Elution of the antigen from sliced gels with 0.1% sodium dodecyl sulfate (SDS) and re-electrophoresis on a 6.5% SDS gel with protein standards revealed that the antigen had a molecular weight of less than 10,000.

An investigation has been undertaken in conjunction with Dr. Henson's laboratory to confirm the presence and distribution of the antigen under study in fresh corneas by indirect immunofluorescent staining. After frozen sections of calf cornea are absorbed with sheep antiserum prepared against soluble calf corneal epithelial proteins the immune sheep serum is then reacted with fluorescein-conjugated rabbit antisheep immunoglobulin G (IgG) to detect the antigen.

A bright diffuse fluorescence was observed in all layers of the epithelium and a less intense staining was seen in the underlying stroma. If, however, the sheep anti-corneal serum was diluted in commercial calf serum before reaction with the tissue and the fluorescein conjugate, the fluorescence present in the corneal stroma was eliminated while the staining reaction in the epithelium persisted.

In separate experiments it had been predetermined before the immunofluorescent studies that the immune sheep serum could react with calf serum proteins but that the precipitin line formed with the epithelial antigen remained intact if the antiserum was preabsorbed with calf serum, calf plasma or bovine liver acetone powder. It was also observed that unabsorbed sheep antiserum failed to react with γ-crystallin purified from pooled calf lenses.

Significance to Biomedical Research and the Program of the Institute: Successful isolation of a soluble antigen from the corneal epithelium in fairly large amounts provides a source of a potential sensitizing agent in which may be employed in experimental graft rejection studies. If such investigations reveal that this macromolecule functions as a transplantation antigen, further research may be directed at modifying its immunogenicity in vivo in order to prolong the survival time of corneal grafts.

Proposed Course: In addition to completing the physical and chemical characterization of the antigen from calf epithelium the isolation procedure for the antigen will be scaled down in order that in vitro metabolic experiments can be performed on a small number of incubated corneas. Modifications in the existing protocol for purification of the immunogenic macromolecule will permit isolation of other corneal epithelial constituents and in turn their influence on the turnover of the tissue antigen can be assessed in the above in vitro studies.

Keyword Descriptors: eye, cornea; tissue, epithelium; immunology, antigens; immunological test and immunoassay, immunofluorescence; glycopeptides; chemical aggregates; calcium; chelating agent, EDTA; mammals, ungulates, cattle.

NEI Research Program: Corneal Diseases-Development, Structure, Function and Degeneration / Trauma and Wound Healing-Including Transplantation

Experimental Subject or Tissue Source: Calf/Rabbit

Research Objective: Etiology

Honors and Awards: None

Publications: None

Project No. Z01 EY 00147-02 LVR
1. Laboratory of Vision Research
2. Section of Biochemistry
3. Bethesda, Maryland

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

Project Title: Mechanism of Herpes Simplex Virus Infection of Corneal Cells

Previous Serial Number: NEI-74 LVR-147

Principal Investigators: Ralph J. Helmsen, Ph.D.
Donald Henson, M.D. (NCI)

Other Investigators: Joseph Metcalf, Ph.D.
Margery Sullivan
Duran Harris
Rachel Levinson

Cooperating Units: Laboratory of Pathology, NCI
Department of Ophthalmology
University of Florida

Man Years:

Total:	0.5
Professional:	0.3
Other:	0.2

Project Description:

Objectives: To isolate individual viral specified membrane proteins on ghosts of rabbit corneal cells derived from tissue culture as well as from primary culture of cells from normal tissue.

Major Findings: Research efforts this year have been directed at shortening the lengthy procedure for preparation of hybrid antibody in order to make more material available for ensuing studies. Two major modifications have been introduced; a) the substitution of sodium sulfate for ammonium sulfate for use in salt precipitation of both human and sheep immunoglobulin G(IgG) and b) the replacement of β_1-mercaptoethylamine with sodium borohydride in the reduction step of the $F(ab^1)_2$ fragments prepared from sheep anti IgG and sheep antiferritin along with elimination of use of the AG 50 x 2 ion exchange column.

This modified method has been successfully employed to localize viral antigens (in the electron microscope) on the surface of herpes infected KB cells.

Significance to Biomedical Research and the Program of the Institute: The use of the hybrid antibody method for localization of new antigens on the surface of viral infected corneal cells represents, as far as can be determined, the first application of this method of immunolabeling to experimental studies directed at control of herpes simplex keratitis. The concept that viral antigens which appear on cellular membranes during herpes infection may be recognized as foreign by the host in a manner analogous to the recognition of foreign histocompatibility antigens in an incompatible tissue graft may prove useful to those investigators involved in formulating protocols for treatment of this ocular disease.

Proposed Course: Attempts will be made to experimentally infect the individual layers of the rabbit cornea in vivo with herpes simplex virus and study the distribution pattern of viral antigens on the surface membranes of the cells in each layer by hybrid antibody labeling. Further efforts will be made to modify the character of the hybrid antibody itself, e.g., replacement of ferritin with a marker which will permit diffusion of the immunoconjugate inside the cell for labeling of internal membranes and enhance the specificity of the hybrid molecule by coupling to it IgG from antisera prepared against individual isolated viral antigens.

Keyword Descriptors: eye, cornea; eye disorders, corneal disorders, keratitis; viruses, herpesviruses, herpes simplex virus; tissue (cell) culture; immunology, antibodies; ferritin labeled antibody technique; immune electron microscopy; mammals, lagomorphs.

NEI Research Program: Corneal Diseases --Inflammatory Diseases-Including Infectious Diseases and Allergy

Experimental Subject or Tissue Source: Rabbit

Research Objective: Etiology and Diagnosis

Honors and Awards: None

Publications:

Henson, D., Helmsen, R., Becker, K.E., Strano, A.J., Sullivan, M. and Harris, D.: Ultrastructural localization of herpes simplex virus antigens on rabbit corneal cells using sheep antihuman IgG antihorse ferritin hybrid antibodies. Invest. Ophthalmol., 13: 819-827, 1974.

Project No. Z01 EY 00135-03 LVR
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

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

Project Title: Biochemical Composition of Photoreceptor, Neuronal and Glial Cells of Normal and Pathological Retina and Brain

Previous Serial Number: NEI-73 LVR 135

Principal Investigator: Helen H. Hess, M.D.

Other Investigators: Julia E. Derr, B.A.

Cooperating Units: Carl T. Hansen, Ph.D., geneticist, VR, DRS

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: The broad aims of the project are (1) to determine the characteristic biochemical composition of photoreceptor, neuronal, glial and pigmented epithelial cells (with emphasis on rod outer segment membranes);(2) to identify and assess the usefulness of biochemical marker substances (or ratios of substances) as quantitative indices of certain histological entities or metabolic routes in retinal tissues; (3) to use indices in conjunction with other chemical constituents and enzymes to study retinal tissues (and vision-related regions of brain) in normal, experimentally-induced and heritable pathological conditions.

Methods Employed: Analyses are being carried out on whole retinas (frog and rat) and on intact retinal rod outer segments of frogs, as well as isolated frog pigmented epithelial cells. Methods in use include spectrophotometry; fluorometry; atomic absorption spectroscopy with graphite furnace; gas liquid chromatography; thin layer chromatography (TLC); and light microscopy.

Major Findings: I. Studies of calcium in pigmented epithelium, rod outer segments and whole retina of frogs: We reported previously that isolated intact retinal rod outer segments of frogs were highly sensitive to small decrements in ionic strength (Na and Ca salts). Frog outer segments prepared from dark adapted retinas by a magnetic stirring technique in a sodium phosphate buffered sucrose medium (ionic strength 0.024) are intact, birefringent organelles that are stable to light and room temperature. However, they show

loss of birefringence and undergo form changes (bending, curling, elongation and disruption) when transferred into lower ionic strength solutions. Addition of Ca^{++} at a concentration of 1.5 mM minimized the form changes. Thus, calcium seemed to be significant in the normal morphology of rod outer segments, as well as having a role in the electrophysiology of these organelles.

Because the normal levels of calcium in rod outer segments and other retinal tissues had not been well-studied previously, we studied this element under different light and dark conditions. The most outstanding result of the study was the discovery that retinal pigmented epithelium (RPE) contains a very high level of calcium.

Calcium was measured by flameless atomic absorption spectrophotometry after solubilization of the tissue in a quaternary ammonium base. The preparatory buffer contained 0.2 M sucrose and sodium phosphates to give a pH of 6.5 The buffer Ca was 2.5 uM by analysis. The Ca of retinal pigmented epithelium was 15 mMoles/Kg wet weight. In comparison, Ca measured in frog red cells was 0.05; it was 0.7 in dark adapted whole retina and 0.6 in dark adapted rod outer segments (equivalent to 0.25 Moles Ca/Mole rhodopsin). The calcium concentration in RPE exceeded that reported for most cells and tissues, including muscle, and equaled that for platelets, considered to be high calcium cells in which Ca plays a role in several cell functions. The high calcium content of RPE may be important in phagocytosis, hydrolytic enzyme activities, and as a source of the ion for regulation of excitation and inhibition of neighboring photoreceptor cells in dark and light adapted states.

II. Studies of glycolipids in rod outer segments and retina: Our previous studies showed that water-soluble glycolipids (gangliosides) were present in frog rod outer segments in lower concentration than in any other tissue studied, and to consist of two species, migrating in TLC like brain gangliosides with 2 or 3 sialic acid molecules per molecule of sphingosine (GD_{1b} and GT_1). This simple pattern contrasts with reports that rat and ox outer segment membranes contain all the ganglioside species present in the respective retinas. The difference probably is due to a greater contamination of rat and ox outer segments with other retinal tissues.

The water-insoluble lipid hexose fraction is also very small in rod outer segments and retina. In rat retina we have found the total concentration of lipid hexose to be of the order of 1 mole for 28 moles of phospholipid, as estimated by the phenol-sulfuric acid method, in the aqueous phase obtained after hydrolysis of the total lipid solids by chloroform:methanol:HCl (30:37:15, v/v).

III. Studies of pigmented hybrid rats from two strains with retinal degeneration: Twenty nine F_1 generation offspring of a cross between the RCS rat and the spontaneously hypertensive (SH) rat have been obtained from Dr. Carl Hansen. Retinal degeneration becomes evident in the RCS rat in the first few weeks after birth, but in the SH rat onset is delayed to 6 months or more. The object will be to determine whether the same rd gene may be present in the two strains of rat. Studies will include ophthalmoscopic examination of the fundus and histopathological study of fixed, stained retina at different ages.

Significance to Biomedical Research and the Program of the Institute: Data on the biochemical composition of normal photoreceptors, neurons, glia and pigment epithelial cells will contribute to an understanding of their function in retina and brain. Calcium may be important in the relationship between the photoreceptor cell and pigment epithelium in light and dark adaptation. Glycolipids may be involved in the cell surface interrelationships of outer segments and pigment epithelium and in the permeability of the outer segment to sodium ions. The possibility that an abnormality in lipid or inorganic cation composition could be a factor in the pathology of some member of the group of heritable retinal degenerations is relatively unexplored.

Proposed Course: Normal biochemical architecture and pathology of retina will be emphasized. Possible changes in Ca concentration of retinal tissues in light and dark adaptation will be studied, and enzymes influenced by Ca will be examined in isolated pigmented epithelium and rod outer segments. Studies of the amounts, types, histological localizations and possible physiological roles of identifiable glycolipids will continue. With completion of a microbalance room and construction of quartz fiber microbalances, a microtome cryostat will be used in microtechniques of frozen section sampling and microdissection to provide samples of the different layers of the retina for analysis. Pathological materials will include an animal model of heritable retinal degeneration (RCS and congenic strains).

Keyword Descriptors: eye, retinal tissues, retinal degeneration, rod outer segments, pigment epithelium, calcium, atomic absorption spectroscopy, glycolipids

NEI Research Program: Retinal and Choroidal Diseases-Development, Structure, Function and Degeneration (Choroid, Pigment Epithelium, and Related Disorders; Developmental and Degenerative Disorders)

Experimental Subject or Tissue Source: Frog/Rat

Research Objective: Etiology

Honors and Awards: None

Publications:

Bass, N.H., Hess, H.H. and Pope, A.: Altered cell membranes in Creutzfeldt-Jakob disease. Microchemical studies. Arch. Neurol. 31: 174-182, 1974.

Hess, H.H. and Derr, H.E.: Assay of inorganic and organic phosphorus in the 0.1 - 5 nanomole range. Analyt. Biochem. 63: 607-613, 1975.

Project No. Z01 EY 00008-04 LVR
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

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

Project Title: Chemistry of Rhodopsin

Previous Serial Number: NEI-71 LVR 008

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: Gary D. Knott, Ph.D., LSMM, DCRT

Cooperating Units: E.T. Adams, Jr., Ph.D., Dept. of Chemistry, Texas A & M University, College Station, Texas

Man Years:

Total:	0.8
Professional:	0.8
Other:	0.0

Project Description:

Objectives: To study the structural and functional aspects of the rhodopsin molecule and to study those biochemical entities which are involved in its function in the visual process.

Methods Employed: Mathematical modeling studies were performed on the DEC-10 computer using the MLAB system. Various interacting systems were studied by analytical ultracentrifugation and by vapor pressure osmometry.

Major Findings: One of the major problems facing an investigator studying interacting systems of macromolecules is the selection of an appropriate model to describe the system. In some cases, such as hemoglobin, this is quite simple, and in others, such as the beta-lactoglobulins, this can be a difficult and controversial problem. In order to attempt to shed some light on this problem, we have undertaken extensive studies using computer modeling techniques to generate the data that would be obtained for different types of systems in sedimentation equilibrium studies in the ultracentrifuge, and have them used non-linear least-squares curve fitting techniques to analyze this data in the presence and absence of randomly distributed error, using both correct and imposter models. It was found that with certain exceptions, it was generally possible to discriminate between true and imposter models even in the presence of normal experimental error provided enough data was obtained from properly designed experiments. The difficulty was found in discriminating between

indefinite associations of the isodesmic type (ie. a stacking type of association with equal free energies of association for the addition of each monomer unit) and a monomer-dimer-tetramer-octamer type of association in a relatively narrow range of values for the equilibrium constant for the isodesmic association. While it was not possible to discriminate between the two types of association on the basis of the quality of the fit, it was noted that the equilibrium constants for the 1-2-4-8 association tended to have a relatively well defined relationship to each other and to the equilibrium constant for the isodesmic case, thus suggesting that discrimination might be made on that basis. In addition, it appears that other experimental techniques might make an absolute discrimination possible in cases of this type. This work was presented at the conference "Fifty Years of the Ultracentrifuge" and will appear in the conference monograph. This conference, which was held February 24-26, 1975 under the joint sponsorship of the Division of Computer Research and Technology and the Fogarty International Center, was co-organized and co-chaired by this investigator and by Dr. George Weiss of DCRT.

Following the demonstration by Moore and Song (Nature New Biology 243: 30-32, 1973) that all-trans retinal appears to undergo a reversible monomer-dimer type of association in ethanol and in other organic solvents, we have undertaken a more detailed study of this association, starting with a study of all-trans retinol in cyclohexane. Ultracentrifugal analysis proved to be impractical because the densities of the solute and the solvent were so close to each other that there was insufficient redistribution of solute in the centrifugal field to permit analysis with sufficient accuracy for our needs. For this reason, vapor pressure osmometry, which measures the number-average molecular weight, was chosen as the method of analysis. Analysis of the resulting data as a monomer-dimer association required the use of a negative virial coefficient to obtain a suitable fit for the data. This indicated that a higher order association was present, and the data was then successfully fit as an isodesmic association with an intrinsic association constant of 15.21, giving a value for the change in standard free energy of association of -1.67 kcal/mole of monomer. While such a free energy is consistant with the long-range forces which are operative in stacking, the present data does not permit deductions concerning the configuration of the stacking, and measurements of the changes in enthalpy and entropy will be required for this purpose.

Significance to Biomedical Research and the Program of the Institute: The studies on the problems of data analysis and modeling in systems involving the self-association of macromolecules has broad biomedical applicability. Their most immediate application to problems of visual biochemistry is in their extension to mixed associations such as the binding of retinol binding protein to prealbumin as well as their direct application to the vapor pressure osmometry studies on the self-association of retinol. Thus, this work is then relevant to an understanding of the basic biochemical mechanisms which are involved in both the normal and pathological aspects of scotopic vision.

Proposed Course: The studies on the self-association of all-trans retinol will be extended to other isomers and derivatives in a variety of solvent systems and under conditions which will permit obtaining enthalpies and entropies as well as free energies of association, and to possibly relate

these properties to the role of retinol in the visual process. Studies on the binding of retinol binding protein to prealbumin have been initiated, and studies on the behavior of rhodopsin in non-aqueous media are planned.

Keyword Descriptors: retinal and choroidal diseases, visual pigments, retina, etiology, analytical ultracentrifugation, vapor pressure osmometry, mathematical modeling, computer data analysis, self-associating systems, retinal, retinol

NEI Research Program: Retinal and Choroidal Diseases-Development, Structure, Function and Degeneration (Visual Pigments, Photoreceptors and Visual Transduction Disorders)

Experimental Subject or Tissue Source: Synthetic

Research Objective: Etiology

Honors and Awards: None

Publications:

Lewis, M.S. and Knott, G.D.: Simulation studies of self-associating systems; descrimination between specific and isodesmic associations. Biophysical Chemistry, (in press).

Lewis, M.S. and Weiss, G.H. (Eds.): Proceding of the conference "Fifty Years of the Ultracentrifuge", Biophysical Chemistry, (in press).

Project No. Z01 EY 00009-04 LVR
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

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

Project Title: Physical Chemistry of Model Gel Systems

Previous Serial Number: NEI-71 LVR 009

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: Jules A. Gladner, Ph.D., LBC, NIAMDD

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To study the physical and chemical parameters of model systems which are pertinent for transparency or opacity of gel systems or which in any way may be of significance to the biochemistry of vision.

Methods Employed: The usual methods of protein preparation, fractionation, purification, and characterization have been employed. In this laboratory particular emphasis has been given to analytical ultracentrifugation and to computer techniques for model simulation, data reduction, and systems analysis as being the most effective means for studying systems of interacting or non-interacting macromolecules.

Major Findings: Studies on the effects of intra- and intermolecular cross-linking of polypeptide chains on gel formation were continued using lamprey fibrinogen. Since the lamprey is the most primitive of the vertebrates, we felt that some significant differences in its cross-linking reactions might be observed. The molecular weights of the α-, β-, and γ-chains were found to be 112,000, 68,000, and 50,000 respectively on SDS-polyacrylamide gels. If there are two chains of each type as in normal vertebrate fibrinogens, this gives a molecular weight for the whole molecule of 460,000. This is in marked disagreement with the value of 326,000 which was originally obtained by sedimentation equilibrium. This discrepancy can be explained by postulating that a α-chain is actually a dimer formed by intramolecular cross-linking, and if this is so, the summation of the SDS-polyacrylamide gel values gives a molecular

weight of 348,000. This postulated presence of an intramolecular cross-link is also consistant with the observation that the α-chains appear to have limited involvement in intermolecular cross-linking, and that the fibrin-lysis time appears to be only a function of γ-chain cross linking. Further studies have shown that the α-chain appears to be particularly susceptible to degradation, and when suitable precautions are taken to inhibit this, sedimentation-equilibrium molecular weight studies have given values of 350,000-360,000, in particularly good agreement with the summation of the weights of the chains.

Significance to Biomedical Research and the Program of the Institute: While the work on fibrinogen contributes to a general knowledge of the factors involved in intra- and intermolecular cross-linking and the formation of gels, it is of more particular importance because of the role of fibrinogen in blood clotting and wound healing, factors which are of importance in those aspects of the treatment of glaucoma and cataract which require surgical intervention.

Proposed Course: We plan to continue studying the general class of proteins which undergo gel formation, with emphasis on the mechanisms of cross-linking and the effects of chemical modification of the proteins on these reactions. Emphasis will be given to those aspects of this general problem which are of greatest significance to visual biochemistry.

Keyword Descriptors: corneal diseases, trauma and wound healing, lamprey plasma, fibrinogen, etiology, treatment, analytical ultracentrifugation, SDS-Polyacrylamide gels, blood clotting, inter- and intramolecular cross-linking reactions

NEI Research Program: Corneal Diseases-Trauma and Wound Healing-Including Transplantation

Experimental Subject or Tissue Source: Lamprey

Research Objective: Etiology

Honors and Awards: None

Publications:

Murtaugh, P.A., Halver, J.H., Lewis, M.S. and Gladner, J.A.: Cross-linking reactions of lamprey fibrinogen and fibrin. Biochim. et Biophys. Acta 359: 415-420, 1974.

Project No. Z01 EY 00015-10 LVR
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

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

Project Title: Synthesis of Sugar-Containing Polymers in Retina

Previous Serial Number: NEI-71 LVR 015

Principal Investigator: Paul J. O'Brien, Ph.D.

Other Investigators: Dean Bok, Ph.D.

Cooperating Units: Jules Stein Eye Institute
UCLA School of Medicine
Los Angeles, California 90024

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.4

Project Description:

Objectives: Many interactions between macromolecules and cell membranes are mediated by the sugar molecules bound to one of the interacting surfaces. In the process of renewal of photoreceptor outer segment disc membranes, rhodopsin, a glycoprotein, must be transported from the inner segment and incorporated into disc membranes with a specific orientation in space. This project was designed to determine where and when sugars are added to the polypeptide and what role they play in the transport and assembly of rhodopsin into disc membranes.

Methods Employed: Ordinary biochemical techniques were used, such as incubation of retinas, cell fractionation, isolation of rod outer segments by density gradient centrifugation, and detergent extraction and purification of rhodopsin by column chromatography.

Major Findings: Both glucosamine and mannose were incorporated into rhodopsin by isolated frog or bovine retinas. A large intracellular pool of sugar nucleotide was found in both which seriously diluted the radioactive glucosamine and retarded its appearance in the endoplasmic reticulum and Golgi complex. An inhibitor of glucosamine synthesis was employed in both bovine and frog retinas to deplete this large pool and permit rapid labeling of both organelles simultaneously, showing that glucosamine is incorporated at two subcellular sites. Further studies with an inhibitor of protein

synthesis showed that glucosamine was incorporated at the Golgi in the absence of protein synthesis but that mannose was not. Consequently, both sugars are added to newly-synthesized opsin polypeptides on the endoplasmic reticulum but only one is added on the Golgi.

Significance to Biomedical Research and the Program of the Institute: The evidence that bovine and frog retinas share very similar pathways of hexosamine metabolism and glycoprotein synthesis supports the earlier conclusion that direct comparison is justified and that the use of either species is valid for making general observations on the details of photoreceptor renewal. The addition of sugars to opsin at different locations in the cell lends credence to the thesis that growth of the carbohydrate chain is associated with the transport of opsin. Failure of these sugar transfer steps could lead to impaired outer segment renewal or disorientation of opsin in the disc membranes resulting in abnormal retinal function.

Proposed Course: Attempts will be made to determine whether any sugars are transferred to opsin or rhodopsin in the outer segments. Efforts will also be directed toward demonstration of specific glycoprotein synthesis in other organelles of the photoreceptor such as mitochondria and synaptic bodies.

Keyword Descriptors: glycoproteins, inhibitors, glucosamine, mannose, rod outer segments, rhodopsin, opsin, endoplasmic reticulum, golgi complex, photoreceptor renewal

NEI Research Program:Retinal and Choroidal Diseases-Development, Structure, Function and Degeneration (Visual Pigments, Photoreceptors, and Visual Transduction Disorders)

Experimental Subject or Tissue Source: Bovine/Frog

Research Objective: Etiology

Honors and Awards: Appointed to the Editorial Board of Experimental Eye Research

Publication: None

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

Project Title: Protein Synthesis in the Retina

Previous Serial Number: NEI-71 LVR 016

Principal Investigator: Paul J. O'Brien, Ph.D.

Other Investigators: Hitoshi Shichi, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.4

Project Description:

Objectives: The renewal of photoreceptor cell outer segments is a continuous process which is impaired in some pathological conditions such as progressive degeneration or developmental anomalies of the retina. The purpose of this project is the elucidation of the biochemical events involved in renewal, especially the distinction between newly synthesized opsin and pre-existing opsin with regard to biochemical alterations in the outer segments.

Methods Employed: Ordinary biochemical techniques were used, such as incubation of bovine retinas, cell fractionation, isolation of rod outer segments by density gradient centrifugation, detergent extraction and purification of rhodopsin by column chromatography.

Major Findings: Newly-synthesized opsin located in bovine photoreceptor outer segments is not selectively phosphorylated upon exposure to light. A small fraction of outer segment opsin is phosphorylated but the basis for this non-random phosphorylation remains unclear. Neither newly-synthesized opsin nor pre-existing opsin can be converted to rhodopsin by incubating retinas or isolated rod outer segments with all-trans retinal or by incubating retinas with retinol palmitate. Published reports claim regeneration of opsin under these conditions, however the generation of 11-cis retinal for these conversions appears to be more complex than originally believed. The synthesis of opsin can be completely blocked with puromycin permitting examination of subsequent biochemical events that might occur in the outer segment, such as the addition of sugars or the chromophore.

Significance to Biomedical Research and the Program of the Institute: The ability to block new opsin synthesis will permit examination of the biochemical changes that occur in the photoreceptor outer segments that depend on newly-synthesized opsin. Since the new opsin molecules are located initially in the plasma membrane before becoming part of the disc membranes, it might be possible to identify those chemical events that involve the plasma membrane opsin specifically. This information could lead to a better understanding of the function of the photoreceptor and permit the design of experiments to assess the nature of the defects in retinas undergoing degeneration.

Proposed Course: Attempts will be made to determine whether the plasma membrane opsin is involved in the selective phosphorylation of opsin. Also efforts will be directed toward demonstrating the location of newly-synthesized opsin in specific structures of the outer segment, such as basal folds and plasma membrane. Attempts will be made to demonstrate membrane protein synthesis in other organelles of the photoreceptor, especially the synaptic body.

Keyword Descriptors: photoreceptor renewal, rod outer segments, opsin, rhodopsin, plasma membrane, disc membrane

NEI Research Program: Retinal and Choroidal Diseases-Development, Structure, Function and Degeneration (Visual Pigments, Photoreceptors and Visual Transduction Disorders)

Experimental Subject or Tissue Source: Bovine

Research Objective: Etiology

Honors and Awards: None

Publications:

Shichi, H., Somers, R.L. and O'Brien, P.J.: Phosphorylation of Rhodopsin: Most Rhodopsin Molecules are not Phosphorylated. Biochem. Biophys. Res. Communs. 61: 217-221, 1974.

O'Brien, P.J. and Muellenberg, C.G.: Synthesis of Rhodopsin and Opsin in vitro. Biochemistry 14: 1695-1699, 1975.

Project No. Z01 EY 00149-02 LVR
1. Laboratory of Vision Research
2. Section on Experimental Pathology
3. Bethesda, Maryland

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

Project Title: Ultrastructure and Function of the Pigment Cells of the Eye

Previous Serial Number: NEI-73 LVR 149

Principal Investigator: W. Gerald Robison, Jr., Ph.D.

Other Investigators: Toichiro Kuwabara, M.D.
David G. Cogan, M.D.

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.1
Other:	0.9

Project Description:

Objectives: Our purpose is to investigate the precise physical and chemical interrelationships that exist between the pigmented cells and the visual cells of the eye. We propose to study how the pigmented cells perform their roles and what specific aspects are absent in various pathological cases.

Methods Employed: We utilized recently developed histochemical techniques and electron microscopy for the precise localization of peroxidase within cells. Correlations between peroxidase activity and lipid metabolism of the retinal pigment epithelium were investigated by studying mice with vastly different levels of serum lipids and cholesterol: 1) mice with a mutation for obesity and high serum lipids; 2) two mouse strains which had undergone strong genetic selection for high serum cholesterol and low serum cholesterol respectively; and 3) mice which had been fed nafenopin, a drug which induces a significant decrease in the amount of serum lipids and serum cholesterol.

We used an hereditary pathological condition as an experimental tool to investigate how the membrane discs of the rod outer segments are digested by the retinal pigment epithelium. The Chediak-Higashi syndrome of humans represents a block in the digestive mechanisms of the cells. The beige mutant of the C57 BL/6N mouse strain serves as a model animal for this disease. Analyses of normal and mutant cells have been carried out by light microscopy and modern techniques of high resolution electron microscopy as well as histochemistry at both levels.

Major Findings: We demonstrated that cell organelles called microperoxisomes, owing to their size and peroxidase content, are abundant in retinal pigment epithelium of humans, monkeys, domestic fowl, frogs, mice, and rats. The number of microperoxisomes in the retinal pigment epithelium increased nearly three fold in animals which had low serum cholesterol and lipids following treatment with nafenopin. Therefore, microperoxisomes may play an important role in lipid metabolism.

We found that microperoxisomes are not related to the development of lysosomes or melanin granules. They are normal in the mouse model for the Chediak-Higashi syndrome even though the lysosomes and melanin granules are defective.

We compared the digestive mechanisms and melanogenesis of the pigment epithelium of beige (bg/bg) and black (+/+) mice of the C57 BL/6N strain. We localized acid phosphatase within the pigment epithelial cells and discovered defects in the packaging of this important digestive enzyme in the mutant (beige) mouse. We found more relation between melanogenesis and lysosome formation than was shown previously.

Significance to Biomedical Research and the Program of the Institute: Studies on pigmented cells of the eye contribute directly to our understanding and managing of many retinal and choroidal diseases. A relation between serum lipids and the number of microperoxisomes in the retinal pigment epithelium, if established by further observations, would provide a means of recognizing, lipid disorders from pathological preparations of ocular tissues.

Proposed Course: Other mutants and techniques will be used for investigating the various roles of the pigmented cells. An attempt will be made to determine those characteristics which are essential to the maintenance of normal vision.

Keyword Descriptors: pathology of retinal pigment epithelium and choroid, function of retinal pigment epithelium, electron microscopy and ultrastructural histochemistry of Chediak-Higashi syndrome.

NEI Research Program: Retinal and Choroidal Diseases-Development, Structure, Function and Degeneration (Choroid, Pigment Epithelium and Related Disorders)

Experimental Subject or Tissue Source: Human/Rhesus Monkey/Fowl/Frog/Mouse/Rat

Research Objective: Etiology

Honors and Awards: None

Publications:

· Robison, W.G.Jr., Kuwabara, T. and Cogan, D.G.: Lysosomes and melanin granules of the retinal pigment epithelium in a mouse model of the Chediak-Higashi syndrome. Invest. Ophthalmol. 14: 312, 1975.

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

Project Title: The Membrane Biochemistry of the Visual Process

Previous Serial Number:

Principal Investigator: Hitoshi Shichi, Ph.D.

Other Investigators: Robert L. Somers, B.S.
Paul J. O'Brien, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.3

Project Description:

Objectives: Photon capture and subsequent generation of the visual signal by the photoreceptor cell are essentially membrane phenomena. The overall objectives of this project are to investigate the light-dark adaptation processes of the retina by means of modern techniques of membrane biology and biochemistry. More specifically, these are (1) identification of a sequence of molecular events initiated by absorption of photons and leading to visual excitation (light process) and (2) elucidation of the biochemical mechanism of regeneration of the photosensitivity of photoreceptor membranes (dark process).

The investigations presented in this annual report deal with two aspects of photoreceptor function, i.e. the phosphorylation of rod membrane protein and the regeneration of rhodopsin. Rhodopsin regeneration: In order to test the previously proposed cycle of rhodopsin regeneration in rod membranes, the transfer of retinal from phospholipid-retinal complex to opsin was investigated. The effect of the fatty acid composition of phospholipid on the reaction cycle was also studied. Phosphorylation of photoreceptor membrane protein: The phosphorylation of rod membrane proteins by ATP was recently reported. The phosphorylated protein was identified with rhodopsin by SDS gel electrophoresis. Since the electrophoretic identification with SDS only demonstrates that the phosphorylated protein has mobility characteristics similar to those of the major protein (rhodopsin) of rod membranes, attempts were made to isolate and identify the phosphorylated membrane protein.

Methods Employed: Biochemical methods such as centrifugation, column chromatography, ultrasonic irradiation, spectroscopic analysis and radioisotope assay.

Major Findings: (1) Rhodopsin regeneration. We have previously suggested a reaction cycle in which the all-trans retinal released from bleached rhodopsin forms a protonated Schiff complex with phosphatidylethanolamine and is photoisomerized to the 11-cis isomer in the complex form. The formation of retinal-phospholipid complex requires the presence of unsaturated fatty acid side chains in the phospholipid; no complex is formed if the phospholipid contains saturated fatty acids only. However, if saturated chain-containing phospholipids are incorporated into the vesicles of unsaturated fatty acids (e.g. linoleic acid and oleic acid) or saturated fatty acids (e.g. caprylic acid) which are fluid at room temperature, the complex can be formed and all-trans retinal is successfully photoisomerized to 11-cis retinal. These findings indicate that fluid environments are essential for retinal-phospholipid complex formation and possibly also for the subsequent photoisomerization reaction. The results are of particular interest in understanding the physiological significance of a high concentration of polyunsaturated fatty chain-containing phosphatidylethanolamine and high fluidity of photoreceptor membranes. The previous studies did not establish whether the 11-cis retinal formed by photoisomerization is directly transferred to opsin from phospholipid or it is once released and reacts with opsin to form rhodopsin. From kinetic studies of the transfer reaction, we now have evidence that retinal is directly transferred to opsin from retinylidne phosphatidylethanolamine. (2) Phosphorylation of photoreceptor membrane protein. The major findings are summarized as follows: (i) Phosphorylation of rod membrane proteins by ATP occurred only in the light. The phosphorylation appeared to be little affected by addition of cyclic AMP or cyclic GMP. The bleached and phosphorylated rod membranes regenerated isorhodopsin by incubation with 9-cis retinal in the dark. (ii) When the phosphorylated and regenerated pigment was extracted and purified on an Agarose column, the phosphorylated protein was found to be separated, although not completely, from the visual pigment. Upon bleaching by light, the visual pigment and phosphorylated protein behaved in an identical manner. (iii) Subsequent purification on ECTEOLA-cellulose columns permitted complete separation of the phosphorylated protein from unphosphorylated pigment. The phosphorylated protein is photosensitive and shows the absorption spectrum of isorhodopsin. The protein incorporates at least 20 phosphate groups per mole of retinal chromophore. The phosphorylated pigment accounts for less than 1% of the total visual pigment; most rhodopsin molecules are not phosphorylated at all under various conditions investigated.

Significance to Biomedical Research and the Program of the Institute: (1) Rhodopsin regeneration is undoubtedly an important dark-adaptation process and is a prerequisite for maintaining unimpaired rod vision (dim-light vision). The present finding indicates that the reisomerization of all-trans retinal to the 11-cis form, a cardinal step in rhodopsin regeneration, requires fluid environments provided by unsaturated fatty chains of phospholipids. Polyunsaturated fatty acids which are found abundantly in photoreceptor membranes are "essential fatty acids" or their derivatives and can not be synthesized by man. The importance of unsaturated fatty acids for biosynthesis (assembly) of photoreceptor membranes was recently demonstrated. Thus, a dietary deficiency

of essential fatty acids has an adverse effect on visual function. (2) Phosphorylation of synaptic membrane proteins was recently demonstrated and suggested to be involved for modulation of ionic permeability (hence, membrane potential) of synaptic membranes. However, the chemical nature of phosphorylated proteins has not been established in any of the known instances of membrane-protein phosphorylation. The present experiment with rod membranes indicates that rhodopsin is the protein phosphorylated and that only a minor fraction of rhodopsin molecules is phosphorylated. Although the significance of this finding has yet to be fully understood, it is probable that the phosphorylation reaction is involved in a biochemical step of visual excitation. Elucidation of the biochemical mechanism of this phosphorylation reaction may help construct a useful model for other sensory processes in which phosphorylation of membrane proteins is expected to play an important role.

Proposed Course: (1) Rhodopsin regeneration. Attempts to elucidate the quantitative aspects of the photoisomerization reaction mediated by phosphatidylethanolamine, including quantum efficiency measurements, will be continued. The physiological significance of the proposed regeneration cycle will be tested with living animals. (2) Rhodopsin phosphorylation: The chemical analysis of phosphorylated rhodopsin will be performed. The cellular localization of the phosphorylated pigment will be determined. The kinetic aspects of the phosphorylation reaction will be investigated under in vivo as well as in vitro conditions.

Keyword Descriptors: bovine eye, photoisomerization of vitamin A aldehyde, rhodopsin regeneration, phosphatidylethanolamine, photoreceptor membranes, protein phosphorylation, ATP

NEI Research Program: Retinal and Choroidal Diseases--Development, Structure, Function, and Degeneration (Visual Pigments, Photoreceptors, and Visual Transduction Disorders

Experimental Subject or Tissue Source: Bovine

Research Objective: Etiology

Honors and Awards: None

Publications:

Shichi, H. and Shelton, E.: Assessment of physiological integrity of sonicated retinal rod membranes. J. Supramol. Struct. 2: 7-16, 1974.

Shichi, H.: Molecular biology of vision. Protein Nuclei-Acid Enzyme 19: 514-526, 1974.

Shichi, H.: A visual pigment in microorganisms? --Bacteriorhodopsin. Chemistry and Biology 12: 724-730, 1974.

Shichi, H. and Somers, R.L.: Is retinal isomerase an enzyme? Federation Proc. 33: (5)1526, 1974.

Shichi, H. and Somers, R.L.: Possible involvement of retinylidene phospholipid in photoisomerization of all-trans retinal to 11-cis retinal. J. Biol. Chem. 249: 6570-6577, 1974.

Shichi, H., Somers, R.L. and O'Brien, P.J.: Phosphorylation of rhodopsin: Most rhodopsin molecules are not phosphorylated. Biochem. Biophys. Res. Comm. 61: 217-221, 1974.

Project No. Z01 EY 00007-01 LVR
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

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

Project Title: The Molecular Pharmacology of the Eye

Previous Serial Number: None

Principal Investigator: Hitoshi Shichi, Ph.D.

Other Investigators: Daniel W. Nebert, M.D.

Cooperating Units: National Institute of Child Health and Human Development

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: Molecular pharmacology, a discipline of biochemistry concerning the action and metabolism of drugs, chemical carcinogens, hormones, and neurotransmitters, has been totally neglected in ophthalmic research. In this project, basic as well as clinical problems that are considered of molecular-pharmacological importance for ocular function will be investigated.

Aromatic hydroxylations of drugs and polycyclic hydrocarbons are catalyzed by the membrane-bound monooxygenases. The importance of the hydroxylation reactions to chemical carcinogenesis, pharmacology, and toxicology is widely recognized. The objective of the experiment described in this report is to elucidate the cellular localization and the functional significance of one such monooxygenase, the aryl hydrocarbon hydroxylase activity in the eye and to relate the findings to preventive and diagnostic measures of ocular carcinogenesis.

Methods Employed: Eyes were collected from inbred strains of mice that had received aromatic hydrocarbons. The aryl hydrocarbon hydroxylase activity of eye homogenates was determined by high-sensitivity fluorospectroscopy.

Major Findings: (1) The aryl hydrocarbon hydroxylase (i.e. benzo(a)pyrene hydroxylase) activity is inducible in the eye with polycyclic hydrocarbons such as β-naphthoflavone, 3-methyl-cholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin (2) The ocular hydroxylase activity is induced only in

inducible mouse strains and is under the same genetic regulation as that in liver. (3) Histochemical examinations of the eyes from induced animals indicate that the hydroxylase activity is apparently associated with the pigment epithelium-choroid layer rather than the retina.

Significance to Biomedical Research and the Program of the Institute: Metabolites of polycyclic hydrocarbons are believed to be involved in carcinogenesis in a variety of tissues. It is therefore possible that a genetic aberration in the regulation of aryl hydrocarbon hydroxylase induction in ocular tissues may set off carcinogenesis in the eye. A recent report shows that susceptibility to bronchiogenic carcinoma in man is highly correlated with the high levels of the lymphocyte aryl hydrocarbon hydroxylase activity. Since the present study demonstrates a common genetic expression in aryl hydrocarbon hydroxylase induction in the eye and other tissues, similar attempts to correlate the lymphocyte hydroxylase activity with susceptibility to retinoblastoma and ocular melanoma may be of prophylactic significance.

Proposed Course: Cellular processes involved in aryl hydrocarbon hydroxylase induction in the eye will be better characterized and a possible function of the hydroxylase activity in the ocular defense mechanism against drugs and carcinogens will be investigated.

Keyword Descriptors: mouse and bovine eye, carcinogens, benzo(a)pyrene, polycyclic aromatic hydrocarbons, aryl hydrocarbon hydroxylase, genetic variance, enzyme induction

NEI Research Program: Retinal and Choroidal Diseases--Development, Structure, Function, and Degeneration (Choroid, Pigment Epithelium, and Related Disorders

Experimental Subject or Tissue Source: Mouse/Bovine

Research Objective: Etiology, Diagnosis

Honors and Awards: None

Publications: None

Project No. Z01 EY 00024-01 LVR
1. Laboratory of Vision Research
2. Section of Biochemistry
3. Bethesda, Maryland

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

Project Title: Intermediary Metabolism of the Cornea

Previous Serial Number: None

Principal Investigator: David R. Whikehart, Ph.D.
(Senior Staff Fellow)

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: This investigation is concerned with the biochemical mechanisms that control corneal hydration (deturgescence). The specific inquiries are: a) significant intermediates of carbohydrate, and possibly amino acid, metabolism that are linked to the ion or water pump(s) and their role (b) relative roles of importance of corneal endothelium vs. epithelium as sites for these pumps (c) possible chemicals or drugs that might control the deturgescent mechanism.

Methods Employed: The major source of tissue has been bovine eyes; rabbit eyes have been used for tissue culture. Extremely sensitive and accurate techniques of assay employing double-beam spectrophotometry and gas liquid chromatography have been employed. These procedures assay intermediates at the nanogram level. Tissue cultures of endothelial cells have been initiated from micro-dissected endothelial/Descemet's layer buttons.

Major Findings: Investigation of endogenous levels of reduced and oxidized glutathione in both epithelial and endothelial tissue of bovine cornea showed remarkable differences in the two tissue types. Epithelial cells have high levels of reduced glutathione as found in brain and retina. The oxidized levels were quite low which is a characteristic of most tissues. Endothelial cells had a lower amount of reduced glutathione compared to epithelial cells, but possessed high amounts of the oxidized form (about 60% of the total content).

The discovery of this high level of oxidized glutathione implies that the hexose monophosphate shunt (highly active in corneal epithelial tissue) may not be very active in corneal endothelium. It points to the existence of a pool of oxidized glutathione in that tissue. The use of standard controls and non-oxidizing media confirmed that the finding could not be attributable to a fault in the assay system. Preliminary results on the levels of ascorbic acid in cornea, indicate that the amount of ascorbate in endothelia is as high as that in epithelia. Such high levels could favor the production of high levels of oxidized glutathione.

Significance to Biomedical Research: Corneal diseases involving disturbances in the proper hydration (deturgescence) of the cornea resulting in cloudy impaired vision are thought to be the result of metabolic dysfunction (degeneration). Such dysfunctions are the result of damage from transplants, storage conditions (cornea banks), inflammatory reactions and inherent dystrophies. Presently, however, the normal metabolic functions associated with the hydration pump(s) and its controls have not been adequately described. Since glutathione is known to strongly promote deturgescence and because it is linked to carbohydrate metabolism (via the pentose shunt), its endogenous level is quite important. The high level of oxidized glutathione, found in the corneal endothelium, implies the existence of a metabolic scheme quite modified from that of corneal epithelium and of a number of other cellular tissues. It has only recently been discovered that the oxidized form is equally as effective as the reduced form in stimulating the pump(s), but it is not understood how this takes place.

Proposed Course: Presently, this study will be involved with levels of metabolites and enzyme activities closely associated with glutathione oxidation/reduction and the pentose shunt. For example, ascorbic acid levels and the activities of glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase will be measured. Corneal endothelial tissue cultures will be used to supplement bovine eyes as a tissue source. Corneal epithelium as well as endothelium will be investigated since the former tissue probably cooperates in some way with hydration control. Other metabolites, such as adenosine, will also be investigated. Ultimately the study will concern itself with possible interactions of these metabolites with Na^+K^+ ATPase and/or other cell wall sulfhydryl interactions. It is at that stage that controls (i.e., drugs, chemicals) might be tested for usefulness in controlling hydration.

Keyword Descriptors: intermediary metabolism, cornea, corneal diseases, function, degeneration, deturgescence, corneal endothelium, corneal epithelium, glutathione, ascorbic acid, hexose monophosphate (pentose) shunt

NEI Research Program: Corneal Diseases-Development, Structure, Function and Degeneration

Experimental Subject or Tissue Source: Bovine/Rabbit

Research Objective: Etiology, Treatment

Honors and Awards: None

Publications:

Whikehart, D.R.: Total and Oxidized Glutathione in Bovine Corneal Epithelium and Endothelium. Experimental Eye Res. 20, (in press), 1975.

Project No. Z01 EY 00138-03 LVR
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

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

Project Title: Photoexcitatory Process in Visual Cells

Previous Serial Number: NEI-73 LVR 138

Principal Investigators: S. Yoshikami, Ph.D.
W.A. Hagins, M.D., Ph.D., NIAMDD
W.E. Robinson, Ph.D., NIAMDD

Other Investigators: None

Cooperating Units: NIAMDD

Man Years:

Total:	2.0
Professional:	2.0
Other:	0.0

Project Description:

Objectives: In the visual receptor cell, the movement of ion across its membrane and the control of this ion flow by light released intracellular excitatory transmitter have been shown by us to be basic in the initiation of the light detection process in the visual system. The nature of this excitatory transmitter, how light and the visual cell control its passage across membranes, and how this transmitter in turn regulates movement of ion across the visual cell membrane are the focal points of our investigations.

Methods Employed: 1. The ionic currents are being studied by electrical means with the aid of microscopy. 2. The driving forces and control of the ionic currents of the visual cell are being determined by measuring its ionic and biochemical contents with the electron microprobe and standard chemical means.

Major Findings: 1. Topology of the visual cell: The fluorescent dansylated amino acid cystine has been discovered to have the unique properties of not being able to permeate through biological membranes but becoming brilliantly fluorescent when bound to them. We have successfully used these features of this non-toxic stain to determine the topology of the rod and cone plasma membranes and to assay the presence or disruption of the plasma membrane envelope in isolated vertebrate rod outer segments.

This dye revealed that in living isolated retinas the outer segment membrane topology of rods differ from those in cones. In cone cells, the membrane of the outer segment is one continuous surface whereas in rods the membrane surface is discontinuous and the plasma membrane is separated from the disc membrane. This finding in conjunction with our other studies show that the plasma membrane of rod outer segment, where the ionic current is controlled by light, is remote from those membranes which contain virtually all of the light absorbing pigment rhodopsin. Consequently there must be some means of conveying information from the light absorbing pigments to the plasma membrane such as a chemical transmitter.

In the past we have reported evidence which support our proposition that calcium may be this chemical messenger. A further test, among others, for calcium as the photoexcitatory medium is whether the visual cell can be desensitized to light by depleting it of calcium or by introducing into the cytoplasm metal ion buffers.

When the isolated rat retina is bathed in a solution where the pCa is changed from 3 to more than 8, the light sensitivity of the rod, as measured by its half-saturating amplitude of the photocurrent, is raised from 30 to as much as 900 photons absorbed rod^{-1} $flash^{-1}$. Part of this desensitization can be attributed to the reduction of calcium stores in the cell and the remainder could be accounted if some non-specific calcium binding sites have become exposed in the cytoplasm which now compete with the ionic current control sites when the calcium activity was thus lowered.

If competing calcium transmitter buffer sites were present in this condition then one should be able to restore part of the sensitivity of the cell to light by saturating these cytoplasmic buffer sites by adding calcium to the cell or by releasing calcium transmitter from the cell's store by the action of a dim light. Indeed when calcium or a dim background light is presented under these conditions the light sensitivity returns and when it is removed insensitivity is restored.

Localized ionic content in vertebrate rod cells was measured with the electron microprobe. Retinas rapidly frozen to liquid nitrogen temperature were studied in the frozen state (<-100°C) for elemental content of sodium, potassium, chlorine, sulfur and phosphorous. The ionic composition of the cell is similar to that of other nervous tissues. It was found in dark adapted rods that there is more sodium in the outer segment than in the inner which is the reverse of what was found for potassium. Currently we are determining the changes in ionic content of the rod cell caused by changes in illumination and metabolic state of the cell.

The determination of soluble biochemicals in the isolated outer segments of rods is now possible with the discovery of the fluorescent stain didansyl cystine as a reporter of the nature of rod plasma membranes. This selective dye makes it now possible to count the number of isolated rod outer segments which have their enveloping plasma membrane and thus cellular content intact.

We have thus measured with luciferin-luciferase the ATP content in freshly isolated frog rod outer segments to be 2-5mM in the cytoplasmic space. The ATP content of these organelles is reduced rapidly by light. Further work is being conducted on ATP and other biochemicals within the rod cell to ascertain their respective participation in the photoexcitatory process of vision.

Significance to Biomedical Research and Program of the Institute: Our understanding of the causes and our ability to prevent and repair many of the numerous visual disorders depends on a clear knowledge of the processes involved in normal vision. Our findings on the importance of calcium and its control in visual cell excitatory process and the tight coupling between photo-excitation and energy metabolism of this cell may help us to realize some of the basis for pathology in the retina.

Proposed Course: The connection between photoexcitation and energy metabolism, in particular the participation of ATP in this process, will receive special attention. Investigation will be continued on how light initiates cellular processes which control movement of ions across visual cell membranes and thus initiates the process of vision.

Keyword Descriptors: photoexcitation, rod, cone, membranes, retina, calcium

NEI Research Program: Retinal and Choroidal Diseases

Experimental Subject or Tissue Source: Rat/Frog/Fish

Research Objective: Etiology and Prevention

Honors and Awards: None

Publications:

Hagins, W.A. and S. Yoshikami. A role for Ca^{++} in excitation of retinal rod and cone. Exp. Eye Res. 18: 299-305, 1974.

Yoshikami, S., W.E. Robinson and W.A. Hagins. Topology of the outer segment membranes of retinal rods and cones revealed by a fluorescent probe. Science 185, 1176-1179, 1974.

Hagins, W.A. and S. Yoshikami. Ionic aspects of excitation in rod outer segments. Ciba Foundation. (in press).

Hagin, W.A. and S. Yoshikami. Ionic mechanisms of photoreceptors. N.Y. Acad. Sci. (in press).

Project No. Z01 EY 00001-05 LVR
1. Laboratory of Vision Research
2. Section on Experimental Embryology
3. Bethesda, Maryland

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

Project Title: Ocular Morphogenesis

Previous Serial Number: NEI-71 LVR-001

Principal Investigator: A. Coulombre, Ph.D.

Other Investigators: J. Coulombre, B.S.

Cooperating Units: Section on Biochemistry and
Section on Experimental Pathology
Laboratory of Vision Research, IR, NEI, NIH

Man Years:

Total:	1.50
Professional:	0.75
Other:	0.75

Project Description:

Objectives: The project seeks to identify and to characterize tissue interactions which control the orderly growth and differentiation of the developing vertebrate eye. Information about the durations, sequence and nature of these interactions is essential for developing bases of accurate diagnoses, for establishing etiologies, and for evolving effective treatments for most congenital eye defects. Such information also contributes to rational programs of prevention by identifying the several restricted periods during which the developing eye is at maximum hazard to specific types of derangements as a result of a variety of teratological influences.

Methods Employed: Routine experimental-embryological procedures were supplemented with techniques drawn from chemistry, microscopy and tissue culture to analyze the development of the eyes in embryos of vertebrates, principally the domestic fowl. Cell-culture methods were developed for clonal passage of pigmented epithelial cells (PE) and neural crest cells from chick embryos. The use of Millipore filters as substrata in culture permitted the collection and processing, by chemical and biochemical means, of extracellular deposits from the cultured cells and led to the demonstration that the pore size of such substrata significantly influences the growth of cell cultures.

Major Findings: During FY 1975 new information was published by this project bearing upon three of the research programs of the NEI.

I. PE of the retina: A. Induction of the avian sclera: 1. Cultures of PE cells, from donors of all ages tested, produced collagen-bearing deposits. 2. When they were freed of living cells, these deposits induced the formation of hyaline cartilage, a constituent of the avian sclera, in clones of neural crest cells and in cultures of competent embryonic head mesenchyme. 3. Deposits from PE cells of donors younger than stage 26 were effective, whereas deposits from PE cells of donors older than stage 37 were not. 4. The potency of deposits was virtually destroyed by treatment with 0.1N HCl, collagenase or neuraminidase. B. Effects of cyclic AMP and of fractions of chick-embryo extract on cloned, retinal, pigmented epithelium in tissue culture (done in collaboration with the Sections on Biochemistry and Experimental Pathology, LVR, IR, NEI, NIH): Seven clonal lines of chick-embryonic PE cells, isolated between stages 23 and 27, were exposed in vitro to "light" or "heavy" Sephadex-G-25 fractions of chick-embryo extract in the presence or absence of dibutyryl cyclic 3',5'-adenosine monophosphate (BcAMP). BcAMP enhanced the initial rate of attachment of the cells to the substratum, depressed cellular proliferation, promoted an epithelial cell morphology (including the elaboration of basement lamina at the basal surface of the cell colonies) and retarded melanin pigmentation of the cells. These findings establish that cultures of normal, developing PE respond to BcAMP similarly to cultures of transformed-cell lines.

II. Cornea: A small colloidal tracer, ruthenium red, was used to assess the permeability of the surfaces of the developing cornea to small macromolecules. A. Endothelium: This marker readily traversed the 30A interspace of the gap junctions between the corneal endothelial cells at all stages in embryonic development. B. Epithelium: Although desmosomal junctions were present between cells of the corneal epithelium of the chick embryo throughout its development, ruthenium red penetrated the intercellular spaces of this epithelium only before, and not after, 14 days of incubation. This finding demonstrates a change in developing epithelium of the cornea which correlates temporally with several other events related to the development of transparency in the cornea (transient appearance of hyaluronidase, disappearance of hyaluronate, deturgensence of the cornea, decrease in corneal sodium, increase in corneal potassium and increase in the number of strata in the corneal epithelium).

III. Abnormal organogenesis in the eye: Three syntheses of findings, derived in part from this project, were published or accepted for publication during FY 1975. These reviews of normal and abnormal morphogenesis of the eye were addressed to those in the biomedical community concerned with the prevention, diagnosis and treatment of such disorders. They dealt with the interpretation of developmental defects of the eye in the light of what is now known concerning the role of tissue interactions in ocular development.

Significance to Biomedical Research and the Program of the Institute: These findings contribute information on development and congenital abnormalities to three NEI research programs. I. Retinal and Choroidal Diseases: A. The demonstration that basement membrane produced by the embryonic PE induces the

sclera clarified the developmental origin of this eye coat and identified an etiological basis for colobomas of the sclera. B. The results of cultivation of the PE suggest that cAMP may be involved importantly in the differentiation of the PE of the retina. II. Corneal Diseases: The demonstration that a barrier to passage of small macromolecules develops in the epithelium of the cornea coincidentally with the emergence of corneal transparency, identifies a new factor in the role of this epithelium in the development and maintainance of the corneal stroma which underlies it. III. Cataract: The involvement of the lens, as well as other ocular tissues, in the genesis of a number of developmental abnormalities of the eye was reviewed for physicians and others concerned with the delivery of health care services.

Proposed Course: Over the years, this project has achieved most of its initial objectives and has grown too large and complex for the single-project format. Accordingly, it has been terminated. Many of the initiatives arising from it will be embodied in separate, independent projects during FY 1976.

Keyword Descriptors: eye, embryology, congenital abnormalities, cornea, sclera, retinal screening pigments, morphogenesis, ruthenium, cyclic AMP

NEI Research Program: Corneal Diseases-Development, Structure, Function and Degeneration /Retinal and Choroidal Diseases-Development, Structure, Function and Degeneration

Experimental Subject or Tissue Source: Domestic Fowl

Research Objective: Etiology

Honors and Awards: None

Publications:

Coulombre, A.: Steps in embryonic development: some implications for developmental pharmacology. In McKhann, G. and Yaffee, S. (Eds.): Drugs and Poisons in Relation to the Developing Nervous System. Public Health Service Publication No. 1791, 1968, pp. 12-23.

Coulombre, A.J. and Coulombre, J.L.: Mechanisms of ocular development. In Zinn, K.M. (Ed.): The Developing Visual System. Int. Ophthalmol. Clin. 15: No. 1, Boston, Little, Brown & Co. 1975, pp. 7-18.

Coulombre, A. and Coulombre, J.: Abnormal Organogenesis in the Eye. In Wilson, J. and Fraser, F. (Eds.): Handbook of Teratology. New York, Plenum Publishing Co., (in press).

Newsome, D.: In vitro induction of cartilage in embryoinc neural crest cells by products of retinal pigmented epithelium. Devel. Biol. (in press).

Newsome, D.: Collagen synthesis in cultured neural crest cells, their derivatives and retinal pigmented epithelium: stimulation of $(\alpha_1)_3$ collagen production. In Slavkin, H. (Ed.): Proceedings of the Second Santa Catalina Island Colloquium: Effects of Extracellular Matrix on Gene Expression. New York, Academic Press, (in press).

Newsome, D., Fletcher, R., Robison, W., Kenyon, K. and Chader, G.: Effects of cyclic AMP and Sephadex fractions of chick embryo extract on cloned retinal pigmented epithelium in tissue culture. J. Cell Biol. 61: 369-382, 1974.

Project No. Z01 EY 00002-01 LVR
1. Laboratory of Vision Research
2. Section on Experimental Embryology
3. Bethesda, Maryland

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

Project Title: Determinants of Collagenous Structure in the Avian Corneal Stroma

Previous Serial Number: NEI-71 LVR 001

Principal Investigator: Jane L. Coulombre, B.S.

Other Investigator: Alfred J. Coulombre, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.50
Professional:	0.25
Others:	0.25

Project Description:

Objectives: Previous work established that the protein collagen (the principal, non-aqueous constituent of the secondary, mature stroma of the avian cornea) is deposited in lamellae of orthogonally-disposed fibers. In the superficial layers of both the secondary stroma and the primary, acellular stroma which foreshadows it, the axes of these stromal lamellae rotate progressively. The direction of axial rotation is the same in both eyes and, therefore, is asymetric about the body midplane.

In this project, we sought to determine experimentally: 1. whether the direction of rotation was obligatory and invariant, or whether it could be modified; and 2. whether the collagenous architecture of the secondary stroma is dictated by that of the primary stroma.

Methods Employed: The study involved: 1. injection of 6-diazo-5-oxo-L-norleucine (DON), an analog of glutamine, by microcatheter into the extra-embryonic blood vessels of 5-day-old chick embryos; and 2. analysis, as revealed by the Gomori silver technique for reticular collagen, of stromal, collagenous architecture in normal embryos and in experimental embryos at several times following the administration of DON.

Major Findings: Modifications of corneal development following the administration of DON differed markedly from those seen in a previous study following the administration of L-azetidine-2-carboxylic acid (LACA); an analog of the amino acid proline). 1. The Golgi apparatuses of the basal cells of the corneal epithelium remained silver-positive following DON, whereas they became silver-negative for about 16 hours after LACA. 2. Collagen continued to be present beneath the epithelium following DON, but was not deposited for about 18 hours after LACA. 3. The corneal endothelium was incomplete or absent for a day or two following DON, and remained continuous following LACA. 4. Cystic clefts of the stroma were common following DON; by contrast, they never ocurred following LACA. 5. The direction of rotation of the axes of the superficial, collagenous lamellae of the stroma was reversed routinely following DON, while no such reversal followed LACA. 6. DON-induced alterations in the collagenous architecture of the primary corneal stroma were copied when the secondary stroma developed within it, whereas LACA-produced lesions healed in a normal configuration.

The results demonstrate that: 1. the direction of rotation of the axes of the superficial lamellae of the corneal stroma is not obligatory and invariant, since it can be reversed experimentally; and 2. the primary stroma dictates the collagenous structure of the secondary stroma deposited within it.

Significance to Biomedical Research and the Program of the Institute: These findings contribute to the Corneal Disease Research Program of the NEI in two ways. 1. They identify the primary stroma as a major determinant of the collagenous structure of the secondary stroma of the cornea. 2. They establish that damage to the primary stroma early in embryonic development can have profound, adverse consequences for the development, later in embryonic life, of the structure of the secondary stroma of the cornea.

Proposed Course: This project accomplished its objectives, has been reported in an article accepted for publication and has been terminated.

Keyword Descriptors: eye, cornea, stroma, endothelium, embryology, 6-diazo-5-oxo-L-norleucine

NEI Research Program: Corneal Diseases-Development, Structure, Function and Degeneration

Experimental Subject or Tissue Source: Domestic Fowl

Research Objective: Etiology

Honors and Awards: None

Publications:

Coulombre, J.L. and Coulombre, A.J.: Corneal development. V. Treatment of five-day-old embryos of domestic fowl with 6-diazo-5-oxo-norleucine (DON). Devel. Biol. (in press) 1975.

Project No. Z01 EY 00129-03 LVR
1. Laboratory of Vision Research
2. Section on Experimental Pathology
3. Bethesda, Maryland

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

Project Title: Anatomical and Pathological Studies of Ocular Tissues

Previous Serial Number: NEI-73 LVR 129

Principal Investigator: Toichiro Kuwabara, M.D.

Other Investigators: David G. Cogan, M.D.
W. Gerald Robison, Jr., Ph.D.
Masakazu Funahashi, M.D.
Machiko Sakuragawa, M.D.
Yasuna, Hamai, M.D.

Cooperating Units: Simmons Lessell, M.D.
Department of Ophthalmology
Boston University
Laurence Avins, M.D., NICHD

Man Years:

Total:	2.7
Professional:	2.1
Other:	0.6

Project Description:

Objectives: A clear understanding of the pathogenesis of many eye diseases is still far beyond our reach. Also, details of normal structure as well as function, of eye tissues are not quite convincingly demonstrated. Constant investigation on anatomical aspects of normal and patholgocial eye tissues, particularly employing advanced techniques, is of basic importance for further understanding of all eye diseases.

Methods Employed: Ocular tissues of various animals and humans were studied by transmitting and scanning electron microscopy. A great number of pathological materials obtained through surgery and autopsy at the NIH Clinical Center was the main source. Also, many unusual clinicopathological materials which were sent for consultation from various parts of the country were studied in this project.

Major Findings: More than one hundred clinicopathological materials have been studied in detail in this project. Studies on these materials have expanded into research projects which require extensive investigations. The

following are parts of such projects which have produced substantial results.

Development of the prenatal rat retina

In the course of study of various congenital diseases of the retina, it is found that details of the early differentiation of the retinal layers have been described only scarcely in literature. The present study, in which a great number of rat fetuses were used, has demonstrated the earliest cellular differentiation of each retinal neural element by electron microscopy. Some of the new findings are: formation of multivesiculated protrusions at the site of axon and dendrite processes; developing mechanism of the inner and outer limiting membranes; elimination of excess neural cells in the early differentiation of the retina. This study has provided several hitherto unknown developing processes of the retina and is directly useful in interpretation of various developmental abnormalities of the retina.

Development of the optic nerve

Fine structural study of the optic nerve in its early developmental stage has been reported infrequently. The present study has dealt with electron microscopic examination of the optic nerve of rat fetuses beginning at the period of optic cup formation. The study has presented several new findings. At the time of optic cup formation, a great number of neuroepithelial cells undergo necrosis and a tubular optic stalk is formed by a few surviving cells. These stalk cells differentiate into the glial cells. Axons which are formed by the retinal ganglion cells extend into the lateral intercellular spaces of the stalk cells. Clarification of the origin of the glial cells and exact location of axonal invasion are the most significant findings in this study. These findings are readily applicable in explanation of congenital colaboma of the eye.

Toxic effect of cyanide to the optic nerve

Albino rats are intoxicated by sodium cyanide and the optic nerves are studied electron microscopically. It is found that the postbulbar portion of the optic nerve degenerates within a few days following the administration of cyanide. Degeneration, which begins in both axons and myelin sheaths, is progressive. The intoxicated optic nerve becomes severely atrophic by 6 weeks. Since the damage in the retinal ganglion cell is considerably minute, the pathological change in the nerve is explained as being initiated by local degeneration of glial cells due to the anoxia caused by cyanide. Similar pathological changes are commonly found in the optic nerve in tabaco amblyopia of the human. This experimental model in the albino rat has demonstrated several heretofore unknown early changes in optic nerve geneneration. This study also has presented information concerning the demyelinating process of the nerve.

Optic neuropathy by para-chlorophenylalanine

Another experiment on optic neuropathy induced by para-chlorophenylalanine has been completed. The demyelinating change in the optic nerve of

the experimental rat by this toxic agent is found to be progressive. This change is an exact demonstration of neuropathic changes in phenylketonuria of the human.

Study of the lens

Demonstration of the whole view of the lens fibers by scanning electron microscopy was pioneered in this laboratory several years ago. Accumulated information was analyzed and reported recently. Lens fibers have complicated shapes which vary greatly by location. Fibers in the superficial zone have knob-socket junctions and ones in the deep zone have fine ridges. The complexity of the shape and the surface structure of the normal lens fibers are apparently to keep the tight engagement between them. Pathological lens fibers (cataract) lose these junctional structures.

Cytological study on congenital cataractous lens has been carried out as a continuation from the last year. The earlier study on Nakano strain has been published in this fiscal year. Similar but more acute cytologic changes have been demonstrated in Fraser strain cataract. The early change in this strain, which occurs on the 13-14th embryonal day, is characterized as swelling of the posterior end of the lens fibers. Cytological detail of this well-known cataractous mouse lens has been demonstrated in literature by electron microscopy for the first time.

Dr. Sakuragawa has demonstrated the three dimensional view of lens fibers of congenital and galactose induced cataracts. The pathological lens fibers swell profoundly in the early stage of the cataract. Swelling of the lens cells in cataract has been demonstrated biochemically by Dr. Kinoshita and many other followers. The present investigation has clearly demonstrated this cataractogenetic mechanism morphologically. This study is significant because the actual location and degree of involvement of cataract become clearly visible by microscopy.

Also, the study on the denucleation process of the lens cells has been completed during this year. Disturbance in the denucleation process is now re-recognized as a pathogenetic factor of certain cataracts, especially induced by various toxic agents.

Study of the pigment epithelium

Importantce of the pigment epithelium has been re-emphasized by several authors in the past few years. However, detailed study of structure of the pigment epithelium of various animals has been reported infrequently. No systematic comparative study in the fine structure of this tissue has been reported. Electron microscopic findings on 20 different animals have been analyzed. Depending upon the constituent of the retinal photoreceptors and the living environment of the animal, the pigment epithelial cells show considerable species differences.

Scanning electron microscopic study on the pigment epithelium of the monkey was completed during the FY75. The study has shown the dense

distribution of microvilli of the pigment epithelium and their firm adhesion with the outer segments of the retina, presenting a different view concerning the retina-pigment epithelium attachment.

Microcystic degeneration of the cornea

Dr. Cogan (Clinical Branch) and Dr. Kuwabara have studied a case of microcystic degeneration of the corneal epithelium by electron microscopy. This condition, first described by the same investigators in 1965, has been reported by several ophthalmologists in recent years. The present study has demonstrated a new pathogenetic theory; the epithelial cells in this disease have lost their normal polar orientation and form abnormal basement membrane. The epithelial cells, which have been trapped beneath the aberrant basement membrane, undergo degeneration.

Peters' Anomaly

A case of Peters' anomaly has been studied extensively by electron microscopy (with Dr. C. Kupfer). Electron microscopy has indicated that the endothelial cell of the cornea is pathologic and Descemet's membrane is poorly formed. Since glaucoma is a common complication in this disease, fine structures of the trabecular meshwork and schlemis canal of this case are carefully examined. The result will be reported shortly.

Eyelid muscle

Ptosis, a dropping of the upper eyelid, is a common symptom of various muscular and neural diseases. However, structure of muscles which control the function of the upper lid has been studied scarcely. Drs. Kuwabara and Cogan have examined many normal and pathological upper eye lids of humans. Their study has corrected the earlier concept on the location of insertion of the levator muscle in the upper lid. No direct connection of the muscle to the tarsus is seen in the present study. Also, the presence of Muller's smooth muscles is re-emphasized.

Pathologic eyelid muscles including many cases of oculopharyngeal dystrophy are studied also. Pathologic changes in the muscle fiber is found to be non-specific. A large variety of cytological changes has been demonstrated in the present cases.

Ciliary epithelium

The effect of intraocular pressure on the ciliary epithelium has been studied by Dr. Okisaka. Perfusion of urea or lactamide into the carotid artery of the rhesus monkey causes a sudden decrease in the intraocular pressure and selective degeneration of the pigmented cells of the ciliary epithelium. This is totally new information to the present knowledge of glaucoma physiology.

Many other ocular tissues of unusual diseases have been studied extensively. Some of these are: Menkes' kinky hair syndrome, Zellweger's disease, Richner Hanhart Syndrome, and Lowe's syndrome. Results of these studies will be reported in the near future.

Significance to Biomedical Research and the Program of the Institute: This section is one of a few laboratories in this country which is capable of carrying out this type of research. The investigations in this project have direct meanings in the understanding of pathogenesis of various diseases of the eye.

Proposed Course: A similar project is actively going on and will be continued in the next fiscal year.

Keyword Descriptors: development of retina, development of neural cell development of optic nerve, optic stalk, development of axon, development of glial cell, cyanide effect on optic nerve, demyelination, amblyopia, phenylketonuria, structure of lens fiber, maturation of lens fiber, congenital cataract, swelling of lens fiber, denucleation of lens cell, topography of pigment epithelium, microvilli of pigment epithelium, species difference of pigment epithelium, microcystic degeneration of cornea, corneal epithelium, Peters anomaly, levator muscle, Muller's lid muscle, ptosis, oculopharyngeal dystrophy, ciliary epithelium, intraocular pressure with hyperosmotic drug.

NEI Research Program: Retinal and Choroidal Disease-Development, Structure, Function and Degeneration (Choroid, Pigment Epithelium and Related Disorders) / Sensory and Motor Disorders of Vision-Congenital, Developmental and Degenerative Abnormalities / Cataract-Lens Structure and Function and Metabolic, Toxic and Traumatic Cataract / Cataract-Congenital Cataract and Developmental Abnormalities of the Lens / Corneal Diseases-Development, Structure, Function and Degeneration / Glaucoma-Developmental Glaucoma / Glaucoma-Primary Glaucoma (Open Angle Glaucoma)

Experimental Subject or Tissue Source: Rhesus Monkey/Rat/Mouse/Human

Research Objective: Etiology, Treatment, Diagnosis

Honors and Awards: None

Publications:

Kuwabara, T. and T.A. Weidman: Development of the prenatal rat retina, Invest. Ophthal. 13: 725-739, 1974.

Kuwabara, T.: Development of the optic nerve of the rat. Invest. Ophthal. In press 1975.

Lessell, S. and T. Kuwabara: Fine structure of experimental cyanide optic neuropathy, Invest. Ophthal. 13: 748-756, 1974.

Avins, L., G. Guroff and T. Kuwabara: Ultrastructural changes in rat optic nerve associated with hyperphenyl-alaninemia induced by para-chlorophenylalanine and phenylalanine. J. Neuropath. Exp. Neurol. 34: 178-188, 1975.

Kuwabara, T.: The maturation of the lens cell. A morphologic study, Exp. Eye Res. 20: 427-443, 1975.

Hamai, Y., H.N. Fukui and T. Kuwabara: Morphology of hereditary mouse cataract, Exp. Eye Res. 18: 537-546, 1974.

Hamai, Y. and T. Kuwabara: Early cytologic changes of Fraser cataract, An electron microscopic study. Invest. Ophthal. (in press), 1975.

Kuwabara, T. and M. Imaizumi: Denucleation process of the lens. Invest. Ophthal. 13: 973-981, 1974.

Kuwabara, T.: Species difference of the pigment epithelium. In Zinn, K.M. and M.F. Marmor Ed. The Retinal Pigment Epithelium, Harvard Press, Cambridge, 1975 (in press).

Sakuragawa, M. and T. Kuwabara: The pigment epithelium of the monkey, Topographic study by scanning and transmitting electron microscopy. Arch. Ophthal. (in press), 1975.

Cogan, D.G., T. Kuwabara, D.D. Donaldson and E. Collins: Microcystic Dystrophy of the cornea. A partial explanation for its pathogenesis Arch. Ophthal. 92: 470-474, 1974.

Kupfer, C., T. Kuwabara and S.J. Walter: The histopathology of Peters' anomaly. A light and electron microscopic study. Am. J. Ophthal. (in press), 1975.

Kuwabara, T. and D.G. Cogan: Structure of the muscles of the upper eyelid. Arch. Ophthal. (in press), 1975.

Johnson, C.C. and T. Kuwabara: Oculopharyngeal muscular dystrophy. Am. J. Ophthal. 77: 872-879, 1974.

Okisaka, S., T. Kuwabara and S.I. Rapoport: Selective destruction of the pigmented epithelium in the ciliary body of the eye. Science 184: 1298-1299, 1974.

Project No. Z01 EY 00130-03 LVR
1. Laboratory of Vision Research
2. Section on Experimental Pathology
3. Bethesda, Maryland

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

Project Title: Effect of Laser on the Retina

Previous Serial Number: NEI-73 LVR-130

Principal Investigator: Toichiro Kuwabara, M.D.

Other Investigators: Shigekuni Okisaka, M.D.

Cooperating Units: Dr. William T. Ham
Department of Biophysics
University of Virginia
Richmond

Man Years:

Total:	1.0
Professional:	0.8
Other:	0.2

Project Description:

Objectives: Photocoagulation treatment of various retinal diseases using lasers and xenon arc has become popular in clinical applications in recent years. However, cytological study of the treated retina has been reported infrequently. Especially, cytological effect of the laser beam on the retinal blood vessel has been scarcely documented.

Methods Employed: Retinas of normal rhesus monkeys were exposed to argon beams using identical doses to those of the clinical treatment. Also, a small amount of blood was injected into the vitreous to simulate the intravitreous neovascularization. Argon laser treatment was applied on the thin blood streaks over the retina. The treated retinas were examined by electron microscopy at various time intervals following the exposure.

Major Findings: The cytological appearances of the retinal damage produced by both ruby and argon lasers are almost identical. Damages are localized in the pigment epithelium and photoreceptor cells and the direct change in the blood vessels is extremely slight. However, at a later stage, occlusion of short segments of blood capillaries is noted in the damaged retina, the photoreceptor cells of which have been eliminated by the direct burn.

Argon laser focused on the blood film often causes severe cellular damage in the ganglion cells and the nerve fibers. The cytological damage in these layers may cause disturbance of the visual function.

Extremely short exposures of YAG laser produce no visible damage in the retina, but severe damage in individual melanin granules of the pigment epithelium is noted. Heat absorption by melanin granules is apparently the first stage of the cellular damage.

Significance to Biomedical Research and the Program of the Institute: The present observation has revealed that laser effect on the blood vessel is secondary rather than direct. This information is helpful in evaluating the effect of treatment of vascular diseases of human retinas. The experiment with YAG laser has demonstrated the precise site of heat absorption in the pigment epithelium. This information will help future development of this new laser for medical application.

Proposed Course: Materials obtained from earlier experiments on rhesus monkeys will be analysed in the next year. The main research will be focused on the effect of multiple lesions on the retina.

Keyword Descriptors: laser photocoagulation, retinal blood vessel vitreal blood vessel, YAG laser, laser damage to retina, laser damage to pigment epithelium

NEI Research Program: Retinal and Choroidal Diseases-Vascular and Circulatory Abnormalities

Experimental Subject or Tissue Source: Rhesus Monkey

Research Objective: Treatment

Honors and Awards: None

Publications:

Ham, W.T., H.A. Mueller, A.I. Goldman, B.E. Newman, L.M. Holland and T. Kuwabara: Ocular hazard from picosecond pulses of ND: YAG laser radiation, Science 185: 362-363, 1974.

Okisaka, S. and T. Kuwabara: The effect of laser treatment of the retinal capillaries, An experimental study. Am. J. Ophthal. (in press), 1975.

Project No. Z01 EY 00131-03 LVR
1. Laboratory of Vision Research
2. Section on Experimental Pathology
3. Bethesda, Maryland

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

Project Title: Light Effect on the Retina

Previous Serial Number: NEI-73 LVR-131

Principal Investigator: Toichiro Kuwabara, M.D.

Other Investigators: Shigekuni Okisaka, M.D.
Masakazu Funahashi, M.D.

Cooperating Units: None

Man Years:

Total:	1.6
Professional:	1.3
Other:	0.3

Project Description:

Objectives: Recent studies by us and many others have demonstrated cytologic similarities between retinas of degenerating diseases of the human and animals and those damaged by light. Human diseases related to this category are retinitis pigmentosa and macula degeneration. Precise cytologic analysis of the effect of light on the retina and pigment epithelium is necessary for the understanding of pathogenesis of these diseases and for evaluation of the effectiveness of the treatment.

Methods Employed: Retinas of normal rhesus monkeys and albino rats were exposed to lights of various natures and densities. The exposed retinas were excised at various time intervals and studied by electron microscopy. Retinas and pigment epithelial cells of hibernating ground squirrels, frogs and bats were studied by electron microscopy.

Major Findings: Retinas exposed to electronic flash for a short period of time showed multiple breakage of the plasma membrane of the outer segment prior to the occurrence of damage in the photoreceptor disc membrane. Groups and individual disc membranes spill out through the broken cell membrane into the subretinal space. These disc membranes are then phagocytized by the pigment epithelium. This change is considered to be a type of photoreceptor turn-over process. Turn-over of cone outer segments, in which no large phagosomes are formed, may be carried out by a similar process.

Experiments on albino rats show that the outer segment turn-over mechanism is profoundly enhanced by the exposure to fluorescent lamps. However, the activated phagocytotic activity in the pigment epithelium is observed only for a short period of time following a relatively short exposure. Severely damaged pigment epithelial cells have no phagocytotic activity.

The cytologic changes in the retina and pigment epithelium are grouped into two categories: photic and thermal. The pathological change produced by photic energy is primarily localized in the photoreceptor elements and the variable cytological appearance of the pigment epithelial cell is due to the degrading process of phagocytosed disc membranes. On the other hand, thermal energy is absorbed by pigment granules and the primary degeneration occurs in the pigment epithelial cell.

For the study of metabolic correlation between the retina and the pigment epithelium, these tissues of hibernating animals were examined. Photoreceptor outer segments of the ground squirrel (predominantly cones) disappear during natural and experimental hibernation and the outer segments of frogs and bats become short. Phagocytized tips of rod outer segments in the pigment epithelial cells of the frog and bat remain unchanged during hibernation. Smooth endoplasmic reticulum of the pigment epithelium, the highly metabolic microorganelles, become sparse during hibernation. Recovery of cytoplasmic constituents of the outer segments and the pigment epithelium is remarkably fast upon arousal of the animal.

Significance to Biomedical Research and the Program of the Institute: The present studies in this project have revealed much heretofore unknown cytologic information of the retina and pigment epithelium which is related to light stimulation and metabolic aspects of these tissues. These findings are directly beneficial in the understanding of pathogenesis and control of various retinal diseases.

Proposed Course: Similar experiments on the retina and pigment epithelium will be continued. The major part of the investigation on the pigment epithelium will be absorbed in Dr. Robison's project in the FY76.

Keyword Descriptors: photic damage to pigment epithelium, thermal damage to pigment epithelium, hibernation, photoreceptor cell during hibernation, pigment epithelium during hibernation

NEI Research Program: Retinal and Choroidal Diseases-Development, Structure, Function and Degeneration (Choroid, Pigment Epithelium and Related Disorders)

Experimental Subject or Tissue Source: Rhesus Monkey/Rat/Ground Squirrel/ Brown Bat/Frog

Research Objective: Etiology

Honors and Awards: None

Publications:

Kuwabara, T. and S. Okisaka: Photo-thermal effects on the pigment epithelium. Chapter in Zinn, K.M. and M.F. Marmor Ed. The Retinal Pigment Epithelium, Harvard Press, Cambridge, (in press) 1975.

Kuwabara, T.: Cytologic changes of the retina and pigment epithelium during hibernation, Invest. Ophthal. 14: 417-429, 1975.

Kuwabara, T. and S. Okisaka: Retinal damage by electronic flashlight. Invest. Ophthal. (n press) 1975.

Project No. Z01 EY 00110-04 LVR
1. Laboratory of Vision Research
2. Section on Neurophysiology
3. Bethesda, Maryland

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

Project Title: Information Processing in the Visual Cortex of the Rhesus Monkey.

Previous Serial Number: NEI-73 LVR 110

Principal Investigator: Bruce M. Dow, M.D.

Other Investigators: None

Cooperating Units: Eleanor Collins, Section on Experimental Pathology, LVR, NEI; Joan Baizer, Ph.D., Robert Wurtz, Ph.D., Laboratory of Neurobiology, NIMH.

Man Years:

Total:	1.1
Professional:	1.0
Other:	0.1

Project Description:

Objectives: To determine whether higher visual processing in the rhesus monkey involves a single hierarchial system (as has been suggested by earlier workers) or multiple parallel systems.

Methods Employed: Extracellular microelectrode recordings have been obtained from single neurons in striate and prestriate cortex of rhesus monkeys. Two quite different experimental preparations have been employed and the results compared. In one instance the monkeys are anesthetized, paralyzed, artificially respired, and provided with contact lenses to focus the eyes on a tangent screen. In the other instance the monkeys are trained to fixate a point on the tangent screen, and recordings are obtained with the animals fully alert. The awake monkey experiments have been done in collaboration with Drs. Baizer and Wurtz, using the facilities of the Laboratory of Neurobiology, NIMH. Under both experimental conditions visual stimuli have been presented onto the tangent screen, and neuronal responses to various shapes, colors, velocities and directions of movement have been examined. Electrode recording sites in both cases have been marked by means of current injection from the electrode tip. The two experimental preparations are complementary in that the acute anesthetized preparation permits more extensive testing on each cell and more complete histological localization, whereas the awake preparation permits correlation of neuronal responses with the animal's

behavior, as well as many more cells per animal.

Major Findings: A number of distinct specialized classes of neurons have been identified. Some cells respond exclusively to the color of a stimulus without regard to its size, shape, or movement. Other cells respond exclusively to the orientation of an elongated stimulus without regard to color or direction of movement. A third class responds in relation to direction and velocity of stimulus movement, without regard to the contrast, color, or orientation of the leading edge. Some cells are activated exclusively by appropriate simultaneous binocular stimulation. Another group responds preferentially to stimuli that trigger an eye movement as opposed to stimuli that the animal has been trained to ignore. There are some differences between the properties of neurons in striate and prestriate cortex, but for the most part these are quantitative rather than qualitative in nature. The results suggest the existence of multiple parallel processing mechanisms, each specialized for the detection of one feature of the visual environment, usually at the expense of other features.

Significance to Biomedical Research and the Program of the Institute: It is essential that we understand the normal mechanisms of information-processing in higher visual centers of the brain before we can expect to define the nature of the visual defect in such conditions as congenital cataract, strabismus, astigmatism, some types of color deficiency, the aphasias, and disorders of reading (dyslexia) in children.

Proposed Course: The project will be terminated due to lack of support.

Keyword Descriptors: rhesus monkey, microelectrode recordings, striate cortex, prestriate cortex, color, orientation, direction, velocity, binocular, eye movement, parallel processing.

NEI Research Program: Sensory and Motor Disorders of Vision-Visual Sensory and Perceptual Disorders (Neural Mechanisms)

Experimental Subject or Tissue Source: Rhesus Monkey

Research Objective: Etiology

Honors and Awards: None

Publications:

Dow, B.M.: Functional classes of cells and their laminar distribution in monkey visual cortex. J. Neurophysiol. 37: 927-946, 1974.

Dow, B.M.: Information processing in the visual system: II. Higher centers. Fed. Proc. (in press).

Project No. Z01 EY 00025-03 LVR
1. Laboratory of Vision Research
2. Section on Neurophysiology
3. Bethesda, Maryland

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

Project Title: Anatomy of Mammalian Retina

Previous Serial Number: NEI-71 LVR 026

Principal Investigator: Edward V. Famiglietti, Jr. M.D., Ph.D.

Other Investigators: Ralph Nelson, Ph.D.
Peter Gouras, M.D.

Cooperating Units: Helga Kolb, Ph.D., NINDS
Julia Lohr

Man Years:

Total:	2.0
Professional:	1.8
Other:	0.2

Project Description:

Objectives: To understand the neuronal circuitry through which visual information is processed at the retinal level.

Methods Employed: Light and electron microscopy. Golgi silver-impregnation methods. Serial-section electron microscopy. Electron microscopy of Golgi impregnations. Intracellular staining with fluorescent dyes.

Major Findings: In the last 15 or 20 years the notion has emerged that birds, fish, amphibia and reptiles process much more behaviorally significant information in their "complex" retinas than mammals in their allegedly "simple" retinas. In support of this view is the well-known fact that at the disposal of the mammalian visual system is a great deal of cerebral cortex, where much behaviorally significant information is processed. In identifying a retina as "complex" particular stress has been laid on the many-tiered stratification of the inner plexiform layer (IPL). It is in the IPL that retinal ganglion cells, which relay visual information to the brain, are contacted by the synaptic terminals of bipolar and amacrine cells. We have shown that mammals share with submammalian species a functionally significant 5-tiered stratification of the IPL. This 5-tiered stratification, dominated by narrowly stratified amacrine and ganglion cells, is obscured, however, in ordinary histological preparations of mammalian retina by the superimposition of a "simpler" system of bi-sublamination, which coincides with the separate distribution of the synaptic terminals

of "flat" and "invaginating" cone bipolars in the outer 1/3 and inner 2/3 of the IPL, respectively. The differing connections of the two cone bipolars with cone photoreceptors suggest that one is concerned with "lightness" and the other with"darkness". Correspondingly, the two sublaminae of the IPL may also be concerned with "lightness" or "darkness". We have found three types of ganglion cell which branch either in the inner 2/3 or in the outer 1/3 of the IPL, and in the cat these constitute a majority of the ganglion cells: the large- and small "tufted" cells and the large-bodied "radiate" cells. The dendritic branching patterns of "tufted" and "radiate" cells have been analyzed in Golgi preparations with the aid of a PDP-12 image-processing computer* in order to give a quantitative basis for the ganglion cell classification. Small- and medium-bodied radiate ganglion cells are of at least 7 types in cat and include the bistratified and "E" type ganglion cells, typical of non-mammalian retinas. Most of the smaller radiate ganglion cells are narrowly stratified in relation to the 5-tiered stratification of the IPL. Preliminary study of the synaptic contacts of retinal ganglion cells in cat reveal that small tufted cells have 70% bipolar and 30% amacrine contacts, while the most common small-bodied radiate cell has far fewer cone bipolar contacts, which make up only 30% of its total synaptic contacts.

Unlike cone bipolars, rod bipolar cells never make synaptic contact directly with retinal ganglion cells, but require an internuncial amacrine cell for this purpose. The type II amacrine cell is a narrow-field, bistratified cell specialized to transmit information from rod bipolar terminals in the inner 2/3 of the IPL to ganglion cells which branch in the outer 1/3 of the IPL. The identity of the amacrine cell which transmits information from the rod system of vision to ganglion cells branching in the inner 2/3 of the IPL is presently being sought. The identities and synaptic connections of other amacrines have been established.

Gap junctions, presumed sites of electrical transmission between nerve cells, have been observed frequently between amacrine cells. Especially prominent are gap junctions between processes of type II amacrine cells, and also between type II amacrine cells and invaginating cone bipolars. Gap junctions also occur among the processes of a single flat cone bipolar terminal and among the processes of a single type II amacrine cell. Ganglion cell dendrites occasionally participate in gap junctions as well. The function of these junctions in the IPL of the retina is not well understood at present, but studies of their possible significance are being pursued by intracellular microelectrode recording from bipolars, amacrines and ganglion cells.

Significance to Biomedical Research and the Program of the Institute: Knowledge of the morphology and connections of retinal neurons is a necessary basis for understanding the pathophysiology of retinal diseases, particularly as regards the natural history of the disease as it disrupts particular neuronal elements. Knowing the normal structure and function of the retina in vision is essential to any rehabilitative program, especially to any hope of developing visual prosthetic devices in the future.

Proposed Course: Preliminary studies on the synaptic connections of different types of retinal ganglion cells will be continued with the object of working out the complete "wiring diagram" of mammalian retina.

Keyword Descriptors: visual information processing, neuronal circuitry, stratification and sublamination of the inner plexiform layer, retinal ganglion cells, synaptic terminals, rod and cone bipolar cells, amacrine cells

NEI Research Program: Retinal and Choroidal Diseases-Development, Structure, Function and Degeneration (Retinal Information Processing and Associated Disorders)

Experimental Subject or Tissue Source: Cat

Research Objective: Etiology

Honors and Awards:

Invited Lecture: Famiglietti, E.V., Structural Organization of the Mammalian Retina, Department of Anatomy, Washington University, St. Louis, Missouri. December 12, 1974.

Invited Lecture: Famiglietti, E.V. and H. Kolb, Ganglion cells and their Connections in the Retina of the Cat. Jules Stein Eye Institute, U.C.L.A., Los Angeles, California. April 2, 1975.

Invited Lecture: Kolb, H. Organization of the cat retina. Department of Anatomy, University of Michigan School of Medicine, Ann Arbor, Michigan. February 14, 1975.

Invited Lecture: Kolb, H., Rod and Cone pathways in the cat retina. Rockefeller University, New York City. March 5, 1975.

Invited Lecture: Kolb, H. Organization of the inner plexiform layer of cat retina. Department of Anatomy, Hershey Medical School, Hershey, Pennsylvania. April 10, 1975.

Publications:

Kolb, H. and Famiglietti, E.V.: Rod and cone pathways in the inner plexiform layer of cat retina. Science 186: 47-49, 1974.

Famiglietti, E.V. and Kolb, H.: A bistratified amacrine cell and synaptic circuitry in the inner plexiform layer of the retina. Brain Res. 84: 293-300, 1975.

Kolb, H. and Famiglietti, E.V.: Stratification of retinal ganglion cells and their connections in the retina of the cat. Anat. Rec. 181: 398-199, 1975.

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

Project Title: Electrophysiological Studies of Mammalian Retina

Previous Serial Number: NEI-71 LVR 005

Principal Investigators: Ralph Nelson, Ph.D.
Peter Gouras, M.D.

Other Investigators: None

Cooperating Units: Helga Kolb, Ph.D., Laboratory of Neurophysiology, NINDS; Astrid Kafka-v. Lützow, M.D., University of Vienna, Austria

Man Years:

Total:	1.1
Professional:	1.1
Other:	0.0

Project Description:

Objectives: To understand the functional organization of mammalian retina and its relationship to disease states.

Methods Employed: Intracellular recording of neuronal responses to light from the in vitro, arterially perfused cat retina; intracellular staining of these neurons with procion dye, and examination of the retina by fluorescence microscopy; comparison with Golgi stained neurons of the cat retina.

Major Findings: We have succeeded for the first time in penetrating mammalian cones and recording their electrical responses to light. Like the cones of lower vertebrates cat cones hyperpolarize by 10 to 20 mv upon illumination. However unlike cones of lower vertebrates 1/3 to 1/2 of the responses from cat cones are generated in neighboring rod cells so that cone responses to light have some of the characteristics of rod responses. This finding places renewed importance of the study of interreceptor contacts in mammals, and provides an explanation for the mysterious rod signals observed in cat horizontal cells. The elegant work of Helga Kolb, Ph.D. had previously shown that horizontal cell bodies are connected only to cones anatomically, and thus the presence of a rod signal in these units was previously thought to be at variance with the anatomy.

Significance to Biomedical Research and the Program of the Institute: Many disease states have their origin at the cellular level and a thorough understanding of the normal functioning of each retinal neuron can only lead to improved insights about abnormal states observed clinically.

Proposed Course: The cat retina contains upwards of 20 different kinds of neurons. Since the experimenter has relatively little control in selecting which neuron his electrode encounters, we intend to advance on a broad front studying all neural types in this retina. In particular we hope to complete work on the properties of the A_{II} amacrine cell, a unit only recently uncovered morphologically by Drs. Kolb and Famiglietti. This amacrine cell receives about 90% of its input from rods, has extraordinary quantum sensitivity, and is apparently a key element in mammalian night vision.

Keyword Descriptors: cat retina, cones, intracellular recordings, procion dye, arterially perfused eye

NEI Research Program: Retinal and Choroidal Diseases-Development, Structure Function, and Degeneration (Retinal Information Processing and Associated Disorders

Experimental Subject or Tissue Source: Cat

Research Objective: Etiology

Honors and Awards: None

Publications:

Nelson, R., von Lützow, A., Kolb, H. and Gouras, P.: Horizontal Cells in Cat Retina with Independent Dendritic Systems. Science (in press).

Project No. Z01 EY 00026-04 LVR
1. Laboratory of Vision Research
2. Section on Physiology
3. Bethesda, Maryland

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

Project Title: Physiology of the Primate Visual System

Previous Serial Number: NEI-71 LVR 026

Principal Investigators: Francisco M. de Monasterio, M.D., D. Sc.
Edward V. Famiglietti, M.D., Ph.D.
Peter Gouras, M.D.

Other Investigators: Eleanor Collins, NEI

Cooperating Units: David Tolhurst, Ph.D., The Physiology Laboratory
Cambridge University, Cambridge, United Kingdom
Helga Kolb, Ph.D., NINDS

Man Years:

Total:	2.5
Professional:	2.5
Other:	0.0

Project Description:

Objectives: To understand the neural organization underlying visual perception in the retina, lateral geniculate nucleus and striate cortex of the rhesus monkey.

Methods Employed: Electrophysiological recordings from single neurons of anesthetized and paralyzed monkeys; correlation of the distribution of single cell varieties and morphological cell types as seen by electron and light microscopy; the use of refined optical stimuli to quantitatively define spatial, temporal and chromatic properties of these cells.

Major Findings: During the past year experiments were completed on the analysis of ganglion cell responses from different areas of the retina. The findings show that there are several types of ganglion cells with different distributions across the retina which can be grouped in three main categories. The first two groups encompass all of the cell subtypes which have been described to date in the monkey's lateral geniculate nucleus, in addition to containing new varieties, suggesting that there is little reorganization of the visual information passing through this nucleus. None of the cells of the third group have been reported in this nucleus, suggesting that they might project to other areas of the visual system. Some of these cells are similar to

those reported in the superior colliculus of the monkey, whereas other cells resemble those described in nuclei of the accessory optic fibre system. In addition, the findings indicate a rather large functional diversity of ganglion cells, which coupled to the results obtained in other species, suggest that differences between species are more quantitative than qualitative.

The chromatic organization of ganglion cells, i.e. the type of input these cells receive from the various types of cone mechanisms, have been studied in detail in colaboration with Dr. David Tolhurst. The results indicate that many ganglion cells receive input from three cone mechanisms (blue-, green- and red-sensitive cone mechanisms), rather than two as previously thought. In most cases the peripheral parts of the receptive field of these cells received input from two synergistic cone mechanisms, whereas the centre of the field received input from the remaining cone mechanism. All cells receiving input from the blue-sensitive cone mechanism were trichromatic and the retinal distribution of cells with trichromatic input parallels the availability of blue-sensitive cones in the retinal area being considered. The results indicate that trichromatic interactions begin in the retina of the monkey and not at cortical levels as previously thought.

The spatial organization of ganglion cells, i.e. the spatial distribution of areas with opponent responses is being studied in detail in the different cell types. The results are being correlated with the response pattern of the cells, i.e. sustained or transient responses. Three main types of spatial organization have been encountered. In one type the opponent areas have non-coincident loci of maximal sensitivity (centre-surround organization), in which one mechanism has maximal sensitivity in the very centre of the receptive field, whereas the other has maximal sensitivity in the periphery of the field. A second type of organization also shows two opponent mechanisms with different distributions, but both mechanisms clearly overlap and have maximal sensitivity at the centre of the receptive field. A third type consists of two opponent mechanisms having similar areas of co-extensive distribution and have maximal sensitivity at the field centre. Cells in which both opponent mechanisms have coincident loci of maximal sensitivity tend to show transient responses, whereas those with non-coincident loci tend to have sustained responses. The results show that supression of the surround mechanism either by chromatic adaptation or by dark adaptation produces sustained centre responses suggesting that centre-surround interaction and its varieties (linear and non-linear) plays an important role in the generation of the response pattern.

Along this line of research, the linearity or non-linearity of ganglion cell responses to a variety of stimuli are being studied, in order to class the cells from the point of view of response linearity. There is no available information of this type in primate retina and such results will be valuable to facilitate comparisons with psychophysical experiments in humans. The studies represent a further step from the previous study of response pattern, since it has been suggested that the linearity of cell responses can be correlated with the response pattern. The results indicate that in many cells these two parameters are not always correlated, and are probably dependent on cell connections before the ganglion cell level.

We have addressed the question of the role of the lateral geniculate nucleus as an intermediate in the transmission of visual information from retina to cortex, by studying the visual receptive field properties of neurons in this nucleus. The position of these cells been determined by extracellular dye injections through the recording electrode, and particular attention paid to the seven-tiered lamination of different cell types. Most of the cells encountered are color-opponent cells and resemble those reported by previous investigators. These cells differ little from retinal ganglion cells except that the receptive field surrounds are "stronger". A few cells, obtained with high impedence electrodes are unlike any cells previously reported in lateral geniculate nucleus, and some of these are different from any of the 6 varieties recently classified in the retina. Several approaches are being tried at present to determine if these unusual cells are the small intrinsic neurons, which may be "strengthening" the surrounds of the more commonly encountered lateral geniculate neurons. Among these techniques is that of intracellular staining with fluorescent and electron opaque dyes. A major effort is now underway attempting the technically difficult task of staining both geniculo-cortical relay neurons and intrinsic neurons in order to confirm their identity and to ascertain their synaptic connections.

Significance to Biomedical Research and the Program of the Institute: These studies should prove valuable for understanding vision and the etiology of pathophysiology of retinal diseases and sensory disorders of vision, programs IA3, VD1 and VD3.

Proposed Course: The actual line of retinal research should be completed at the beginning of the next fiscal year. At that time it is contemplated a joint project with Dr. Ralph Nelson to study intracellular responses of various cell types in the isolated and perfused monkey eye. To pursue attempts to stain intracellularly geniculocortical relay cells and intrinsic neurons in the lateral geniculate nucleus, and to better characterize the intrinsic neurons electrophysiologically.

Keyword Descriptors: cone mechanism: spectral sensitivity of any of the three types of cones as detected at the ganglion cell level. Three types of cones have been described to date: blue-, green- and red-sensitive; receptive field: the retinal area subserved by a single cell, in which modifications of illumination modify the activity of the cell; chromatic adaptation: technique using relatively intense coloured lights, which tend to desensitize selectively one type of cone mechanism; dark adaptaion: removal of any background illumination and the use of dim stimuli which bring a change in the level of activity of the receptors. In this conditions it is possible to study input from rods. Lateral geniculate nucleus: part of the sensory "thalamus" of the brain which received information from retinal ganglion cells of both eyes and transmits information to visual cortex; lamination: the lateral geniculate nucleus (lgn) is made up of 7 laminae of cells, sorted according to eye dominance and other functional properties; intrinsic neurons: like other thalamic nuclei, the lgn contains some neurons, the processes of which do not leave the bounderies of the nucleus. These neurons have strictly local, and presumed inhibitory functions; synaptic connections: neurons transmit information from one to another across such connections: intracellular staining: as an aid to

identification of neurons at a later time in tissue sections, dye can be injected into the cell through a micropipette recording electrode.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders (Neural Mechanisms)
Experimental Subject or Tissue Source: Rhesus Monkeys

Research Objective: Etiology

Honors and Awards:

P. Gouras. Awardee of the Alexander von Humboldt Society, Germany, 1974--Visiting Professor Neurologische Universitats Klinik mit Abteilung fur Neurophysiologie - Freiburg, West Germany.

Publications:

De Monasterio, F.M. and Gouras, P.: Ganglion cell types in different regions of primate retina. Assoc. for Research in Vision and Ophthalmology, Spring Meeting, Sarasota Florida, Abstracts, p. 74 (1974).

De Monasterio, F.M. and Gouras, P.: Functional properties of ganglion cells of the rhesus monkey retina. J. Physiol. (London), (in press), (1975)

De Monasterio, F.M., Gouras, P. and Tolhurst, D.J.: Trichromatic colour opponency in ganglion cells of the rhesus monkey retina. Assoc. for Research in Vision and Ophthalmology, Spring Meeting, Sarasota Florida, Abstracts, p. 51 (1975).

De Monasterio, F.M., Gouras, P. and Tolhurst, D.J.: Trichromatic colour opponency in ganglion cells of the rhesus monkey retina. J. Physiol. (London), (in press), (1975).

De Monasterio, F.M., Gouras, P. and Tolhurst, D.J.: Concealed colour opponency in ganglion cells of the rhesus monkey retina. J. Physiol. (London), (in press) 1975 .

Gouras, P.: The remapping of visual space. Invest. Ophthal., (in press) 1975 .

OFFICE OF BIOMETRY AND EPIDEMIOLOGY

ANNUAL REPORT
OFFICE OF BIOMETRY AND EPIDEMIOLOGY
July 1, 1974 - June 30, 1975

REPORT OF THE ACTING CHIEF, OFFICE OF BIOMETRY AND EPIDEMIOLOGY

Fred Ederer

During the year the staff of the Office was engaged in a wide variety of studies relating to methods of diagnosis, measurement of the extent of disease, identification of factors related to risk, objective comparison of alternative treatments, and statistical methodology. These involved a combination of direct research activities by the staff and assistance or consultation to others in vision research. Emphasis on clinical trials has increased, with the initiation of studies of evaluation of early vitrectomy in diabetic retinopathy, and of the effectiveness of urokinase and heparin in preservation of visual function in central retinal vein occlusion.

Harold A. Kahn, at his request, was relieved of the duties of Chief, Office of Biometry and Epidemiology (OBE) in order that he could give full time and attention to the successful completion of the Framingham Eye Study. A replacement is being recruited. Dr. J. Theodore Schwartz requested and obtained reassignment to the Division of Hospitals, Health Services Administration, DHEW. Other staff changes included the addition of Karen Yuen, Ph.D. and David C. Allen, O.D., M.A. Dr. Frederick L. Ferris will begin an ophthalmology residency training program at Johns Hopkins University in July 1975.

The effort to educate the vision research community in epidemiological and biostatistical methods continued with several publications, including the NEI Workshop on Clinical Trials.

An OBE seminar series was begun to benefit both OBE staff and clinical vision research workers. Both OBE staff and outside speakers are participating in this series.

Publications (related to projects completed last year):

Kahn HA: Comparison of localized treatments for bilateral disease. Invest. Ophthalmol. 13:634-635, 1974.

Kahn HA: Letter to the Editor. Br. J. Ophthalmol. 58:634, 1974.

Kahn HA, Bradley RF: Prevalence of diabetic retinopathy for age, sex and duration of diabetes. Br. J. Ophthalmol. (in press).

Kahn HA, Hiller R: Blindness caused by diabetic retinopathy. Am. J. Ophthalmol. 78:58-67, 1974.

Project No. Z012 EY2 00102-042 OBE
1. Office of Biometry & Epidemiology
2.
3. Bethesda, Maryland

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

Project Title: Framingham Eye Study (Contract NIH-NEI-72-2112)

Previous Serial Number: NEI 72 OBE 102

Principal Investigator: Harold A. Kahn (for NEI aspects of the study)

Other Investigators: None

Cooperating Units: Biometry & Epidemiology Branches, NHLI
Department of Ophthalmology & Preventive Medicine, Boston University
Department of Preventive Medicine, Harvard University

Man Years:

Total:	1.9
Professional:	0.9
Other:	1.0

Project Description:

Objectives: The aim of this investigation is to identify individuals among the Framingham Heart Study cohort who at the present time have a disease or condition related to one or more of the four most common causes of adult blindness, i.e. senile cataract, senile macular degeneration, chronic simple glaucoma, and diabetic retinopathy. In addition to determining the prevalence of these diseases, we hope to be able to relate past measurements to present disease status in an effort to identify risk factors.

Methods Employed: An ocular examination according to a standard protocol (with replications by an OBE ophthalmologist to control observer error) was carried out under contract with Boston University on the survivors of the original Framingham Heart Study cohort to identify individuals with these diseases. Additional information will be obtained from data accumulated over the previous 20 years on members of this group by the National Heart and Lung Institute.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: The four eye diseases under consideration are the most frequent causes of adult blindness in this country today. As a guide to prevention of these eye diseases, it will be very helpful to identify risk factors associated with them.

The study has been designed with this objective in mind. Prevalence data for this age group (53-83) in this community will be a useful by-product.

Proposed Course: Patient examinations were completed in February 1975 with 2,679 individuals examined (subject to edit correction). This includes 85% of the cohort still resident in the Framingham area. Coding of all clinical records has been completed and computer editing is in process. Six Boston University ophthalmologists have been trained in a fundus photograph reading protocol. Each of the six has independently read the same trial set of fundus photos for 53 persons. Interobserver error is now being evaluated and the decision as to whether to begin definitive reading of the accumulated photographs, or to schedule additional training, will soon be made.

Through the cooperation of the Biometry and Epidemiology Branches of the National Heart and Lung Institute, we have been given two computer tapes of data they have collected on this population for use in our analyses. These tapes have been copied in a form suitable for reading by our data processing subcontractor (Harvard University Department of Preventive Medicine) and have been transmitted to Harvard.

Detailed plans for the initial computer tabulations have been made and programming is well under way. The first substantive reports are scheduled for completion by December 1975. When these are done, detailed plans will be drawn up for future tabulations and for sharing the ophthalmic data collected with other interested investigators (with respect to the latter point some requests have already been received).

Keyword Descriptors: epidemiology, senile cataract, senile macular degeneration, chronic simple glaucoma, diabetic retinopathy, prevalence, risk factor, Framingham Heart Study, blindness

NEI Research Program: Retinal and Choroidal Diseases--Macular Diseases; Cataract--Degenerative (Senile) Cataract; Glaucoma

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

Honors and Awards: None

Publications:

Kahn HA, Leibowitz H, Ganley JP, Kini M, Colton T, Nickerson R, Dawber TR: Standardizing diagnostic procedures. Are they reproducible? Am. J. Ophthalmol. 79:768-775, 1975

Section on Clinical Trials and Natural History Studies

Office of Biometry and Epidemiology

The Section on Clinical Trials and Natural History Studies has continued its active involvement in and support of the national collaborative Diabetic Retinopathy Study. Particular emphasis, during the past year, has been given to the control of performance at the clinics by the establishment of the Clinic Monitoring Committee, of which both Mr. Ederer and Dr. Ferris are members.

In the meantime, staff of the Section has participated in the development of detailed protocols for the national collaborative Diabetic Retinopathy Vitrectomy Study and of the NEI intramural Urokinase Central Retinal Vein Occlusion Trial.

A contract was awarded to the Statistical Center of the Cooperative Glaucoma Study to undertake an analysis of the Study's data collected over 13 years. Under the direction of Mr. Dean Krueger, an experienced biostatistician, the study data have been edited, corrected for errors, and reclassified to achieve standardization; also, detailed tabulation plans for data analysis have been prepared.

Publications:

Ederer F: Letter to the Editor. N. Engl. J. Med. 291:1143, 1974.

Ederer F: Patient bias, investigator bias, and the double-masked procedure in clinical trials. Am. J. Med. 58:295-299, 1975.

Ederer F, Hiller R: Clinical trials, diabetic retinopathy, and photocoagulation. A reanalysis of five studies. Survey Ophthalmol. 19:267-286, 1975.

Ederer F: Practical problems in collaborative clinical trials. Am. J. Epidemiol. (in press) (August) 1975.

Ederer F (ed): The randomized controlled clinical trial. Am. J. Ophthalmol. 79:752-789, 1975.

Ederer F: Why do we need controls? Why do we need to randomize? Am. J. Ophthalmol. 79:758-762, 1975.

Lectures:

Dr. David C. Allen delivered three lectures on Epidemiology and Biostatistics to optometry students at the Pennsylvania College of Optometry, Philadelphia, Pennsylvania.

Mr. Fred Ederer presented a seminar on regression toward the mean at the School of Public Health, University of North Carolina, Chapel Hill, N.C.

Dr. Frederick L. Ferris presented a lecture on clinic monitoring in clinical trials to the staff of the DRS Clinic, University of Wisconsin, Madison, Wisconsin.

Consultations:

Mr. Fred Ederer consulted with Dr. Bradley R. Straatsma, UCLA, on a clinical trial of intraocular lenses; with Dr. Max Miller, Case Western Reserve University, on the analysis of fundus photographs of the University Group Diabetes Project.

Dr. Frederick Ferris consulted with the following: Dr. Daniel Eichenbaum and Dr. Steven Charles, of the NEI, on the analysis of the results of vitrectomies performed at the Clinical Center of the NIH, the Vitrectomy Advisory Group on the reporting of the results and complications of 263 vitrectomies for complications of diabetic retinopathy, and Dr. Robert Frank, of the NEI, on the design of a clinical study to evaluate platelet aggregation in diabetics.

Dr. David Allen and Dr. Frederick Ferris consulted with Dr. Carol Kollarits, of the NEI, on the design and development of a clinical trial of urokinase and heparin in central retinal vein occlusion.

Membership:

Dr. Frederick Ferris was appointed as an ex officio member of the National Research Council's Working Group #39 on Standards for Testing Visual Fields and Visual Acuity.

Project No. Z012 EY2 00105-042 OBE

1. Office of Biometry & Epidemiology
2. Section on Clinical Trials & Natural History Studies
3. Bethesda, Maryland

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

Project Title: Collaborative Diabetic Retinopathy Study (Contract NIH-NEI-73-2129, 2130 and others)

Previous Serial Number: NEI 72 OBE 105

Principal Investigators: Fred Ederer and Frederick L. Ferris (these are the National Eye Institute investigators only)

Other Investigators: Investigators from fifteen Clinical Centers, the Reading Center, and the Coordinating Center

Cooperating Units: Sixteen medical centers, all in the United States

Man Years:

Total:	1.5
Professional:	1.5
Other:	0.0

Project Description:

Objectives: This is a cooperative clinical trial to determine whether photocoagulation can delay the onset of blindness in proliferative diabetic retinopathy. The National Eye Institute participates in the planning, coordination, and overall direction of the study.

Methods Employed: Planning for the study began late in 1968 and a detailed protocol was evolved over a 3-1/2 year period. Only patients with bilateral disease are eligible for study. One eye is randomly selected for treatment, the other is an untreated control. One of two treatments is randomly selected: argon laser or xenon arc.

The study's operations are directed by the Executive Committee, composed of Diabetic Retinopathy Study investigators. The Policy Advisory Group, composed of senior scientists who are not investigators in the study, is monitoring progress and advising both the National Eye Institute and the Executive Committee on the conduct of the study. A Data Monitoring Committee periodically reviews the accumulating data for evidence of positive and negative treatment effects, and has the responsibility for reporting significant effects to the Policy Advisory Group along with recommendations for changes in the study protocol. National Eye Institute staff participates actively on all committees.

Progress to Date: In 1971, ten Clinical Centers were selected to participate, and eight were added in 1972. Three Clinical Centers eventually dropped out. Patient recruitment began at four Centers in June 1972, at three Centers in September 1972, and at eight Centers in 1973. Early recruitment was far below expectations, and a minimum quota of five patients per month per clinic was established to achieve a total of some 1,500 study patients by June 30, 1974; eventually the target was revised to 1,600, and the deadline extended until June 30, 1975. Through the end of March 1975, 1,521 patients had entered the study.

An Editorial Committee has been established, and writing teams have been named to prepare papers on the design of the study, the baseline findings, and the natural history of diabetic retinopathy.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy is one of four major causes of adult blindness and differs from the other three in that it affects a younger population. There is a real need for finding a treatment which delays the onset of blindness. Although photocoagulation is extensively used as a treatment for diabetic retinopathy, the value of the treatment is uncertain.

Critical to the success of the study, in addition to an adequate number of patients, are adherence to protocol and the prevention of dropouts (patients who fail to return for periodic examinations and those who receive treatment in the control eye). These aspects of the study will be closely watched by the Clinic Monitoring Committee.

Keyword Descriptors: diabetic retinopathy, photocoagulation, argon laser, xenon arc, collaborative clinical trial

NEI Research Program: Retinal and Choroidal Diseases--Vascular and Circulatory Abnormalities

Experimental Subject or Tissue Source: Human

Research Objective: Treatment

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00101-042 OBE

1. Office of Biometry & Epidemiology
2. Section on Clinical Trials & Natural History Studies
3. Bethesda, Maryland

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

Project Title: National Health and Nutrition Survey

Previous Serial Number: NEI 72 OBE 101

Principal Investigator: David C. Allen

Other Investigators: Helen Moorhead

Cooperating Units: Division of Health Examination Statistics, National Center for Health Statistics, HRA, DHEW

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To determine the prevalence of visual disorders in a random sample of the U.S. population. Associations of eye problems with nutritional defects and systemic diseases are also being studied.

Methods Employed: A random sample of 60,000 persons, from 128 geographic areas in the continental U.S., between the ages of 1 and 74, was offered an examination according to a standard protocol. An ophthalmological examination conducted with the help of the National Eye Institute was included as part of the overall examination for the first two years of the project. During the time that NEI participated in this project, 10,126 of a random sample of 14,147 persons in 33 geographic areas were examined, a response rate of 71.6%. House staff and research fellows from various academic institutions performed the ocular examinations after receiving instruction in the protocol. In addition to the ocular history and examination, data were gathered on medical history, dietary history, physical examination, hematologic studies, blood chemistries, and urine chemistries. To obtain an estimate of reproducibility, NEI staff ophthalmologists and field ophthalmologists independently performed replicate eye examinations of a sub-sample of individuals. During the past year coding was completed on the ophthalmic data.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: This is the first attempt to determine the prevalence of visual disorders in the U.S. population based on examination according to fixed protocol. In addition, the study will provide (1) a measure of the status of ocular health care, and (2) directions for future areas of ophthalmic research.

Proposed Course: The ophthalmology examination ceased to be a part of the Survey at the completion of the first year in October 1972. This was necessitated by an inability to obtain examining ophthalmologists. NEI has completed editing the ophthalmology examinations and coding the history and diagnoses obtained from them. Plans for editing the data by computer are now being developed.

Keyword Descriptors: National Health and Nutrition Survey (HANES), prevalence, epidemiology, measures of reliability

NEI Research Program: Retinal and Choroidal Diseases; Corneal Diseases; Cataract; Glaucoma; Sensory and Motor Disorders of Vision

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00028-012 OBE

1. Office of Biometry & Epidemiology
2. Section on Clinical Trials & Natural History Studies
3. Bethesda, Maryland

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

Project Title: Controlled Clinical Trial to Evaluate Early Vitrectomy in Diabetic Retinopathy

Previous Serial Number: None

Principal Investigators: Frederick L. Ferris, Fred Ederer, David C. Allen

Other Investigators: Will include investigators from Clinical Centers, a Reading Center, and a Coordinating Center

Cooperating Units: Will include Clinical Centers, Reading Centers, and a Coordinating Center, all in the United States

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: This is a cooperative clinical trial to determine whether vitrectomy done in the first six months after vitreous hemorrhage secondary to diabetic retinopathy can reduce the ocular morbidity compared to the standard technique of waiting twelve months after vitreous hemorrhage prior to vitrectomy. The initial objective is to obtain a workable protocol, develop a request for proposal, and initiate work by the collaborative group on the development of a final manual of operations. Further objectives are to assure adequate control of the study by the Chairman, Executive Committee, Coordinating Center, Policy Advisory Group, and National Eye Institute; improve methods of patient recruitment; develop research procedures to minimize or eliminate sources of bias and to quantify any residual bias; insure uniformity of terminology and definitions and standardization of methodology; assess reproducibility of vision examinations; monitor adherence tc protocol and completeness of patient studies and follow-up; advise on data editing, monitoring and analysis.

Methods Employed: Planning of the study began in March 1974, and a draft protocol and request for proposals were developed by March 1975. Only patients with vitreous hemorrhage secondary to diabetic retinopathy, which is severe and has been present for less than six months, are eligible for the study. Eyes are randomly selected to have either early vitrectomy (within six months

of initial hemorrhage) or routine delayed vitrectomy (at least 12 months after initial hemorrhage). National Eye Institute staff participates in the development of the protocol and operations manual, in site visits to evaluate clinic performance, adherence to protocol, and reproducibility of vision tests, and through membership on the Executive Committee, Data Monitoring Committee, and Policy Advisory Group.

Detailed administrative structure is in the planning stage, but will be similar to that of the Diabetic Retinopathy Study.

In June 1975 Clinical Centers, a Reading Center, and a Coordinating Center will begin work on a detailed manual of operations. Patient recruitment is not expected to begin before December 1975.

Critical to the success of the study, in addition to an adequate number of patients, are adherence to protocol and the prevention of dropouts (patients who fail to return for periodic examinations and those who receive treatment in the control eye). These aspects of the study will be closely monitored.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy is one of four major causes of adult blindness and differs from the other three in that it affects a younger population. A major cause of this blindness is vitreous hemorrhage. Vitrectomy has been shown to be of benefit in individuals who have had a severe vitreous hemorrhage for at least one year. Diabetic blindness may be further reduced if the vitrectomy is performed at an earlier date, and these early vitrectomies are becoming increasingly common. This presents an ideal opportunity for the National Eye Institute to organize scientific and research talents to answer a major medical care delivery question.

Proposed Course: A detailed manual of operations should be developed by about December 1975. Following this, patient recruitment will begin and the number of clinics involved will expand to between 10 and 20. Recruitment will continue for two years and each patient will be followed for four years. The Coordinating Center will continually process and analyze results, which will be reviewed periodically by a Data Monitoring Committee. Expected completion date, including data processing and report writing, is 1983.

Keyword Descriptors: diabetic retinopathy, vitrectomy, vitreous hemorrhage, collaborative clinical trial

NEI Research Program: Retinal and Choroidal Diseases--Vascular and Circulatory Abnormalities--Retinal Detachment and Vitreous Abnormalities

Experimental Subject or Tissue Source: Human

Research Objective: Treatment

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00029-012 OBE

1. Office of Biometry & Epidemiology
2. Section on Clinical Trials & Natural History Studies
3. Bethesda, Maryland

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

Project Title: Development of a Standardized Visual Acuity Measuring Device

Previous Serial Number: None

Principal Investigators: Frederick L. Ferris, III, M.D.
Charles J. McCarthy, Biomedical Engineering and Instrumentation Branch, Division of Research Services

Other Investigators: None

Cooperating Units: Biomedical Engineering and Instrumentation Branch, Division of Research Services

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: The purpose of this project is to develop a standardized visual acuity viewing box which could be used in each of the 15 Diabetic Retinopathy Study clinics. The viewing box will have a standard level of illumination which can be maintained over prolonged use and is relatively independent of room illumination. The design is such that its use will aid in maintaining a constant technique of measuring the visual acuity by convenient arrangement of visual acuity charts. The use of this box is projected not only for the Diabetic Retinopathy Study, but also for other collaborative clinical trials or other areas where a standardized visual acuity system would be necessary.

Methods Employed: The Diabetic Retinopathy Study calls for between 75 and 125 foot-candles of incident illumination on a Snellen type visual acuity chart, which is to be viewed from 20 feet. After research into the best possible lighting arrangement, several fluorescent lights were purchased and a dummy visual acuity box was set up. Using a Weston visual and cosine-corrected light meter, a workable system was developed in which there would be even illumination across the entire chart of approximately 100 foot-candles. Using this prototype as a model, exact blueprints of such a system are being developed. Using these blueprints an exact prototype will be built, and if this

proves satisfactory the National Eye Institute will consider a contract to have one of these built for each of the Diabetic Retinopathy Study clinics.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: The development of such a standardized visual acuity system will aid greatly in maintaining quality of visual acuity measurements in the Diabetic Retinopathy Study, as well as in other cooperative clinical trials. Experience from site visits in the Diabetic Retinopathy Study has shown how complicated and variable the measurement of visual acuity can be. This system will assure that the lighting is even across the chart and constant, and make it convenient to do the examination according to the protocol.

Proposed Course: Careful blueprints will need to be made of this system. With an adequate set of blueprints a prototype model will be developed. The prototype will be modified as needed after careful testing. The final phase of this project will be to provide one of these visual acuity systems for each of the Diabetic Retinopathy Study clinics.

Keyword Descriptors: visual acuity, diabetic retinopathy, collaborative clinical trials, standardization of observations

NEI Research Program: Retinal and Choroidal Diseases; Sensory and Motor Disorders of Vision

Experimental Subject or Tissue Source: Human

Research Objective: Etiology, Treatment

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00040-012 OBE

1. Office of Biometry & Epidemiology
2. Section on Clinical Trials & Natural History Studies
3. Bethesda, Maryland

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

Project Title: Development of a Visual Acuity Examination Technique to Evaluate Patient Bias

Previous Serial Number: None

Principal Investigators: Frederick L. Ferris, III, M.D.
Fred Ederer
David Allen, O.D., M.A.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: The purpose of this study is to develop a visual acuity examination technique by which a useful estimate of patient bias can be determined.

Methods Employed: A prototype system is being developed in which the patient is asked to read a single letter which he sees for only a short period of time. Through use of a polaroid system, the patient will have difficulty knowing with which eye he is seeing the letter.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: It is important to attempt to evaluate the effect of patient bias in any clinical study, since it is well known that patient bias can affect the outcome of such a trial. The patient's visual acuity is the major response variable in the Diabetic Retinopathy Study. Because the patient knows which eye has been treated by photocoagulation, there is no way to prevent the patient from exercising any bias he or she may have during the visual acuity exam, as this is a subjective examination. Since bias cannot be eliminated, it is extremely important to attempt to measure this bias. If this technique is successful, the result will be an estimate of the extent of the patient bias, as well as

its effect on visual acuity measurements. This system may also prove useful for identifying malingerers.

Proposed Course: Further development of this prototype system will continue. Upon completion it will undergo clinical testing to evaluate its usefulness and reliability. If this phase proves successful, it is proposed that this system be used in the Diabetic Retinopathy Study to evaluate possible patient bias.

Keyword Descriptors: visual acuity, patient bias, examination techniques, Diabetic Retinopathy Study

NEI Research Program: Retinal and Choroidal Diseases; Sensory and Motor Disorders of Vision

Experimental Subject or Tissue Source: Human

Research Objective: Etiology, Treatment

Honors and Awards: None

Publications: None

Section on Ophthalmic Field and Developmental Research

Office of Biometry and Epidemiology

During the past year, Dr. J. Theodore Schwartz, Section Head, requested and obtained a Public Health Service transfer to the Division of Hospitals and Clinics. Mrs. Doris Collie, Ophthalmic Technician, the other technical staff member of this Section, was reassigned to the Clinical Center, NEI.

Within the past year, Dr. Schwartz authored or co-authored eight new manuscripts for publication. Details appear with individual project reports for this Section.

Under the auspices of the U.S. Special Foreign Currency Program, a site visit was conducted by Dr. Schwartz to review a PL480 project proposal by Dr. Mohyi-Eldin Said, Professor of Ophthalmology, University of Alexandria, Egypt. The proposed project, a study of chronic simple glaucoma, has two objectives, namely, an appraisal of the advantages and disadvantages of tonometric screening for glaucoma as a potential public health practice in Egypt, and further definition of the ensuing natural history of individuals who exhibit ocular hypertension but whose eyes are otherwise normal. This site visit provided a valuable preliminary assessment of the feasibility of undertaking the proposed project from the point of view of available manpower and local logistic issues.

Project No. Z012 EY2 00041-062 OBE

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

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

Project Title: Twin Register for Eye Examinations (TREE)

Previous Serial Number: NEI 70 OBE 001

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: Doris J. Collie

Cooperating Units: None

Man Years:

Total:	0.15
Professional:	0.05
Other:	0.10

Project Description:

Objectives: To provide a local register of twins as a resource for investigations on the heritability of ocular characteristics, case-control studies and studies of the early natural history of chronic disorders.

Methods Employed: Prior to its transfer from the National Institute of Neurological Diseases and Stroke to the National Eye Institute, this Section compiled a register of over 700 pairs of monozygotic and dizygotic twins for the purpose of ophthalmic investigations. These twins reside in the metropolitan Washington, D. C. area. A description of this register and the data originally collected was given in earlier reports.

Major Findings: This register has provided a source of subjects for numerous direct and collaborative studies described in this and previous annual reports.

Work undertaken during the past year which originated through collaboration with other institutions included a study of genetic involvement in the formation of normal palmar crease patterns, simian and Sydney patterns and a newly classified interdigital pattern. This work suggested a strong genetic contribution to the development of some palmar crease patterns and further supported the usefulness of the newly proposed classification. This work (HD-CP 14) was undertaken in collaboration with the Children's Diagnostic and Study Branch, National Institute of Child Health and Human Development and the Department of Pediatrics, Medical University of South Carolina.

Significance to Biomedical Research and the Program of the Institute: Comparison of agreement among monozygotic and dizygotic twins with regard to physical characteristics can provide an assessment of the relative roles of heredity and environment in the expression of these characteristics. This register serves as a resource to identify appropriate populations for such studies as well as investigations on therapeutic effectiveness.

Proposed Course: None

Keyword Descriptors: twin register, heritability, genetics, palmar crease patterns

NEI Research Program: Glaucoma; Sensory and Motor Disorders of Vision

Experimental Subject or Tissue Source: Human

Research Objective: Etiology, Treatment

Honors and Awards: None

Publications:

a) Investigations initiated by other units:

Plato CC, Schwartz JT, Wertelecki W: Dermatoglyphic investigations in twins and siblings. Acta Geneticae Medicae et Gemellologicae. (in press).

Feinleib M, Christian JC, Borhani NO, Rosenman RJ, Garrison RJ, Wagner J, Kannell WB, Hrubec Z, Schwartz JT: The National Heart and Lung Institute Twin Study of Cardiovascular Disease Risk Factors: Organization and Methodology. Acta Geneticae Medicae et Gemellologicae. (in press).

Plato CC, Wertelecki W, Schwartz JT: Normal and aberrant palmar creases in twins and siblings. Acta Geneticae Medicae et Gemellologicae. (in press).

b) Investigations initiated by this Section:

These publications appear under other individual project reports for this Section.

Project No. Z012 EY2 00042-062 OBE

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

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

Project Title: Effect of Treatment on the Progression of Myopia

Previous Serial Number: NEI 70 OBE 004

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: Roy C. Milton, Ph.D.

Cooperating Units: Section on Mathematical Statistics and Computer Application, OBE

Man Years:

Total:	0.55
Professional:	0.20
Other:	0.35

Project Description:

Objectives: To assess the effect of a specific treatment in retarding the progression of myopia.

Methods Employed: This was a three-year study among a population of 25 pairs of young, monozygotic twins who are similarly myopic. One co-twin received standard spectacle correction as the control; the other was managed using specially prescribed bifocal spectacles and topical, short-acting cycloplegic eye drops instilled upon retiring at night. There is no question about the safety of this regimen and it has no influence upon normal day time vision. The essential advantage in working with MZ twins in this investigation lies in the complete match on genetic constitution for the treated twin and his co-twin control. Key biologic variables of age, race, sex, period of gestation and maternal age are inherently controlled as are certain environmental factors common to their shared domicile. The study population was selected from our Twin Register for Eye Examinations.

At the outset of this investigation, historical data including maternal, perinatal, growth history, family history, diet, development and past medical and ophthalmic history were obtained and detailed general ocular examination was undertaken. Clinical measurements include refraction, corneal curvature, corneal thickness, anterior chamber depth, anterior lens curvature, posterior lens curvature, lens thickness, vitreous length and overall axial length.

Participating twins were reexamined at six-month intervals. The data collection phase was completed during the past year. A description of this co-twin control study design was presented at the First International Symposium of Twin Studies, Gregor Mendel Institute, Rome, Italy.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: Myopia is by far the world's most common cause of defective vision. Among environmental factors of suggested etiologic importance, one widely held theme, recurrent throughout the literature, relates the progression of myopia to prolonged use of the eyes for near tasks. Methods of treatment have been directed toward limiting accommmodation and the effort of near work. Published data regarding the effect of strong cycloplegic medications are promising. Such agents, however, produce side effects which influence the daytime function of the eyes. This study will provide a careful appraisal of the effectiveness of a clinically acceptable method of controlling accommodation.

Proposed Course: Data analysis will be undertaken in collaboration with the Section on Mathematical Statistics and Computer Applications, OBE.

Keyword Descriptors: myopia, monozygotic twins, cycloplegic medication, bifocal spectacles

NEI Research Program: Sensory and Motor Disorders of Vision--Congenital, Developmental, Degenerative Abnormalities

Experimental Subject or Tissue Source: Human

Research Objective: Etiology, Treatment

Honors and Awards: None

Publications:

Schwartz JT: A monozygotic co-twin control study of a treatment for myopia. Acta Geneticae Medicae et Gemellologicae. (in press).

Project No. Z012 EY2 00159-022 OBE

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

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

Project Title: The Influence of Methodologic Differences on Measurements of Cup/Disc Ratio

Previous Serial Number: NEI 74 OBE 159

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.15
Professional:	0.10
Other:	0.05

Project Description:

Objectives: To provide a general assessment of the average effect of overall interobserver differences on measurements of cup/disc ratio.

Methods Employed: All frequency distributions of cup/disc ratio published up to the present time were assembled and converted to a common graphic format. The extent to which observed disparity is likely to reflect true differences in cup/disc ratio among the study samples was determined by epidemiologic analysis of available descriptive data. The remaining disparity among distributions of cup/disc ratio was attributed to methodologic differences.

Major Findings: Marked differences in measurement of cup/disc ratio appear attributable to overall interobserver differences. In a clinical context, the value of horizontal cup/disc ratio > 0.3 which has been suggested as "suspicious" with respect to glaucoma surveillance would classify as glaucoma suspects over half of the clinically normal eyes represented by four of the seven distributions published in the literature.

Significance to Biomedical Research and the Program of the Institute: Central cupping of the optic nerve head is associated with glaucoma. A linear ratio of the horizontal diameter of the central cup to the horizontal diameter of the optic nerve head is commonly used by contemporary ophthalmologists in clinical and investigative applications. This study documents the existence of striking interobserver differences among those interested investigators who

have published frequency distributions of the measurement. The results indicate that methodologic differences need to be attended before appropriate screening or "suspect" measurements of cup/disc ratio can be developed.

Proposed Course: A manuscript describing this study entitled "Influence of methodologic differences on measurements of cup/disc ratio. An epidemiologic assessment," was completed and submitted for publication. Study is completed.

Keyword Descriptors: cup/disc ratio, interobserver measurement differences, glaucoma surveillance, optic nerve head cupping

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: None

Research Objective: Etiology

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00160-022 OBE

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

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

Project Title: Frequency Distribution of Horizontal Cup/Disc Ratio and Relationship Between Cup Size and Other Clinical Variables

Previous Serial Number: NEI 74 OBE 160

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: Frank H. Reuling, M.D.
Robert J. Garrison

Cooperating Units: Epidemiology Branch, NHLI

Man Years:

Total:	0.15
Professional:	0.10
Other:	0.05

Project Description:

Objectives: To determine the frequency distribution of a horizontal cup/disc ratio among a sample of normal subjects and to examine the association between cup size and variables such as age, sex, race, intraocular pressure and refractive error.

Methods Employed: The size of the physiologic cup of the optic nerve head of 160 normal subjects was estimated as a horizontal cup/disc ratio by slit lamp examination. The association between cup size and sex and race was analyzed by t-test; the association between cup size and age, intraocular pressure and refractive error was examined by multiple regression analysis.

Major Findings: This is the first frequency distribution of a horizontal cup/disc ratio based on data obtained by clinical examination using the biomicroscope. The size of the physiologic cup was found to be significantly associated with prevailing intraocular pressure among normal subjects, and, contrary to current teaching, it was also associated with age.

Significance to Biomedical Research and the Program of the Institute: The finding of a significant association between size of the physiologic cup and age raises the possibility of an acquired increase in cup size among normal subjects, which should be taken into consideration in the diagnosis of chronic simple glaucoma.

Proposed Course: A manuscript describing this study was prepared and accepted for publication. Study is completed.

Keyword Descriptors: cup/disc ratio, optic nerve head cup

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

Honors and Awards: None

Publications:

Schwartz JT, Reuling FH, Jr., Garrison RJ: Sources of acquired cupping of the optic nerve head in normotensive eyes: Br. J. Ophthalmol. (in press)

Project No. Z012 EY2 00161-022 OBE

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

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

Project Title: Association Between the Ocular Hypertensive Response to Topical Dexamethasone and Other Clinical and Laboratory Measurements

Previous Serial Number: NEI 74 OBE 161

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: Robert J. Garrison
Manning Feinleib, M.D.
Frank H. Reuling, M.D.
Doris J. Collie

Cooperating Units: Epidemiology Branch, NHLI

Man Years:

Total:	0.05
Professional:	0.05
Other:	0.00

Project Description:

Objectives: To determine the statistical association between ocular hypertensive responsiveness to topical dexamethasone among healthy subjects and variables such as cup/disc ratio, plasma cortisol, refraction, glucose tolerance, protein bound iodine, blood lipid fractions, hematocrit, uric acid and blood pressure.

Methods Employed: Measurements of the ocular hypertensive response to topical dexamethasone obtained in a heritability study among a sample of 63 twin pairs (see Project # NEI 74 OBE 002) are being compared with clinical and laboratory measurements obtained among the same sample of subjects.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: The ocular hypertensive response to topical corticosteroids is a phenomenon which has gained considerable attention and emphasis in the field of ophthalmology. In addition to being considered by some as a predictor of chronic simple glaucoma, the phenomenon is said to be associated with thyroid function, diabetes mellitus, cup/disc ratio and myopia. This study was undertaken to

reexamine the relationship between steroid responsiveness and parameters of these disorders and other clinical and laboratory variables.

Proposed Course: This study will be continued by the original collaborators.

Keyword Descriptors: ocular hypertensive response, topical corticosteroids, dexamethasone

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00162-022 OBE
1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

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

Project Title: Twin Study on the Inheritance of Normal Levels of Intraocular Pressure

Previous Serial Number: NEI 74 OBE 162

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: Frank H. Reuling, M.D.
Manning Feinleib, M.D.
Robert J. Garrison
Doris J. Collie

Cooperating Units: Epidemiology Branch, NHLI

Man Years:

Total:	0.05
Professional:	0.05
Other:	0.00

Project Description:

Objectives: To assess the role of genetic factors in determining normal levels of intraocular pressure.

Methods Employed: A sample of 80 pairs of monozygotic and like-sex dizygotic twins of age 15 years and older was examined. Prevailing intraocular pressure was measured using the Goldmann applanation tonometer. Zygosity was determined by blood serotyping.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: Elevated intraocular pressure is regarded as one of a triad of diagnostic signs associated with chronic simple glaucoma. Understanding the determinants of normal variation in levels of intraocular pressure is important to our ultimate understanding of the determinants of pathologic elevation in intraocular pressure. The present study examines the inheritance of normal levels of intraocular pressure using a genetic model not employed heretofore.

Proposed Course: This study will be continued by the original collaborators.

Keyword Descriptors: twins, heritability, intraocular pressure, normal variation, genetic model, chronic simple glaucoma

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00163-022 OBE

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

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

Project Title: Twin Heritability Study of Horizontal Cup/Disc Ratio in Normal Eyes

Previous Serial Number: NEI 74 OBE 163

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: Manning Feinleib, M.D.
Frank H. Reuling, M.D.

Cooperating Units: Epidemiology Branch, NHLI

Man Years:

Total:	.20
Professional:	.10
Other:	.10

Project Description:

Objectives: To assess the role of genetic factors in determining size of the physiologic cup of the optic nerve head as measured by a horizontal cup/disc ratio.

Methods Employed: A sample of 80 pairs of monozygotic and like-sex dizygotic twins of age 15 years and older were examined. A horizontal cup/disc ratio was estimated clinically by biomicroscopic examination using the Allen-Thorpe contact lens with the Haag-Streit Slit Lamp. Zygosity was determined by blood serotyping.

Major Findings: Analyses of these data suggest a highly significant contribution of genetic factors in determining the size of the central cup of the optic nerve head in normal eyes. This finding is consistent with recent findings of inheritance of the size of the physiologic cup as reported on the basis of studies using other genetic models.

Significance to Biomedical Research and the Program of the Institute: Cupping of the optic nerve head is regarded as one of the triad of diagnostic signs associated with glaucoma. Understanding the determinants of normal variation in size of the optic cup is important to our ultimate understanding of the determinants of pathologic change. The present investigation provides confirmatory evidence of a significant hereditary effect based on a genetic model not employed heretofore.

Proposed Course: A summary report on genetic aspects of this study was presented at the First International Symposium of Twin Studies in Rome, Italy, and a full report on ophthalmic aspects was prepared for publication. Study is completed.

Keyword Descriptors: twins, heritability, cup/disc ratio, genetic model, optic nerve head cup, normal variation

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

Honors and Awards: None

Publications:

Schwartz JT, Reuling FH, Feinleib M: Heritability study on size of the physiologic cup of the optic nerve head. A summary report. Acta Geneticae Medicae et Gemellologicae. (in press).

Schwartz JT, Reuling FH, Feinleib M: Nature, nurture and size of the physiologic cup of the optic nerve head. Arch. Ophthalmol. (in press).

Section on Mathematical Statistics and Computer Applications

Office of Biometry and Epidemiology

The main efforts of the Section on Mathematical Statistics and Computer Applications in its second year have been in consultation with and support of laboratory and OBE investigation in methodologic studies, and in epidemiologic identification of risk factors.

Karen Yuen, Ph.D., joined the Section in August under the provisions of the Intergovernmental Personnel Act by agreement with the University of California at Los Angeles.

The Section provided consultation and collaboration with several investigators in the Clinical Branch of NEI: Dr. Douglas Gaasterland on studies of parameters of aqueous humor dynamics; Dr. Kenneth Foon on lymphocyte transformation inhibition; Dr. Robert Frank and Dr. Elmer Ballintine on platelet aggregation and capillary fragility in diabetic retinopathy; Dr. Ballintine in the development of a contract for a photogrammetric fundus camera.

Rita Hiller participated in the contract development and review for the contract "Summary and Critique of Available Data on Prevalence and Economic and Social Costs of Vision Disorders and Disabilities," and in consultation with Westat, Inc., the contractee. This contract replaces an effort previously supported by this Section.

Rita Hiller also represented the NEI at the American Foundation for the Blind seminar on "Birth Defects/Blindness and Severe Vision Impairment," and at the meeting of the Steering Committee of the Total Ophthalmic Community when she spoke on "Reliability of Statistics."

Karen Yuen participated in the Computer Science and Statistics 8th Annual Symposium on the Interface, and in a UCLA-sponsored workshop on use of computers in biomedical analysis.

Roy Milton has continued to provide assistance to the Office of Extramural and Collaborative Programs of NEI in the planning and development of a computer-based information system for scientific program analysis of research grants, with similar assistance to NHLI.

As part of an ongoing effort to promote awareness and cooperation among the statistical computing community at NIH, and to improve the local availability of statistical software, Roy Milton has organized a Statistical Computing Seminar. He also coordinated the NIH Probability and Statistics Seminar.

Project No. Z012 EY2 00119-032 OBE

1. Office of Biometry & Epidemiology
2. Section on Mathematical Statistics and Computer Applications
3. Bethesda, Maryland

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

Project Title: Accuracy and Repeatability of Reading Fundus Photographs

Previous Serial Number: NEI 73 OBE 119

Principal Investigator: Roy C. Milton, Ph.D.

Other Investigators: James P. Ganley, M.D,, Dr. P.H., University of Arizona
Rodney Lynk, M.D., University of Florida
Harold A. Kahn

Cooperating Units: Department of Ophthalmology, University of Wisconsin

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: The purpose of this study is to investigate the use of trained non-ophthalmologists and non-physicians in reading stereo fundus photographs according to an established protocol, and subsequently to examine the accuracy and intra- and interobserver variability associated with this reading, from the viewpoint of professional (physician) readers, non-professional (technician or clerk) readers, and expert (standard) readers.

Methods Employed: The modification of the Airlie House Classification of diabetic retinopathy used by the collaborative Diabetic Retinopathy Study is the standard by which the fundus photographs are evaluated. This standard classification consists of 15 stereo photographs by which the following 17 types of lesions found in diabetic retinopathy are evaluated: hemorrhages, microaneurysms, hard and soft exudates, venous, arteriolar and intraretinal microvascular abnormalities, arteriovenous nicking, macular edema, neovascularization both within one disc diameter of the disc and elsewhere in the fundus, fibrous proliferation within one disc diameter of the disc and elsewhere, plane of proliferation, retinal elevation, and preretinal and vitreous hemorrhage.

The lesions on the patient photograph are compared to the standard photograph for the degree of the particular abnormality under consideration. A detailed protocol, suitable for use by both lay and professional readers, has been developed by the physician investigators describing each lesion in detail (e.g. color, size, shape, etc.) and how they are to be read according to the modified Airlie House Classification.

Two lay readers (a secretary and a medical coding clerk) have been taught to read stereo fundus photographs for specific lesions according to the developed protocol. A teaching set of stereo photographs was used during the training period by both the lay readers and the two physician readers (one a non-ophthalmologist) in order to familiarize themselves with the methodology.

The study group of diabetic stereo fundus photographs, obtained from Dr. James Harris of the Department of Ophthalmology at the University of Wisconsin, is a group of 14 eyes of individuals with moderate to severe diabetic retinopathy which have previously been graded elsewhere. Eight eyes from normal volunteers complete the study group, which consists of 148 stereo slides. Each reader graded the slides twice from a random ordering of the slides, for each of the 17 lesions.

Major Findings: Analysis of intraobserver variability (repeatability) showed the physician readers to exhibit somewhat less variability than the lay readers, but not for all lesions and seldom to a meaningful extent. Intraobserver variability, which may be interpreted as a measure of accuracy, varied by lesion, from most and unacceptable variation for intraretinal hemorrhage to least variation for macular edema. Assessment of contribution to variation by individual readers was possible and permits future training directed at reducing variability to acceptable values in potential production applications.

Significance to Biomedical Research and the Program of the Institute: Increasing use is being made of fundus photography as a means of documenting clinical pathology in therapeutic trials, in multiphasic screening programs, in epidemiologic studies, and in clinical follow-up of patients. Studies of accuracy and variability are essential steps in the development and acceptance of this use. Lay readers can be comparable to physician readers in terms of accuracy and variability, and they may be utilized to free the physician from this expensive and time-consuming procedure without loss of quality.

Proposed Course: A manuscript, "Repeatability and accuracy of grading stereo fundus photographs," will be submitted for publication this year, and the project is completed.

Keyword Descriptors: fundus photography, diabetic retinopathy, observer variability

NEI Research Program: Retinal and Choroidal Diseases--Vascular and Circulatory Abnormalities

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00035-012 OBE

1. Office of Biometry & Epidemiology
2. Section on Mathematical Statistics & Computer Applications
3. Bethesda, Maryland

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

Project Title: Blindness from Glaucoma

Previous Serial Number: None

Principal Investigators: Rita Hiller
Harold A. Kahn

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.1
Other:	0.2

Project Description:

Objectives: To analyze data on blindness due to glaucoma with respect to trends and factors related to risk.

Methods Employed: The data from the Model Reporting Area for Blindness Statistics (MRA) were used to study demographic factors such as age, sex and race in relation to glaucoma blindness. The rate of register incidence for blindness from glaucoma was also calculated for each of the years 1963 through 1970 for a constant group of states in an effort to assess the recent trend of blindness due to glaucoma.

Major Findings: Primary glaucoma continues to be one of the leading causes of blindness in the U.S.

An overall ratio of eight to one was observed for nonwhite to white rates of glaucoma blindness. We have fragmentary evidence suggesting that the ratio for underlying disease, that is, nonblinding glaucoma or intraocular pressure differentials, may be closer to two to one than eight to one as between nonwhites and whites. It is likely that the remaining four-fold differential is explained by a combination of more complete reporting for nonwhites, reporting for medical care later in the disease than for whites, and poorer response to treatment for nonwhites compared to whites.

The trend of new blindness from glaucoma for recent years is shown to be stationary in the U.S. and in England and Wales.

Significance to Biomedical Research and the Program of the Institute: Nonwhites have been identified as a high risk group for glaucoma blindness.

Proposed Course: Project completed.

Keyword Descriptors: glaucoma, blindness, risk factor, Model Reporting Area (MRA), white nonwhite incidence for blindness from glaucoma

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

Honors and Awards: None

Publications:

Hiller R, Kahn HA: Blindness from glaucoma. Am. J. Ophthalmol. (in press).

Project No. Z012 EY2 00036-012 OBE

1. Office of Biometry & Epidemiology
2. Section on Mathematical Statistics & Computer Applications
3. Bethesda, Maryland

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

Project Title: Sunlight Exposure and Cataract

Previous Serial Number: None

Principal Investigators: Rita Hiller
Luigi Giacometti, Ph.D., Scientific Programs Branch, NEI

Other Investigators: Karen Yuen, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To investigate sunlight as a risk factor for cataract.

Methods Employed: Determinations were made, using a map prepared by the Department of Commerce, of annual hours of sunlight by county for Model Reporting Area for Blindness Statistics (MRA) and cities for National Health and Nutrition Survey (HANES). The cataract data available from these two studies were then grouped according to annual hours of sunlight. A suitable control group will be chosen from among other eye diseases. From these data, we can then see if there is an additional risk of cataract for locations where there is a large amount of sunlight. The analysis will be done by age, sex and race.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: This is a pilot study to investigate sunlight exposure as a possible risk factor for cataract, a major cause of blindness in the United States. Little is known about the epidemiology of cataract. It is hoped that this study will provide clues about possible environmental risk factors in cataract development.

Proposed Course: This project will continue into the next FY.

Keyword Descriptors: cataract, sunlight, risk factor, Model Reporting Area (MRA), National Health and Nutrition Survey (HANES)

NEI Research Program: Cataract

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00037-012 OBE

1. Office of Biometry & Epidemiology
2. Section on Mathematical Statistics & Computer Applications
3. Bethesda, Maryland

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

Project Title: Senile Cataract Extraction and Diabetes

Previous Serial Number: None

Principal Investigator: Rita Hiller

Other Investigators: Harold A. Kahn

Cooperating Units: Washington Hospital Center, Washington, D. C.
Hospital Discharge Survey, NCHS
Household Interview Survey, NCHS

Man Years:

Total:	0.7
Professional:	0.1
Other:	0.6

Project Description:

Objectives: To ascertain whether or not presence of diabetes increases the probability of development of senile cataract, where extraction is used as an index of development.

Methods Employed: Hospital discharge data were obtained from the U.S. Hospital Discharge Survey (1972) and Washington Hospital Center, Washington, D. C. area (July 1971 through June 1973), from which information was collected about the presence or absence of diabetes among patients having cataract extraction and among controls (patients with fracture, sprain or strain). Age-race specific odds ratios were used to study the relationship of diabetes to the probability of senile cataract extraction. The validity of choice of control group was tested in various ways and found satisfactory.

Major Findings: The odds ratio for cataract extraction was four to one comparing diabetics to non-diabetics for ages 40-49, two to one for ages 50-69 and approximately one to one for ages 70 and above. This suggests that diabetes greatly enhances the probability of cataract extraction in age group 40-49, is less important in ages 50-69 and not important in ages above 70.

Significance to Biomedical Research and the Program of the Institute: Diabetes was identified in this study as a moderate risk factor for development of senile cataract in the U.S. A previous British study reported a risk

ratio of about seven at ages 50-69, suggesting a much greater importance of diabetes. Reasons for preferring the results of the present study as an appropriate assessment of diabetes as a risk factor are in a paper submitted for publication.

Proposed Course: A self-coded questionnaire was designed for a study to help determine whether the severity and/or duration of diabetes is a factor in the natural history of senile cataract. The questionnaire also is designed to determine whether there is a difference in post-operative visual acuity between diabetics (with and without retinopathy) and non-diabetics. Development of a study using this questionnaire is being considered. A manuscript, "Senile cataract extraction and diabetes," has been submitted for publication.

Keyword Descriptors: senile cataract extraction, diabetes, risk factor, odds ratio, hospital discharges

NEI Research Program: Cataract--Degenerative (Senile) Cataract

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00031-012 OBE

1. Office of Biometry & Epidemiology
2. Section on Mathematical Statistics & Computer Applications
3. Bethesda, Maryland

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

Project Title: Robustness of Some Sequential Procedures

Previous Serial Number: None

Principal Investigator: Karen Yuen, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To investigate the robustness of four sequential procedures against departures from assumptions of normality and independence.

Methods Employed: Computer simulations were applied to study the performance of three sequential t-tests (open plan, restricted plan, wedge plan) and a non-parametric sequential signed-rank test under the following non-normal distributions: uniform, scale contaminated normal, and Cauchy. Secondly, these four test procedures were investigated for normal distributions with a specified correlation structure.

Major Findings: The three sequential t-tests are discovered to be quite robust against a short-tailed uniform distribution. However, these tests become conservative when the underlying distribution is long-tailed. The signed-rank test is recommended to be used when the underlying distribution is believed to be intrinsically long-tailed or contaminated with outliers. The open scheme is superior in power and has the smallest average sample size if the underlying distribution does not deviate too much from normality. However, if one is concerned about the unlimited nature of the maximum sample size, the wedge procedure is a good alternative. All four tests are "optimistic" if the observations are serially correlated. The signed-rank test seems to be most vulnerable among the four in terms of empirical Type I error. With consideration also of the mean sample size, the open or wedge scheme is recommended for this situation.

Significance to Biomedical Research and the Program of the Institute: Sequential analysis is a common statistical procedure used in biomedical research, clinical trials, toxicity studies, etc. The result of this study gives an indication of how sensitive these procedures are to deviations from assumptions on which the theories are based. Thus, this knowledge will guide design and analysis of future experiments.

Proposed Course: A manuscript, "Robustness of some sequential procedures," has been submitted for publication.

Keyword Descriptors: statistical methodology, simulation, computer, sequential procedures, robustness

NEI Research Program: (Statistical methodology)

Experimental Subject or Tissue Source: None

Research Objective: Etiology, Treatment

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00032-012 OBE

1. Office of Biometry & Epidemiology
2. Section on Mathematical Statistics & Computer Applications
3. Bethesda, Maryland

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

Project Title: Comparison of Two Survival Curves

Previous Serial Number: None

Principal Investigator: Karen Yuen, Ph.D.

Other Investigators: M. Ray Mickey, Ph.D., Health Sciences Computing Facility, University of California, Los Angeles

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To set forth a new statistical procedure for the comparison of two survival curves, and subsequently, to evaluate this new technique against several existing methods.

Methods Employed: The proposed procedure is substantiated both theoretically and by Monte Carlo method. The comparison of techniques is based on computer simulation.

Major Findings: Under the exponential survival model with no censoring, the proposed procedure yields the anticipated Type I error. When compared with Mantel's procedure, the new technique is more powerful as long as the number of time intervals is not excessive.

Significance to Biomedical Research and the Program of the Institute: This research will provide an additional tool to the statistician in analyzing survival data. The statistical problem arises in clinical trials comparing two treatments, where the observation for each patient is often time to failure or time to withdrawal.

Proposed Course: Work is being concentrated on the case when there is censoring. Again the exponential model is used, and several patterns of censoring are considered. The new procedure will also be compared with the Wilcoxon text, permutation test, Mantel's test, and the test by Berger and Gold.

Keyword Descriptors: survival curves, simulation, computer, statistical methodology

NEI Research Program: (Statistical methodology)

Experimental Subject or Tissue Source: None

Research Objective: Treatment

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00033-012 OBE

1. Office of Biometry & Epidemiology
2. Section on Mathematical Statistics & Computer Applications
3. Bethesda, Maryland

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

Project Title: Small Sample Behavior of Some Non-Parametric Multi-Sample Location Tests in the Presence of Dispersion Differences

Previous Serial Number: None

Principal Investigator: Karen Yuen, Ph.D.

Other Investigators: None

Cooperating Unit: None

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: This is a comparative study of the small and moderate sample behavior of four non-parametric statistics that were proposed to test the equality of several location parameters without assuming the equality of scales. The objectives of this investigation include, 1) how well the asymptotic null distribution of the statistic approximates the small sample distribution of the test, 2) the comparison of validity among the tests and, 3) the comparison of powers among these statistics under normal distribution.

Methods Employed: Monte Carlo sampling experiments were done on the computer.

Major Findings: With respect to the size and power of these non-parametric test statistics under heteroscedasticity, the studentized Wilcoxon and the studentized Mood test are the most likely choices in the 2-sample and c-sample situation respectively for moderate sample sizes. When sample sizes are small (e.g. 10), none of the statistics considered here are satisfactory except for the studentized Wilcoxon case when the number of samples is four.

Significance to Biomedical Research and the Program of the Institute: Testing the equality of means (or medians) is a basic procedure in biomedical investigations. When there is a lack of homogeneity of scales (dispersions) among groups, the existing non-parametric tests may produce Type I errors that

are greatly different from the nominal value. Hence, Sen proposed several statistics for this situation and showed that they are distribution-free when the sample sizes approach infinity. This project aims at studying the validity of his tests for finite sample sizes so that we can evaluate whether they can be applied to real life data. Results of this research reveal to us which tests are the most likely choices.

Proposed Course: A manuscript, "Small sample behavior of some non-parametric multi-sample location tests in the presence of dispersion differences," has been submitted for publication.

Keyword Descriptors: statistical methodology, non-parametric tests, location parameters, heteroscedasticity, simulation, computer, multi-sample tests

NEI Research Program: (Statistical methodology)

Experimental Subject or Tissue Source: None

Research Objective: Etiology, Treatment

Honors and Awards: None

Publications: None

Project No. Z012 EY2 00034-012 OBE

1. Office of Biometry & Epidemiology
2. Section on Mathematical Statistics & Computer Applications
3. Bethesda, Maryland

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

Project Title: Statistical Packaged Programs Design and Development

Previous Serial Number: None

Principal Investigator: Karen Yuen, Ph.D.

Other Investigators: None

Cooperating Unit: Health Sciences Computing Facility
University of California, Los Angeles

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To design and develop statistical packaged programs in the UCLA BMDP series.

Methods Employed: Literature search, methodology research and the coding of the programs in Fortran.

Major Findings: 1) Sixty-five robust estimates of location which were investigated in the Princeton Study have now been incorporated into BMDP7D. 2) A new life-table program with the capability of handling competing risk was proposed to UCLA. They have expressed definite interest in this program and developmental work is now in progress.

Significance to Biomedical Research and the Program of the Institute: This is an effort to make statistical procedures easily available to users in solving real medical problems. Newly developed statistical techniques and computing algorithms are being incorporated into these programs to better meet the users' needs. The life-table program that was proposed is useful in analyzing clinical trial data. It has enough options in it to provide sufficient flexibility for different projects.

Proposed Course: Major emphasis will be on the programming part of the life-table program. Other statistical programs will also be proposed and developed as future needs arise in NEI.

Keyword Descriptors: computer, statistical methodology, life table, robust estimation

NEI Research Program: (Statistical methodology)

Experimental Subject or Tissue Source: None

Research Objective: Etiology, Treatment

Honors and Awards: None

Publications: None

CONTRACT NARRATIVE

NEW YORK MEDICAL COLLEGE (NIH-NEI-72-2113)

Title: Steroid Induced Glaucoma

Current Fund Allocation: $115,000

Objective: This contract is directed toward increasing our knowledge of the repeatability and heritability of the intraocular pressure response to topical corticosteroids.

Progress to Date: Verified data on test-retest comparability and on intra-pair variance among twins have been received. Data on other aspects of the contract have not yet been received and the contractor has been requested to supply these.

Significance to NEI Programs and Biomedical Research: The intraocular pressure response to seroids was reported as a monogenic inherited trait closely related to glaucoma. Recent work in this office has cast doubt on the heritability of this response and the present contract is intended to clarify the matter.

Proposed Course: When the full set of data is on hand and has been reviewed, a meeting will be held with the contractor and project staff to discuss their preparation of a formal final report.

Keyword Descriptors: glaucoma, topical corticosteroids, intraocular pressure, heritability, twins

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

OFFICE OF SCIENTIFIC REPORTS AND PROGRAM PLANNING COORDINATION

ANNUAL REPORT
OFFICE OF SCIENTIFIC REPORTS AND PROGRAM PLANNING COORDINATION
NATIONAL EYE INSTITUTE
July 1, 1974 - June 30, 1975

SCIENTIFIC REPORTS

The Office engaged in a variety of activities during the year which broadened the scope of communications with the biomedical scientific community, the general public, and special interest audiences. While a number of these projects were initiated directly by the Office, many were carried out in response to growing public awareness of and an interest in vision care and vision research. Such initiation and responsiveness on the part of the Office served as forerunners of and were in line with subsequent recommendations of the National Advisory Eye Council's Vision Research Program Planning Report. The Report calls for expanded communication, not only with members of the medical and vision research communities, but also with the general public, in order to improve the overall level of vision care in the United States.

Scientific Communications

Direct communications with the biomedical scientific community were enhanced by a number of special projects throughout the year. Early in October 1974, representatives of the Office accompanied Institute scientific staff to the annual convention of the American Academy of Ophthalmology and Otolaryngology in Dallas, Texas. In particular, the Office assumed primary responsibility for planning, coordinating, and staffing the NEI exhibit highlighting the Institute's research activities for the Academy meeting. To supplement the exhibit, the Office also prepared and distributed a more detailed brochure to members of the Academy.

In conjunction with the NIH Alumni Reunion in April, the Office coordinated the planning and design of another exhibit. Prepared specifically for a scientific audience, this exhibit represents entramural research efforts in cataract and glaucoma.

The Institute was asked, by special invitation, to participate in the American Medical Association convention in Atlantic City in June. For this meeting, the Office redesigned, supervised the refurbishing of, and again staffed, the program exhibit for this meeting.

In April, the Institute hosted--for the first time--an NIH Lecture and invited Torsten N. Wiesel, M.D., of Harvard Medical School, to address a scientific audience at NIH. The Office undertook full responsibility for the publicity efforts in conjunction with this lecture, including posters, a press release, notices in the NIH Record, and other publicity materials. As a result, the lecture on Visual Deprivation and Its Effects on the Monkey Striate Cortex was well attended by NIH staff members, as well as those from other medical institutions.

The Office contributed five full-length articles to a new NIH publication, Research Advances, which was widely disseminated to medical schools and other grantee institutions throughout the country. The NEI portion of this award-winning publication, written by the Office staff, required extensive coordination and collaboration with grant-supported researchers, other NEI offices, and central NIH representatives.

During the spring of 1975, the Office published the fourth and final experimental issue of 20/20, the Institute's newsletter for the vision research community. Before another issue can be published, the newsletter must be established as a permanent periodical publication. In this regard, the Office arranged, under private contract, for a readership survey of the newsletter. Results of the survey were very favorable, and the Office is now attempting to establish the newsletter on a regular basis.

Other scientific communication activities in which the Office participated included arranging interviews with Institute staff for broadcast on the Physicians Radio Network, conducting tours of NEI facilities for visiting physicians and scientists, and assisting in the dissemination of information regarding various scientific meetings, seminars, and workshops hosted by the Institute's scientific staff.

These activities required substantial coordination among various concerns and brought the Office into direct contact with numerous members of the vision research community throughout the year. The rapport developed in this collaboration will be continued in the coming year and will serve as a foundation for expanded scientific communication activities.

Consumer Education

A number of new activities were undertaken during the year which were designed to promote better public understanding of vision research and of the visual system in health and disease.

Probably the most unique among these consumer education activities was the Office's involvement in the weekend-long NIH Open House in April. The event marked the kickoff for the Agency's participation in the Nation's Bicentennial celebration. The NEI booth, planned and designed by the Office staff, attracted a large portion of the 35,000 people who visited the campus for the first such Open House in more than 20 years. The Office staff, along with members of the Clinical Branch, provided information about the activities of the Institute, vision and visual care, and eye diseases to the general public.

In conjunction with the Bicentennial, the Office also revised the Alumni Reunion exhibit for the lay audience. The exhibit will remain standing at NIH for the duration of the 18-month celebration.

Yet another aspect of the Office's participation in the Bicentennial, on behalf of NEI, involves representation in three aspects of the NIH exhibit at the Department of Health, Education, and Welfare. The Office

assisted in planning and coordinating presentations on research activities, improved treatment modalities, and answers to commonly asked questions about the eye and vision.

A new channel of communications with a specialized segment of the general public was opened when the Institute participated in the NIH Minority and Women's Opportunity and Resources Conference this past spring. The Office helped plan, coordinate, and execute the NEI briefing which was designed to achieve improved communications with faculty and students from minority and women's educational institutions.

In the area of general consumer education information, the Office prepared a series of four articles concerning vision and the visual system in health and disease for the NIH column, Search for Health, which is distributed by the NIH Office of Information to weekly newspapers across the country.

Publications

The Office revised and reprinted two fact sheets--Diabetic Retinopathy and Glaucoma--in its series of publications on vision disorders.

During the year, the Office distributed the following number of publications, including dissemination of a large number during the NIH Open House:

Cataract	4,800	Retinal Detachment	1,562
Retinitis Pigmentosa	1,850	Corneal Diseases	1,678
Refractive Errors	3,648	Glaucoma	3,861
Diabetic Retinopathy	4,100	Macular Degeneration	1,597
Statistics on Blindness in the Model Reporting Area, 1969-1970			401
Security is an Eye Patch			34,563
U.S. News and World Report, Interview with Dr. Kupfer			172
Evaluation of the Treatment of Diabetic Retinopathy, A Research Project, Reprint from the Sight Saving Review			825

Public Inquiries

This year, as last, approximately 800 letters of inquiry from the general public required individual written responses from the Office. The number of telephone inquiries continued to increase, with nearly 3,000 calls handled during the year. Inquiries concerning cataract research and treatment, glaucoma, and diabetic retinopathy continued to be principal areas of interest. In addition to these, the Office also handled numerous inquiries regarding less common causes of blindness and visual disability.

The Office responded to approximately 60 written Congressional or other controlled inquiries, and to 48 telephone calls from Congressional offices.

Press Relations

During the year press releases announcing the NIH Lecture delivered by Dr. Torsten N. Wiesel, the selection of Dr. William R. Raub as Associate Director for Extramural and Collaborative Programs, the appointment of four new members to the National Advisory Eye Council, and the publication of the Council's Vision Research Program Planning Report were prepared. In addition, 5 stories were prepared for the NIH Record, and 10 articles were edited for News and Features from NIH, which is distributed to approximately 500 science writers in the professional and general press. An announcement was also written for distribution to the vision research journals concerning the NEI Information Suites hosted by Institute staff at the spring Association for Research in Vision and Ophthalmology (ARVO) meeting in Sarasota, Florida.

The Office assisted press representatives from the McGraw-Hill Publishing Company, National Observer, National Enquirer, Boston-Herald American, Mr. Peter Weaver's Consumer Column, U.S. News and World Report, the Drug Research Report (Blue Sheet), U.S. Medicine, Medical World News, Family Health Magazine, Clinical Trends, and a number of free-lance writers. In addition, a representative from the New York Times spent several days at the Institute doing extensive research and interviewing NEI staff for a lengthy article scheduled to appear in mid-July in the New York Times Magazine. A reporter from a Soviet Union magazine, Ogonyok, also visited the Institute for an interview with Dr. Kupfer.

Press Seminar

The most significant activity involving press relations undertaken by the Office this year was the Institute's first science writers seminar held in mid-June. The seminar served as a five-year report on the growth and development of the Institute and provided a forum for discussion of significant advances in vision research. Reporters from approximately 20 publications, representing both specialized and mass media, attended the briefing on research progress in cataract, retinal degenerative diseases, and diabetic retinopathy. A presentation on the publication of the Council Report, Vision Research Program Planning, added a perspective to the current status and future course of vision research. Speaking at the briefing were Drs. Carl Kupfer, Jin H. Kinoshita, Alan M. Laties, Matthew D. Davis, and Bradley R. Straatsma, and Mrs. Mary Lasker.

The Office assumed total responsibility for the planning and execution of the seminar including the advance publicity, coordination of the scientific presentations, preparation and distribution of appropriate background information, liaison with the various university public relations offices, and all necessary follow-up activities. The briefing was well received by the press, and publication of subsequent articles is expected.

Miscellaneous

The annual Save Your Vision Week and White Cane Safety Day Presidential Proclamations were prepared by the Office, as well as a presidential message

to the 77th Annual Congress of the American Optometric Association. A message was also prepared for the Secretary, DHEW, to the American Optometric Association Conference on the Future of Optometric Practice. The Office also coordinated the Institute's contributions to the NIH Annual Report, NIH Quarterly Communications Report to the White House, NIH Scientific Directory and Annual Bibliography, Freedom of Information Act Annual Report to the Congress, and the Scientific Information Exchange of the Smithsonian Institution. The Office also wrote the NEI portion of the NIH Almanac.

In response to Congressional requests, the Office prepared a number of reports relating the Institute's information activities in three areas: scientist-to-scientist communication, scientist-to-physician communication, and scientist-to-general public communication. As in previous years, the Office continued its liaison with various voluntary and professional organizations including the National Society for the Prevention of Blindness, Inc., Fight for Sight, Inc., Research to Prevent Blindness, the American Association of Ophthalmology, and the American Optometric Association.

Assistance was provided to the Director in the preparation of presentations before the Pan American Congress of Ophthalmology in San Juan, Puerto Rico, the American Academy of Ophthalmology and Otolaryngology, the Association for Research in Vision and Ophthalmology, and the Prevention of Blindness Society of Metropolitan Washington.

PROGRAM PLANNING COORDINATION

The Office continued coordinating the National Advisory Eye Council's Vision Research Program Planning Committee workshops. The Committee met with the consultants for the Glaucoma and Sensory and Motor Disorders of Vision programs in early July and assisted in the drafting of the last two program analyses indicating the major needs and opportunities for research in these fields in 1975. These program analyses were combined with the already completed analyses for Retinal and Choroidal Diseases, Corneal Diseases, and Cataract to form the principal component of Vision Research Program Planning. This 250-page documentation of the present state of vision research in America was one of the most important tasks of the Office in FY 1975. The Office wrote or rewrote several passages within the Report; edited and formated the entire Report; supervised all typing, proofreading, and preparation of the artwork; and designed the layout of over 70 tables documenting all vision research known to be funded by public or private organizations in the United States. The Office also supervised printing of the Report and its distribution to the vision research community, practicing ophthalmologists, members of Congress, others within the Federal government, and interested members of the general public who requested copies or made inquiries easily answered by the Report.

Besides being responsible for formating and publishing this NEI Annual Report, the Office also produced or collaborated with other elements within NEI on the production of a number of other evaluative and/or planning documents. The NEI portion of the NIH Forward Plan, which covers Fiscal Years 1976-1980, was prepared by this Office. The Plan addresses itself to the NEI mission, program balance and strategy, opportunities for research progress in each of the five NEI research programs, and the impediments to research progress for each of these as well as to intramural research and research management and program services.

The Office wrote a proposed NEI Expanded Mission Statement which includes expansion into those disease control and demonstration activities which would provide a suitable setting for testing the application of research results to the improved delivery of eye care and rehabilitation. In addition, a draft Expanded Mission of NIH was written in cooperation with the National Institute of Child Health and Human Development.

During the past year, the Office responded to several requests for information concerning grant and contract mechanisms from the President's Biomedical Research Panel by assembling data from several components of NEI. The Office also responded to requests from the NIH Office of Program Planning and Evaluation and various similar Institute-level offices concerning such disparate topics as: NEI support related to the National Heart, Blood Vessel, Lung and Blood Program; NEI research efforts which have significance for prevention and intervention aspects of DHEW Early Periodic Screening, Diagnosis, and Treatment efforts affecting children; future NEI implementation of Health Aspects of Nutrition Policy in the HEW Forward Plan for Health; and NEI activities applicable to the Handicapped Individuals Survey for the DHEW Office for Handicapped Individuals.

The Office answered a request from the Division of Resources Analysis, OPPE, concerning NEI efforts to improve Indian health which was incorporated in the annual Indian Health Service Survey.

The Office prepared speeches for the Director, NEI, concerning topics related to program planning. Perhaps the most important examples were the Opening Statement for the Congressional Budget Hearings and the slide show and discussion provided for Dr. Kupfer's presentation to the Association for Research in Vision and Ophthalmology in April.

Finally, the Office is responsible for managing an evaluation contract with Westat, Inc., which is attempting to identify and acquire secondary data on the prevalence and cost of visual disorders in America. Despite delay in clearance of survey forms by OMB, the contractor has made satisfactory progress during FY 1975 and a final report is expected in early 1976.

EXTRAMURAL AND COLLABORATIVE PROGRAMS

ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1974 - June 30, 1975

REPORT OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL AND COLLABORATIVE PROGRAMS

William F. Raub, Ph.D.

Fiscal Year 1975 was marked by continued maturation of all National Eye Institute programs. Despite considerable uncertainty during the first three quarters of the year as to the availability of funds, the NEI was able not only to sustain the level of momentum of vision research that had been reached during the previous year but also to enrich its portfolio of sponsored activities with a wide array of new and important high quality research projects. The interests and accomplishments of the vision research community span the spectrum from basic biomedical science to health care delivery. It is noteworthy that an ever-increasing number of the grantees and contractors of the NEI are found at the forefront of essentially all of the different research areas that comprise this spectrum.

1. Vision Research Program Planning

A major event during Fiscal Year 1975 was the publication of the report of the special Vision Research Program Planning Committee of the National Advisory Eye Council. This report surveys the entire field of vision research and identifies areas in which there is special need and opportunity for a broadened investigative effort. These recommendations should be an important stimulus to scientists who are working in or planning to work in the vision research field. The report undoubtedly will have value as a conceptual framework for the management and administration of NEI extramural programs.

2. Funding of Research Grants[1]

The amount available for NEI research grants during Fiscal Year 1975 was $29,979,000, an increase of $1,950,000 over the preceding year. This funding level made it possible for the NEI to award 536 grants distributed as follows:

	Number	Total Awarded (in thousands)
Prior Year Commitments	300	$18,258
Competing Renewals	90	5,376
New Awards	146	6,345

The combined number of competing renewals and new awards represents over 75% of all applications that had been recommended for approval by the National Advisory Eye Council.

[1] As the writing of this report occurred prior to the completion of final FY 1975 funding decisions, this section of necessity reflects projected figures as well as actual obligations.

The funding data for NEI research grants can be broken down further in two complementary ways: by scientific program area and by funding mechanism. Tables for each of these representations are given below.

NEI Research Grants by Program Area

	Number	Total Awarded (in thousands)
Retinal and Choroidal Diseases	197	$10,962
Corneal Diseases	84	5,297
Cataract	55	2,887
Glaucoma	54	4,007
Sensory and Motor Disorders of Vision	146	6,826

NEI Research Grants by Funding Mechanism

	Number	Total Awarded (in thousands)
Project Grants	473	$26,261
Special Visual Sciences Research Awards	18	149
Core Center Grants	9	1,605
Specialized Clinical Research Center Grants	6	1,233
Research Career Development Awards	24	576
Academic Investigator Awards	6	155

It should be noted that the foregoing breakdown by funding mechanism includes one type of award not available to the vision research community in previous years--the Academic Investigator Award. This new funding mechanism is designed to help young faculty members in academic settings mature as independent investigators. The six awards made in Fiscal Year 1975 and those to be made in future years should do much to increase the number and quality of vision research scientists fulfilling the dual roles of investigator and educator.

3. Research Training Support

During the past year, the National Research Service Award Program was inaugurated. This Program includes both individual fellowships and institutional fellowships, the latter being somewhat comparable to the traditional training grants now in a phase-out status. While many details remain to be resolved regarding the nature and scope of future NEI research training activities, there is good reason for optimism that the National Research Service Awards will provide a firm basis for equipping motivated young people with the research skills they will need to advance the frontiers of vision science. Of particular interest is the potentiality of these fellowships for bringing the tools and methods of biochemistry, immunology, pharmacology, physiology, epidemiology, biomathematics, and many other scientific disciplines to bear on problems associated with visual system disorders. Projected

NEI expenditures for research training during Fiscal Year 1975 are listed below. This tabulation includes both fellowships and training grants activated in previous years as well as the new National Research Service Awards.

	Total Awarded (in thousands)
Postdoctoral and Special Fellowships	$ 109
Weinberger Fellowships	70
Graduate Research Training Grants	2,000
National Research Service Awards (Individual)	860
National Research Service Awards (Institutional)	1,064

4. Research Contracts

The NEI budget for research contract activities during the past year was $2,322,000. The bulk of these funds was allocated for the support of two cooperative clinical trials: a continuing study of photocoagulation therapy in diabetic retinopathy and a new study of vitrectomy surgery in diabetic retinopathy. These two studies should do much to determine what is the best care for patients afflicted with this dread chronic disease. These studies also illustrate how NEI's research contracting authority can complement the portfolio of research grants, supporting important studies whose scope and complexity make the research grant mechanism impractical, if not impossible, to use.

5. Data and Analysis Unit

The Data and Analysis Unit for NEI Extramural and Collaborative Programs continued its development during the past year. A substantial amount of financial and other management information about NEI's portfolio of awards now is available in computer-manipulable form and can be accessed to produce a wide array of reports and analyses. Within recent months a pilot effort involving the storage and retrieval of research grant abstracts has yielded promising results. If expansion of the scientific content of the data file continues to prove feasible, the resulting information system should be invaluable not only to NEI staff but also to consultants participating in the program planning efforts of the National Advisory Eye Council.

6. Staff Changes

During the past year, there were several changes in key positions concerned with NEI Extramural and Collaborative Programs.

a. Dr. William F. Raub replaced Dr. George T. Brooks as Associate Director for Extramural and Collaborative Programs. Dr. Brooks became the Associate Director for Extramural Program Activities, National Institute of Arthritis, Metabolism, and Digestive Diseases.

b. Dr. Israel A. Goldberg, Program Director for Sensory and Motor Disorders of Vision, left the NEI to join the National Institute of Arthritis, Metabolism, and Digestive Diseases. Dr. Wilford L. Nusser, Chief of the Scientific Programs Branch, has assumed Dr. Goldberg's responsibilities pending selection of a successor.

c. Dr. Samuel C. Rawlings joined the NEI as Program Director for Glaucoma and for Research Training Activities.

RETINAL AND CHOROIDAL DISEASES

DISORDERS OF THE RETINAL PIGMENT EPITHELIUM

In the course of embryological development, the pigment epithelium and neural retina approximate each other. At later stages of development of the sensory retina, it is suggested that the interdigitation of photoreceptors with microvilli of the pigment epithelium coincides with a physiological interdependence. The role of the retinal pigment epithelium in the nutrition of the sensory retina and in the excitatory process is an important area of investigation.

Cytoplasmic inclusion of melanin granules are evident in the normal pigment epithelium. In the pigmentary degenerative disorders, it appears that the ophthalmoscopic observation of pigment proliferation in the equatorial region is accompanied by areas of sparse pigmentation in other areas of the retina. However, it is generally believed that the obvious pigmentary changes of the pigment epithelium are secondary to its more subtle metabolic changes which may influence the degenerative changes of photoreceptors. It appears that technological advances will permit further investigation of the retinal pigment epithelium and thereby contribute to the understanding of the possible causes of a number of clinically evident disorders of the visual system. Because of the unique location and possible role of the retinal pigment epithelium, its pathological alterations may lead to retinal detachments, retinitis pigmentosa, macular degeneration, and retinal damage from overdoses of light energy.

Roy H. Steinberg et al.[1], University of California, San Francisco, have been concerned with functional relationships involved in maintaining retinal viability. Their approach is to study passive and active ion transport at basal, apical ends as well as intercellular membrane functions of retinal pigment epithelial cells. These investigators are able to make direct evaluations of the electrophysiological properties of the cells. Changes in membrane conductance, diffusion or rate of active transport may produce a hyperpolarization of retinal pigment epithelial cells when photoreceptors respond to light. The cone-pigment epithelium relationship is evidenced as the c-wave in the electroretinogram. The special relationships between apical ends of pigment epithelial cells and photoreceptors have been demonstrated by use of scanning electronmicroscopy; however, the functional significance of this interaction is not fully appreciated at this time.

Intracellular recording has been restricted to large cells due to the limitations of electrodes. Kenneth T. Brown et at.[2], University of California, San Francisco, have improved methods of intracellular recording by refining techniques for beveling micropipette-electrodes. These investigations showed that precise bevels with sharp cutting edges can be produced. Beveled electrodes penetrate cells more easily and permit an increase in the range of intracellular experiments possible, in the use of smaller cells, and in a wider choice of cells and species for study.

Work done on the domestic cat has been extended to humans by Michael J. Hogan et al.[3], University of California, San Francisco, who have found that

cytoplasmic extensions of the retinal pigment epithelium ensheath the cone outer segments. Phagosomes which contain cone outer segments can be observed. Hence, it appears that the pigment epithelial processes can phagocytize the tips of cone outer segments. This study strongly suggests that cone discs also are continuously renewed. Previously, phagosomes were looked for in the pigment epithelial cell body region only. The intimate contact of these specialized processes with cone outer segments may indicate other physiological functions.

The basement membrane of the retinal pigment epithelium rests upon the membrane (basal lamina or Bruch's membrane) which separates the epithelial cells from the choriocapillaris. This membrane is derived from both the choroid and the pigment epithelium. Because of its critical position, it should be considered in discussions of dysfunctions of the retinal pigment epithelium.

An effect of high intensity light is to produce a reversible disruption of photoreceptors and alterations in the retinal pigment epithelium. This phenomenon is known as "photic maculopathy" and has been described by Mark O. M. Tso et al.[4] of the Armed Forces Institute of Pathology, Washington, D.C. One eye of each of two monkeys was exposed to the light of an indirect ophthalmoscope for two hours. This exposure produced an ophthalmoscopically evident macular lesion and reduction of visual acuity. Approximately one year later, histopathologic and electron microscopic examination of tissue revealed that photoreceptor cell at the injury site had regenerated, as did visual function. However, an irregular depigmentation and a proliferation of retinal pigment epithelial cells, as well as a focal obliteration of the choriocapillaris, had developed. The animals did have a return of relatively good visual acuity by the end of the year. The early changes were characterized by edema and whitening of the retina, followed by a subsiding of the edema and a coarse pigmentation.

Another short term study by Tso et al.[5] involved four patients whose eyes were scheduled for enucleation because of melanoma. These individuals volunteered to participate in a sun gazing program. Histologic examinations were performed two days after light exposure. The retinal pigment epithelium, Bruch's membrane and choriocapillaris showed damage. Visual acuity returned to normal within one to two days after sun gazing. The retinal pigment epithelium showed some loss of melanin granules, lipofuscin aggregated around melanin granules and nodules (drusen-like) in Bruch's membrane. The light injury also caused fluid leakage in the foveal region. Therefore, the accumulated evidence suggests repeated photic insults may lead to pathological changes in retina, pigment epithelium, Bruch's membrane and choriocapillaris and may produce a clinical picture similar to that of senile macular degeneration.

The interaction between retinal pigment epithelium, glial cells and Bruch's membrane is also important in the problem of redetachment of retina following use of xenon arc photocoagulation as a treatment for retinal detachment. Tso et al.[6] have produced lesions in monkeys which are comparable in size and intensities to those produced for prophylactic purposes in man. He found new retinal adhesions between retinal glial cells and proliferated retinal pigment epithelial cells or between glial cells and Bruch's membrane.

The destruction of Bruch's membrane and a process of cellular differentiation appears to be associated with the formation of new retinal adhesions.

Lynette Feeney et al.[7], University of Oregon Medical School and Hadassah University Hospital, Israel, have developed methods for the isolation and preparation of good yields of retinal pigment epithelial cells from cattle retinas. The bovine cells resemble those of other species and are suitable for structural and biochemical analyses. Accurate information about the chemical composition of the retinal pigment epithelium is important for the understanding of its role in normal retinal function. In this study, chemical composition comparisons were made between retinal pigment epithelium, whole retina, and liver. Both retinal pigment epithelium and retina contain much less protein than does liver. The phospholipids and fatty acid composition of retinal pigment epithelium are different than those of photoreceptors. Specific unsaturated fatty acids have been readily identified and quantitated; differences have been noted, but the functional significance of the data is not yet appreciated. Two forms of vitamin A have been quantitated as vitamin A alcohol and vitamin A aldehyde ester in retinal pigment epithelium. The total content is much higher than in whole retina or in liver. These data support the concept that the retinal pigment epithelium serves as a depot for vitamin A alcohol used for visual pigment synthesis. Although the high content of nucleic acids (DNA) measured in retinal pigment epithelium would suggest multiple nuclei, cyotophotometric analyses showed normal diploid nuclei. These data serve as a base-line for further investigation.

Joe G. Hollyfield et al.[8], Columbia University, have shown that embryonic retinal rudiments are dependent upon the presence of the retinal pigment epithelium for development of outer segments and of the photoreceptor synaptic complex in tissue culture. In addition, the retinal pigment epithelium must be present for the cultured tissue to produce electroretinograms. These investigators continue to explore the culture of the amphibian eye rudiments. The methodology offers an opportunity to explore the interrelationships of retinal pigment epithelium and photoreceptors. In retinal degenerative disorders studied in animal models, rod outer segment debris accumulates between the retina and the pigment epithelium. Hollyfield et al.[9,10,11] believe that in pathological situations, the retinal pigment epithelium may alter its specificity and its phagocytic properties. Frog embryos and tadpoles were used to demonstrate that the material presented to the retinal pigment epithelium may be a determining factor in whether or not that material will be phagocytized. It was found that the retinal pigment epithelium will engulf polystyrene spheres, whereas pasturized micrococci are not engulfed. The methods available were further exploited as a means of altering the composition of phagosomes found in the retinal pigment epithelium. Phagosomes which contain polystyrene spheres showed the presence of lysosomal acid phosphatase surrounding the spheres. These results suggest that the phagosomal membrane and not its contents may be an essential component for recognition of a phagosome by a lysosome. It is anticipated that these studies will permit further evaluation of the contribution of the retinal pigment epithelium to the development and function of photoreceptors.

Further support of the concept that the fundamental alteration which permits an accumulation of photoreceptor debris in retinal dystrophies lies in the

photoreceptor and less in the retinal pigment epithelium has been provided by Dean Bok et al.[12], University of California, Los Angeles. Preliminary experiments indicate that the retinal pigment epithelium of dystrophic rats (RCS strain) will engulf carbon particles but not its own outer segment debris. These investigators have preliminary experimental results which indicate that the retinal pigment epithelium of the dystrophic rat can recognize normal outer segment fragments. These data are of value in view of ongoing amphibian studies and will be interpreted as more information becomes available. The question remains whether normal retinal pigment epithelium will engulf outer segment fragments from dystrophic photoreceptors and whether the primary pathological site may be localized in the membranes of retinal pigment epithelium or in the photoreceptor outer segments.

In recent publications, Richard W. Young et al.[13,14], University of California, Los Angeles, point out that the role of the retinal pigment epithelium in the process by which lipids are utilized for membrane synthesis is unknown. Acetate has been used as a nonspecific precursor for lipid synthesis. Autoradiographic and radiobiochemical methodologies provide data which suggest that acetate can be localized in a small molecule in the retinal pigment epithelium. The published studies do demonstrate that lipids in retinal pigment epithelium are continually revewed and that the epithelial cells are involved in fatty acid metabolism by storing fatty acids which have been carried to the retinal pigment epithelium by the blood and by esterifying vitamin A with the fatty acids for utilization by the photoreceptors.

Glycerol is a small molecule which has also been useful in the study of lipid synthesis by retinal pigment epithelium by use of radioautographic methods; Young et al. have concluded that photoreceptor membrane renewal by molecular replacement is more rapid for its lipids than for its proteins. However, the radioactive label from injected glycol will accumulate in the oil droplets found in frog retinal pigment epithelium. These data suggest that complex lipids are stored for utilization by photoreceptors and support conclusions that the retinal pigment epithelium has a significant role in this process.

Unesterified retinol (vitamin A alcohol) is carried in the blood stream in combination with a specific binding protein which is bound to prealbumin of the blood plasma proteins. There is some evidence that other proteins may also bind retinol. Nevertheless, the retinol-binding protein is useful in the study of the metabolism of visual pigments. It is possible to remove retinol from its binding site and to substitute other chromophores. Joram Heller et al.[19], University of California, Los Angeles, have found that the choroidal side of the retinal pigment epithelium has a high affinity for the retinol-binding protein. No binding is found on the photoreceptor side of the epithelial cells. These investigators suggest that there is a control mechanism for the delivery of retinol from the blood through the pigment epithelium to the photoreceptors. Furthermore, some retinal dystrophies which manifest a visual pigment deficiency may have their biochemical lesion at the level of specific retinol-receptor sites on the choroidal side of the retinal pigment epithelium rather than due to a lack of retinol or its binding protein.

Further evidence that the retinal pigment epithelium has an essential role in the maintenance of photoreceptors has been provided by Albert M. Potts

et al.[16], University of Chicago and the University of Louisville. It is known that drugs in the classes of the quinolines and phenothiazines will bind to melanin. Pigmentary changes in retina have been observed and supporting data have shown that the primary site of the drug-induced retinopathy may be in the retinal pigment epithelium. Pharmacological levels of chloroquine and related derivatives react, as do known inhibitors of protein synthesis, and have been shown to inhibit the incorporation of amino acids into retinal pigment epithelial protein, in vitro. The biochemical lesion is probably not in the amino acid transport mechanism but at the level of direct toxic effect on cellular protein synthesis. It is suggested that the adsorption of drugs on melanin and a gradual release into the epithelium is a chronic contributing factor to the biochemical lesion which damages the retinal pigment epithelium and its dependent retinal tissue.

DISORDERS OF THE PHOTORECEPTORS

(a) Night Blindness:

The night blinding disorders are problems of retinal dysfunction. In order to understand the causes, it is essential to study the problems of photoreceptor degeneration, photopigments, visual cycle and transduction.

The nature of the problems concerning retinal tissue is variable. The approaches deal with a range of problems from general metabolism to metabolism of cellular components. Liane Reif-Lehrer et al.[17], Boston Biomedical Research Institute, have been concerned with biochemical control mechanisms in retinal tissue. Her investigations have dealt with induction of the retinal enzyme glutamyl transferase (GT) by steroids. A recent study was carried out to investigate the uptake of the steroid cortisol by chick embryo retinas as a function of age. Induction of GT by cortisol is minimal prior to 10 days of embryonic development, and increases from that time on. However, the embryonic uptake of cortisol is higher at 8 days than at 15 days. The explanation may be that there are steroid receptor sites in the retina which may be occupied by endogenous steroids of different degrees of affinity. These receptor sites may be both specific and nonspecific, within retinal cells. The exact location of the enzyme and physiological function within the retina is unexplained, but the fundamental knowledge obtained from this study may assist in the biochemical manipulation of retinal function.

An abnormality develops in the immature C3H mouse retina before the onset of photoreceptor degeneration. Richard N. Lolley et al.[20], University of California, Los Angeles, are developing biochemical information which will be applicable to the problem of retinitis pigmentosa. These investigators have found an abnormal deficiency in the enzyme cyclic nucleotide phosphodiesterase in photoreceptor cells. This study will describe the relationship of the enzyme deficiency to photoreceptor cell death. Whether the enzyme is synthesized in an aberrant form or is completely lacking, the results point to a possible fault in protein synthesis in photoreceptors before the onset of the physical degeneration. Changes in levels of tissue components, such as cyclic nucleotides and related enzymes, are evidence of metabolic adaptations which are closely associated with photoreceptor degeneration. The interpretation of data suggests that the metabolism of surviving retinal tissue is significantly modified in the unexplained retinal degenerative process.

The study of photoreceptor pigment kinetics is an active field of investigation in retinal degenerative disease. Mathew Alpern et al.[21], University of Michigan, have been studying the photosensitivity of human rhodopsin in vivo which is essential to understanding rod photoreceptor functions. In their night blindness work, they have been studying the bleaching and regeneration kinetics of rhodopsin as well as cone photoreceptor visual pigments. They measure action spectra of rods and cones by psychophysical methods and determine the extent to which kinetic constants are due to the possibility that visual pigments are different in a variety of retinal diseases. This work will increase knowledge and permit in-depth comprehension of the nature of visual defects.

Ronald E. Carr and Harris Ripps et al.[22,23], New York University Medical Center, have been investigating the relationship between visual photopigments and retinal function in congenital night blindness. They have had the opportunity to study patients with fundus albi punctata, a form of of night blindness which is characterized by punctate, whitish lesions which dot the fundus. These investigators have developed a fundus reflectometer apparatus which utilizes on-line computer analyses, magnetic tape storage and a fast display capability and permits them to examine the bleaching and regeneration of rhodopsin as well as the appearance and disappearance of some of the photoproducts. The results obtained from two patients showed that rhodopsin is slow to regenerate after bleaching but electrical properties approach normal after substantial amounts of visual pigment returns. It is possible to detect the presence of a photoproduct in the abnormal patient long after it has disappeared in the normal eye. The long term presence of a photoproduct is a factor for further study, as is the slow dark adaptation and pigment regeneration in this retinal disease.

The mechanism of visual excitation is not well understood, but it is clear that the absorption of light energy does produce a number of measurable events. The photopigments are located in specialized membranes of the rod outer segments. The sequence of events are not well understood; however, among the early events following absorption of light energy is a photochemical isomerization of rhodopsin and changes in permeability of photoreceptor membranes. The evidence is based on studies of isolated photoreceptors and electrical recordings from photoreceptors in situ and in ion replacement studies. Sodium conductance has been shown to decrease in vertebrate photoreceptors and to contribute to the receptor potential. Thomas G. Ebrey et al.[24], University of Illinois, have tested the effect of a cyclic nucleotide (cAMP) on receptor potential and ion permeability of rods. These investigators argue against the hypothesis that cAMP is involved in visual excitation; however, cAMP may be involved in the recovery of ion permeability during dark adaptation, in some indirect manner. The approach of this laboratory will provide a basis for study of retinitis pigmentosa, effects of strong light, and disorders in dark adaptation processes.

The physiological processes linking photochemistry of rhodopsin with the ration of receptor potentials continue to be explored. For vertebrate photoreceptors, a current hypothesis is that light leads to an increase in intracellular calcium ions which may in turn lead to the inactivation of sodium ion conductance. Joel E. Brown et al.[25], Vanderbilt University, support the hypothesis that calcium is the intracellular transmitter for the excitation process. Although the evidence is indirect and depends upon changing extra-

cellular calcium concentration, they are able to record receptor potentials in an in vitro preparation while the ionic composition of the extracellular medium is rapidly changed. These investigators believe that capture of a photon by rhodopsin leads to a rise of intracellular calcium and the subsequent decrease in sodium permeability of the cell membrane. Further modifications and study will be required to test the "calcium-hypothesis" in vertebrate photoreceptors. This type of study will lead to an understanding of the events which link initial photochemical reactions to membrane permeability changes in photoreceptors.

Study of the regulatory mechanisms which control the biogenesis of photoreceptor membranes will involve numerous components which include carbohydrates, proteins and lipids. Richard W. Young et al.[13], University of California, Los Angeles, have hypothesized that the phospholipids found in the rod outer segments are synthesized in the myoid region of the inner segment of the photoreceptor. The lipids are transported by unknown mechanisms to the outer segments of photoreceptors where they are assembled into the membranes. These investigators believe that the phospholipids combine with preexisting membranes as a molecular replacement phenomenon. The photoreceptors contain relatively large amounts of phospholipids which are rich in polyunsaturated fatty acids. Robert E. Anderson et al.[26], Baylor College of Medicine, have made the important observations that they can alter the biosynthesis of photoreceptor membranes and their electrical response to light by dietary manipulations. If these investigators can retard the uncontrolled growth of rods in experimental animals through manipulation of essential fatty acids in diets, information may be applicable to problems of retinal degeneration in humans. These investigators reason that it is important to have base-line data for the phospholipid content of frog rod outer segments. This experimental animal has been used for numerous biochemical and electrophysiological studies of visual mechanisms. In addition, these investigators find that the phospholipids of photoreceptor membranes of frogs, cattle, and rats are very similar. They predict that the phospholipid pattern from most vertebrates will be similar. This means that a model for the study of photoreceptor degeneration in man may be selected from a number of vertebrate animals.

The processes whereby the retina carries out the attachment of its carbohydrate chains in the biosynthesis of visual pigment are being investigated by Edward L. Kean et al.[18], Case Western Reserve University. Evidence indicates that lipid-activated carbohydrates are involved in the biosynthesis of some glycoproteins in retina. These investigators have placed special emphasis on the attachment of mannose to rhodopsin, a glycoprotein. The reaction in which mannose is transferred to endogenous acceptors is catalyzed by the enzyme mannosyl transferase which is being characterized in this laboratory. It appears that a larger amount of mannose is incorporated into glycoproteins other than those of rhodopsin in bovine retinal preparations.

The general topic of the biosynthetic incorporation of sugars into rhodopsin is being studied by Dean Bok and Michael O. Hall et al.[27], University of California, Los Angeles. They are using biochemical and autoradiographic techniques in order to study the cellular organelles involved in and the time course for the incorporation of glucosamine into rhodopsin by the frog retina. Their data show that the amino sugar is acted upon in the ribosomes and then in

the Golgi apparatus of myoid region of the photoreceptors. The sugar molecule exists as part of a protein complex (glycoprotein) prior to its assembly into the growing disc membrane. The sugar moiety appears to serve in the orientation of rhodopsin in the disc membrane where the sugar group is on the hydrophilic side of the disc membrane. The sugar moiety role in the orientation of rhodopsin is probably important in the process of capturing light energy.

Search for improved animal models for the human disease of retinitis pigmentosa has continued. The clinical syndrome is characterized by normal childhood vision followed by the onset of night blindness and a disordered pigment distribution in the retina. Retinal degeneration in the mouse model is characterized by a failure in postnatal development of photoreceptors. Therefore, temporal factors are different in the human than in the mouse model. The rat with hereditary retinal degeneration does go through a brief normal postnatal period of development prior to the onset and rapid completion of photoreceptor degeneration. David Papermaster et al.[28], Yale University, have been able to obtain an inbred strain of rats, designated Wag/Rij, which show temporal and structural characteristics of retinal degeneration which may serve as an improved model for human retinital dystrophies. Rats at ages 3, 6, 9, and 12 months were used to establish the pathogenesis of the lesion. The retina appears normal at birth and has only focal photoreceptor degenerations at 3 months, which are then followed by progressive changes in the outer nuclear layer. By 6 months of age, the destruction of outer nuclear layer and loss of photoreceptor cells are more evident and significant. The degeneration appears to begin in the cell body of the photoreceptor. The outer segment debris does not accumulate and phagocytic activity of the retinal pigment epithelium is present until late stages in the process.

Strains of rodents used for the study of retinal dystrophies require the use of controls from normal strains in order to make comparisons. This is true of ongoing studies; however, an ideal research protocol would use experimental and control animals which are littermates. Matthew M. LaVail et al.[29], Children's Hospital Medical Center, Boston, have developed a mouse strain which has both genetic uniformity and a linked skin pigmentation gene. Within the same litter will be found animals which are normal and those which are abnormal with regard to retinal development. The animals with a linked skin pigmentation marker permit separation of normal and abnormal mice. These investigators are examining the genetic properties of heritable factors which may control the retinal dystrophies. Cytological and autoradiographic data suggest that in this mouse model, retinal degeneration may be a photoreceptor disorder and not a retinal pigment epithelium dysfunction. It is important to continue the search for animal models which more nearly approximate the human disorder.

(b) Day Blindness:

Differences in protein and amino acid nutritional requirements of rods and cones are being investigated by Kenneth C. Hayes et al.[30,31], Harvard School of Public Health. These investigators will define a nutritional model for retinal degeneration. The approach is through the feeding of purified diets which will permit the study of nutritional factors required by the retina. Monkeys were deprived of vitamins A or E for more than two years. In the case of vitamin E deficiency, a massive, focal disruption of photoreceptor outer segments was

produced. There was an accumulation of extracellular debris, phagocytized outer segments and increased pigment granules in the retinal pigment epithelium. It is speculated that the pathological changes are attributable to lipid peroxidation of unsaturated fatty acids in lipoproteins. The focal lesions were not detected by full-field electroretinography which seems to be normal. Vitamin A deficiency did not develop significant new information compared to that previously reported. It appears that the retinal degeneration was most advanced in the macular region, occurred in the cones first and progressed to the region around the macula. In general, it has been observed that the macular region is more susceptable to metabolic imbalances.

Dogs can be selectively bred for day blindness (hemeralopia). These animals can be studied to determine the onset and progression of cone photoreceptor degeneration. In dogs with hemeralopia, cones begin to mature and then degenerate in the postnatal period. Gustavo D. Aguirre et al.[32], University of Pennsylvania, are using ultrastructural and electroretinographic methods to characterize this disease. Cone degeneration is preceded by functional hyperactivity and structural outer segment degeneration. However, they find that neurofibrillar degeneration occurs after the onset of cone dysfunction. There are no clinically visible abnormalities, but there is complete daylight blindness with a return of visual function in dim light in the animal model used by these investigators. Histological changes in dogs with fully developed hemeralopia are difficult to detect by routine examination; nevertheless, there is a statistically significant reduction in the number of cone nuclei which indicates a loss of cones. These investigators will continue to explore the retinas of hemeralopic dogs in order to determine time course of transient functional phases and morphological changes in late states of cone degeneration. There are difficulties encountered in making exact comparisons with human cases, because the human material was poorly prepared, examined by routine histological methods, and complicated by other diseases. However, reduction in cone nuclei, loss of photoreceptors in the macular region and similar pathological changes make comparison of the human cone dysfunction and that of this animal model desirable, although difficult at this time.

By careful screening of adult domestic cats, Roy W. Bellhorn et al.[33], Montefiore Hospital and Medical Center, New York City, and Gustavo D. Aguirre et al., University of Pennsylvania, have observed a condition referred to as feline central retinal degeneration (FCRD). This is a bilateral symmetrical lesion of the area centralis (maculopathy). It is associated with a generalized cone dysfunction which may serve as a model for the study of cone dysfunction syndromes in man. Two types of retinopathy have been observed in the affected cats with visible degeneration of the area centralis, those with widespread cone outer segment abnormalities and abnormal cone electroretinographic responses and those with normal rod and cone structure and electroretinographic responses outside of the area centralis. Cats with FCRD are being bred and observed in anticipation that the progeny may show a hereditary pattern.

VASCULAR AND CIRCULATORY DISORDERS

Problems of importance are the rate of blood flow and oxygen supply to the retina, blood pressures within the vessels, the anatomy and physiology of the vessels per se, permeability of the blood vessel walls, vascular occlusive

disease and the complications of diabetic retinopathy.

Paul Henkind et al.[34], Montefiore Hospital and Medical Center, New York City, have been using clinical and pathological materials and animal models in the study of human vascular parameters. These investigators point out that retinal vascular disorders are leading contributors to human visual impairment. It is their thesis that the retinal vasculature can respond to the need for a continuity of blood supply in a limited physical manner. The vascular response are: "neovascularization" which is new vessel formation within or adjacent to the retina; "collaterals" which arise from capillaries and link veins to veins and arteries to arteries; and "vascular shunts" which link arteries and veins. These vascular entities represent developmental and spontaneous alterations in retinal blood vessels which can be visualized by ophthalmoscopic or angiographic techniques. The retinal vasculature can be viewed in the living subject and information with regard to continuity of vessels can be obtained by use of fluorescein angiography which has become an important technique for evaluation of the retinal vasculature. The choroidal circulation is not clearly revealed due to the optical properties of retinal tissue and pigments of the pigment epithelium.

The qualitative characteristics of flow, velocity, oxygenation and transfer of metabolites in retinal tissue of normal subjects and of patients with retinal vascular disorders are being studied by Charles E. Riva et al.[36], Retina Foundation, Boston. They have modified an ocular fundus reflectometer to measure emitted fluorescent light from small areas of the fundus and choroidal vessels after a series of injection of small amounts of fluorescein and indocyanin green. Their goals are to establish normal time courses for the change of dye concentration and to determine the effects of fundus pigmentation on dilution curves. Normal human volunteers have provided dye dilution curves in individual blood vessels of optic disc tissue[37] and choroidal circulation behind the fovea centralis[38]. Two dyes are injected simultaneously into the circulatory system. Indocyanin green does not diffuse out of the choriocapillaris because it binds to blood albumins; however, fluorescein does permeate choroidal tissue. Mathematical analysis of the data permits the fluorescein dye to be used as a tracer to characterize how metabolic exchanges are affected by disease. This is a new field of investigation, and they intend to concentrate on problems of diffusion of fluorescein which will broaden knowledge gained from fluorescein angiography.

The investigation of the separate contributions of choroidal and retinal circulations is of importance in understanding the maintenance of retinal nutrition. Arnall Patz et al.[39], Johns Hopkins University, are mapping the oxygen tension at the retinal surface. The arterial-venous (A-V) differences in oxygen tension in different retinal areas are an index of metabolic activity and may give an indication of ischemia and susceptibility to related retinopathies, such as neovascularization. This group of investigators has developed a method of simultaneously but separately photographing the retinal and choroidal circulations by use of a multispectral fundus camera after a single intravenous injection of fluorescein and indocyanin green dyes. The apparatus consists of beam splitters and filters on a standard fundus camera which can record fluorescein fluorescence, indocyanin green fluorescence and adsorption of light energy by indocyanin green. Thus, these investigators can obtain angiograms which provide

information about the blood flow in retinal vessels, and in choroidal arteries, veins and capillaries. Routine observations of blood flow in retinal and choroidal circulatory systems will help to understand the pathogenesis of vascular diseases and permit early diagnosis.

Study of experimental models for papilledema has been in progress at the Armed Forces Institute of Pathology under the direction of Mark O. Tso et al.[40] They have been investigating circulatory dynamics of the optic disc by following the passage of horseradish peroxidase (a protein with molecular weight of 40,000) from the blood stream through vessel walls. Electron micrographs show that blood vessels of the retina are similar to those in the central nervous system in that they have tight junctions between their endothelial cells. In the experimental study, rhesus monkeys were injected with horseradish peroxidase and the optic disc studied. They have demonstrated that in certain regions of the optic nerve head, the tracer can move directly from the blood stream; nevertheless, the tracer does not pass through most of the blood vessels in the retina. This study presents proof that there is a diffusion pathway in the optic disc which has been confirmed in human eyes by intravenous injection of fluorescein immediately before enucleations scheduled for other reasons. In addition, these observations reveal the important role of diffusion and exchange of nutrients from the choriocapillaris, retinal blood vessels and nerve fibers in the optic disc.

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FUNCTIONS AND DYSFUNCTIONS OF THE CORNEAL CELL LAYERS

The corneal endothelium is the cell layer most responsible for keeping the cornea clear, and there are a whole host of degenerative diseases, many of them occurring with normal aging, which have never really been understood and are often difficult to diagnose clinically. The following paragraphs highlight research relevant to these problems.

It is well established that the corneal endothelium is responsible for most of the fluid transport out of the stroma in the cornea. In all biological systems water movement is coupled to ionic transport; thus metabolically dependent fluid transport (such as in the cornea) is probably coupled to active ionic transport. An indication of ionic transport is electrical activity. Per Barfort and David Maurice[1] at Stanford University were able to isolate an endothelium-stroma preparation from rabbit corneal tissue in a chamber and make simultaneous measurements of the electrical activity and water transport. In spite of the small magnitude of the potential difference, a good correlation between these two parameters was found. These results support the idea that ion transport is the link between metabolic energy and water transport by the endothelium.

Jorge Fischbarg and Jong J. Lim[2] at Columbia University found that an inhibitor of carbonic anhydrase (ethoxyzolamide) decreases the rate of fluid transport of the endothelium by 60% without affecting the potential difference. Fischbarg and Lim propose a model in which a $Na^+ - H^+$ pump is present in the stromal side of the endothelium and the classical $Na^+ - K^+$ pump in the aqueous humor side. The passive movement of ions (down the gradients created by the pumps) will be the driving force for fluid transport. These studies may help to provide a physiological basis for the prevention and treatment of cornea edema and blindness of corneal origin.

Bullous keratopathy, one of the manifestations of chronic edema, is characterized by various degrees of corneal clouding due to stromal swelling and epithelial bullae. The most common causes of bullous keratopathy requiring corneal transplantation are Fuchs' dystrophy and corneal edema following cataract surgery. This latter condition may be associated with endothelial dystrophy or may be the result of operative complications or vitreous adhesion to the endothelium. It is known that excrescences or warts in Descemet's membrane (cornea guttata) precede the development of corneal edema, and bullous keratopathy frequently occurs in eyes with cornea guttata after uncomplicated intraocular surgery.

Scanning electron microscopy (SEM) technology was used by Frank Polack[3], University of Florida, to study the endothelium of corneas affected by Fuchs' dystrophy. Polack has shown that endothelial cells acquire the morphology and function of fibroblasts. Because these cells have been found only in aphakic eyes, it is possible that some fibroblastic cells are actually fibroblasts or macrophages of uveal origin, and that the vitreous plays a major role in their access to the cornea and in their proliferation or transformation from endothelial cells to fibroblasts. Fibroblastic cells have

been seen in regenerating endothelial injuries, in retrograft membranes, and after chemical injuries. Once the stimulus for fibroblastic cell activity or transformation disappears, these cells may revert to their original endothelial aspect. Polack has seen such fibroblastic cells in SEM specimens of retrocorneal membrane formation and graft failures and in clinical specimens of rejected grafts.

Extended survival of the endothelium, an advance directly applicable to longer-term storage for eye bank tissue, was achieved by new techniques involving the addition of a polymer to the storage medium. Bernard E. McCarey and Herbert E. Kaufman[4] , University of Florida, developed a media which permits the storage of corneal tissue in healthy living condition considerably longer than was previously possible when the cornea was kept on the refrigerated eye. This method involves the use of a tissue culture medium (199) and is now in use by several eye banks throughout the United States. A study at Johns Hopkins University by Walter J. Stark et al.[5] strongly suggests that not only is it possible to use corneal tissue more efficiently, and to operate during convenient hours with little risk of damaging the tissue in the storage process, but also, and most important, that "tissue failures" or graft transplant failures (thought to be due to some tissue cell death) are minimized by the use of this technique. The value of the present medium is already apparent to clinicians, but further studies on corneal physiology, and on improving the medium even further, are necessary. In addition, it is likely that keeping the cornea in a medium such as this will wash out antigens which may be foreign to the recipient, and thereby reduce the likelihood of corneal transplant rejection. Although this is not established at present, there is much indirect evidence to suggest that it may be true.

More recently Takashi Sakimoto et al.[6] University of Florida, reported a simpler method for corneal preservation using an "interface moist chamber." The solutions used are simpler than those used for the tissue culture solution immersion method and the endothelium shows little change from normal, even over a two-week period. "Vitreous touch" is a common complication of cataract extraction, occurring when the intact vitreous face bulges through the pupil to contact the posterior surface of the cornea. Clinically, the "vitreous touch" syndrome is first manifested by decreased transparency of the central cornea and a resultant reduction in visual acuity. The exact mechanism of this syndrome is controversial because it is not clear whether the dense semi-fluid vitreous in contact with the corneal endothelium has a direct adverse effect or whether the endothelium itself is already diseased before the vitreous contact. Jorge Fischbarg[7] at Columbia University assessed the ability of rabbit corneal endothelium to pump fluid when its normal bathing medium (aqueous humor) was replaced by vitreous humor. Fischbarg found a definite adverse effect on the corneal endothelial pumping and the solid, collagenous elements of the vitreous appears to interfere with the endothelial function.

A new ophthalmic solution developed by Ethel Anderson et al[8] at Columbia University has recently attracted some attention. These investigators found that oxidized gluthathione has a beneficial effect on the transendothelial fluid transport in the cornea. Evidence has been provided that the oxidized

gluthathione is responsible for the increased stromal thinning and fluid transport of the cornea. A patent application for the use of this solution has been filled in behalf of Columbia University.

Another improvement to preserve the integrity and functionality of the corneal endothelium is a new solution used during irrigation in vitrectomy. It was not until the development of the surgical procedures of phacoemulsification and pars plana vitrectomy (both requiring large volumes of irrigation solutions for extended periods of time) that corneal edema began to occur as a major problem following intraocular surgery. Individuals undergoing vitrectomy have stressed corneas and are most susceptible to corneal endothelial breakdown and edema if stressed further with the currently available intraocular irrigating solution. Henry F. Edelhauser et al.[9] at the University of Wisconsin, through various perfusion studies, have shown that the corneal endothelium can best be maintained physiologically, biochemically, and structurally by a salt solution containing glutathione, bicarbonate, and glucose. It has therefore become imperative to provide a more complete intraocular irrigation solution for these surgical procedures. Edelhauser's research has provided the ophthalmologist with a scientific basis for a more rational use of intraocular irrigation solution.

A new class of polypeptide hormones, derived from the submaxillary and pituitary glands, has been proposed to be the regulator of cell growth in the intact animal. It has been postulated that these polypeptide hormones derive their specificities from specific receptor sites on the target cells. Included among these proposed new hormones are epidermal growth factor and several mesodermal growth factors which have been isolated and partially purified by Virginia L. Weimar and Kenneth H. Haraguchi[10] of the University of Oregon. Weimar and Haraguchi[10,11] described three different growth factors from the submaxillary gland which markedly stimulate corneal stromal cell growth. One of these had been previously described as a muscle-dedifferentiating factor and the other as a thymocyte-transforming factor. The third, also very potent, is new and previously undescribed.

Patrick C. Ho et al.[12] Vanderbilt University, evaluated the influence of epidermal growth factor on the healing of epithelial wounds in the cornea. Epidermal growth factor, given topically four times daily increased the healing rate over that of the controls to a statistically significant degree. No signs of toxicity were detected either clinically or histologically. The work of these investigators indicates that the enhancing effects of epidermal growth factor on the rate of corneal healing might augment current therapeutic measures for nonhealing epithelial defects and erosion. Ho et al.[12] urge caution in proceeding with human trials because epidermal growth factor is a polypeptide derived from a xenogenic source. This problem exists with all of the polypeptide hormones of high molecular weight. However, if the mechanism(s) by which these polypeptide growth factors regulate cell growth can be determined, it may become possible to bypass these hormones with smaller compounds. For instance, these hormones might attach by specific receptors to the target cells and activate a common mechanism, the product of that activity being determined by the cell type. Once the mechanism becomes known, one might find various steps further along in the reaction sequence which could be stimulated by intermediate reaction products.

In connection with the stimulation and inhibition of cell growth, Weimar, et al.[13] found that cornea fibroblasts appear to form an inhibitor of DNA synthesis after they reach the monolayer stage in tissue culture. DNA synthesis in stimulated cultures can be inhibited up to 80 per cent by media from confluent cultures of corneal fibroblasts. The nature of the inhibitor is unknown, but it is in the media and can be transferred from one culture to another. If this inhibitor(s) can be isolated, it might be of importance in several research areas for inhibition of cell growth.

Another important development concerns the use of glucocorticoids as anti-inflammatory agents. It has recently been suggested that an important role of the glucocorticoids is the control of cell growth. It has long been recognized in ophthalmology that the rate of wound healing of the corneal stroma is substantially decreased even when therapeutic doses are used. Juan Sanchez and Frank Polack[14] of the University of Florida evaluated the effect of dexamethasone on the healing of endothelial wounds. A decrease in the rate of healing occurred in the first four to five days. Then the lesion healed at about the same rate as the untreated corneas. Glucocorticoids are potent inhibitors of fibroblast growth, apparently binding to cytoplasmic receptors. Progesterone, among other hormones, strongly interferes with the binding of dexamethasone to the cytoplasmic receptors. These observations suggest that a combination of an anti-inflammatory glucocorticoid and one of its antagonists might permit fibroblast growth without interfering with the anti-inflammatory action. Much depends on the mechanism of the anti-inflammatory action of the glucocorticoids. Another aspect of this problem concerns those instances in which it is desirable to suppress fibroplasia - for instance, prevention of fibroblastic overgrowth of surgically prepared aqueous outflow channels in the treatment of glaucoma. It might be possible to enhance the growth inhibition, for example, by using some other steroid which enhances the binding of the glucocorticoid.

Surface properties of cells are known to play a major role in many physiological and pathological phenomena and are increasingly studied in cells of various tissues and organs. In corneal research, these studies were almost exclusively dominated in the past by physiological studies on the ion transport across various corneal layers. Recent developments in the study of cell surface phenomena in the cornea are related to the use of immunological methods. These studies have led to a new concept of the fluid mosaic model of the cell surface, and changes in the cell surface composition were demonstrated in association with viral transformation, carcinogenesis, etc. A particularly interesting study, of possibly great significance in this field, is the demonstration by Wladyslaw Manski and Theresa Whiteside[15], Columbia University, of metabolically dependent cell surface receptors to antigenic determinants which are present only on the surface of actively metabolizing and/or proliferating cells but not on the surface of resting cells. The study of cell surface receptors on corneal epithelial and endothelial cells by Manski and Whiteside revealed changes in the cell surface associated with increased metabolic activity occurring, e.g. in corneal regeneration or experimentally in tissue culture. These processes led to the unmasking of antigenic determinants which in the resting tissue are present in a masked state.

The above differences between active and resting corneal cells were demonstrated by the fluorescent antibody technique and by the occurrence of cytotoxic effects after exposure to anti-corneal antibodies. The metabolically dependent cell surface receptors unmasked only in metabolically active and/or dividing cells were found to be different for the corneal endothelium and epithelium. The metabolically dependent antigens present in the corneal epithelial surface occur similarly on cells of other ectodermal tissues, whereas metabolically dependent endothelial antigens were found on cells of mesodermal tissues. The demonstration by Manski and Whiteside of the unmasking of antigenic receptors in metabolically active states and in proliferating states suggests that still other receptors may be unmasked and may play a role in growth regulation. Epidermal growth factor, for example, is said to stimulate only fetal or embryonic tissues and stimulates the adult epithelium only during epithelial regeneration, losing its effect as soon as healing is complete. Perhaps this effect of the epithelial growth factor is due to alterations in the cell surface where it is thought to be located. Cell surface receptors, unmasked during wound healing, might alter the binding of drugs during therapy.

CORNEAL PROSTHESIS, TRANSPLANTATION, AND OTHER SURGICAL PROCEDURES

After 14 years of research on prosthokeratoplasty, the Columbia-Harkness Eye Institute research team published an apparently successful technique to restore vision in previously hopeless causes of blindness, such as seen in advanced vascularized corneas after chemical burns or in pemphigoid eye and bullous keratoplasty. Hernando Cardona, Arthur DeVoe and Anthony Donn were primarily involved in this development. The new procedure of prosthokeratoplasty, developed and tested mainly in 1974, is called the "Through and Through Technique" [16], in which the optical prosthesis is supported by a periosteal graft. The extraordinary improvement and the future possibilities for blind people was shown in 52 prosthokeratoplasty cases after chemical burns; with no extrusions occurring. In contrast, the extrusion rate with the previous technique was about 45%. Based on these results, the same technique was employed in the pemphigoid eye, and in conjunctivitis sicca, diseases for which no treatment has previously succeeded. Thirteen patients have been treated with the new method, and except for the first case, the twelve remaining cases had a restoration of vision ranging from light perception of 20/40 to 20/100. For bullous keratopathy, a new prosthesis called the "Nut and Bolt" is now being used as a primary procedure by Donn[16]. In 33 consecutive cases, 85% derived significantly improved vision during follow-up periods of up to 6 years.

Thermokeratoplasty was developed about three years ago as a unique and fascinating approach to the treatment of keratoconus. It involves the application of a specially controlled heated probe through the bulging conical cornea. The heat could produce shrinkage of the bulging cone with little scarring, and could save the patient the risks and morbidity of surgery. One of the questions raised at that time was whether the cone would tend to recur and whether this kind of procedure, although potentially valuable, might be only

a very short-lived expedient. A follow-up of those initial patients was done and published this year by Antonio Gasset and Herbert Kaufman[17] of the University of Florida, and it is clear that in the three years of follow-up, there is no tendency for the cones to recur and the effect of the very brief thermokeratoplasty procedure seems long-lived.

Of potentially great significance both from a basic point of view as well as for the practice of corneal transplantation is the finding of DeVoe and co-workers[18] of a transmission of slow virus infection by a corneal transplant.

The whole field of corneal transplantation has been changed by the use of soft contact lenses. It has recently been suggested that patients whose sight was damaged, apparently permanently, from chemical burns might be successfully treated with corneal transplantation. The experience at the University of Florida this year has provided the first confirmatory study in a large scale test of these findings[19]. Before soft contact lenses were available, the success rate of corneal transplantation in eyes with acid and alkali burns was almost zero. Following the use of soft contact lens and with the proper management of these cases, between 70% and 80% of patients have clear grafts with good vision at least a year after surgery. Many of these were in people who were considered "hopelessly blind" for many years. Soft lenses have changed this problem from one of nearly hopeless prognosis to one in which conventional keratoplasty has reasonably good results. The crucial variable responsible for this change is clearly the soft contact lens, which also permits successful keratoplasty in many other conditions which would otherwise damage the surface of the new graft such as dry eyes, skin diseases with damaged lids, etc.

Infections of the cornea by pseudomonas often results in a rapid destruction of the corneal stroma. It was generally assumed that the liquifaction of the stroma was effected by a collagenase produced by pseudomonas. Stuart Brown, et al.[20], University of Pittsburgh, have shown that the protease was not a collagenase but rather a proteoglycanolitic enzyme capable of degrading proteolyglycan (a major noncollagen component of the cornea). Na EDTA, 10^{-2}M inhibited the enzyme activity and has obvious clinical importance.

Herpes simplex keratitis is a viral infection that can produce ulceration, opacification, and perforation of the cornea. During the last year, several topical antivirals have become available as alternatives to iododeoxyuridine (IDU). There are a significant number of patients who have become allergic to IDU, or unresponsive to it. Adenine arabinoside has been shown to be as effective (although no more effective) than IDU. Most important, however, it is effective in patients allergic to IDU and in patients whose virus is resistant to IDU. Another antiviral phosphonoacetic acid studied by Roger Meyer, et al.[21], University of Florida, falls into the same category. Unlike adenine arabinoside and IDU, it has little or no potential for being mutogenic or teratogenic if systemically absorbed, and may be safer than the antimetabolite antiviral drugs. It is important also because it opens up a new field of drug types which may be valuable in treating viruses. At present, the best topical antiviral remains trifluorothymidine. It has the disadvantage of being an antimetabolite, but the advantage of being more potent and more effective than the other drugs, and active in patients who cannot take IDU.

The pathogenesis of corneal inflammation due to herpes simplex virus is being studied extensively. Roberta Meyers and Thomas Pettit[22], University of California at Los Angeles, reported that corneal inflammation can result from intracorneal injection of herpes simplex virus (HSV) antigens in sensitized rabbits and the resulting corneal opacity provides an important experimental model for human disciform keratitis. Meyers and Pettit showed that the necessary components for a humorally induced immune reaction which leads to tissue damage appear to be present in experimental stromal herpes keratitis in the rabbit. It is reasonable to assume that there exists a similar mechanism in stromal herpes in man and that the pathogenesis may be in part related to tissue damage caused by the interaction of the antiviral antibody with herpes virus antigen in the corneal stroma, a reaction which attracts complement and polymorphonuclear (PMN) leukocytes. These results lend support to the hypothesis that the persistence of viral antigen or virus-transferred cell neoantigens may cause the chronicity of herpes keratitis by chronically stimulating a local antibody response which perpetuates the chain of events leading to ocular inflammation.

Meyers and Pettit[23] also reported that the interaction of antiviral antibody and herpes virus antigens activates the complement sequence and causes the release of mediators able to attract PMN and mononuclear leukocytes. The PMN found in the inflammatory infiltrate in Herpes Simplex Virus (HSV) stromal keratitis are chemotactically attracted to the cornea by factors liberated from HSV-induced cell lysis releasing intracellular proteases, HSV antigen-antiviral antibody complexes, complexes of neoantigens induced on the cell surface after HSV infection, virus-sensitized lymphocytes releasing mediators of cellular immunity such as a PMN chemotactic factor, and non-specific inflammation causing cell damage with the release of proteases and collagenolytic enzymes. In the presence of serum as a complement source, all of these factors are able to generate leukotactic factors which lead to PMN infiltration, and this in turn leads to a cascade of chemotactic activity perpetuated by the release of leukotactic products from the PMN. Most of the chemotactic activity in stromal herpes is a result of this cascade rather than of chemotaxis due to the presence of the virion.

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The function of the lens in the visual process is to transmit light to and focus it on the retina. Light must pass through the lens without distortion. To accomplish this the lens must be a medium with flexible optical properties. This medium is the result of protein-protein and protein-water interactions which are now just beginning to be understood. In 1971 George B. Benedek and Judith Jedziniak of The Massachusetts Institute of Technology found that protein aggregation in the lens leads to loss of lens transparency because of the scattering of light by these aggregates. Later, Abraham Spector of Columbia University showed that such aggregates are indeed present in solutions of proteins extracted from the nuclear fraction of aging normal and cataractous human lenses. Since these studies were conducted with materials extracted from lens and separated biochemically, it was important to determine whether such aggregates exist and can be demonstrated in the intact lens.

Confirmation that such apparently is the case was provided by Benedek and Toyoichi Tanaka[1]. By measuring the spectrum, or the auto-correlation function of laser light scattered from the lens, Benedek and Tanaka observed the Brownian movement of the proteins in the lens. From the diffusion coefficient, the mean size of the different proteins was shown to be as large as that determined in the lens extract. This demonstration by Benedek and Tanaka that the size of proteins in the intact lens can be determined optically provides the means to conduct in vivo investigations of the status of proteins in normal as well as cataractous lenses. Thus it is now possible with this laser technique to demonstrate the presence and size of the protein aggregates in the intact lens and to move toward studying the inception and progress of the development of the cataract in patients. Furthermore, one can now examine at the molecular level the action of cataract-inducing or cataract-reversing biochemical reagents which can be introduced by diffusion into the intact lens.

During embryonic development, the eye and the early central nervous system (the neural tube) develop similarly, folding inward to form a pit (eye) or a groove (spinal cord) that subsequently must close and pinch off from the embryonic ectoderm. Johan Zwaan and Richard Hendrix[2] at The Children's Hospital Medical Center in Boston have continued to elucidate the mechanism of lens formation by the embryo as well as the influence of cell shape and cell division on cellular specialization. Zwaan[3] has recently demonstrated that the appearance of delta-crystallin, the fiber crystallin of the chick's embryo, occurs at the latter part of the DNA-synthetic period of the cell cycle. This finding indicates that the optic cup exerts an inductive influence at this stage of lens cell development. Induction is an embryological process responsible for most of the specialization of tissues of the body, and the demonstration of a direct influence of the optic cup at a crucial stage of lens development is an important finding. This may be related to the observed migration of the cell nucleus to the base of the elongating cell at the beginning of the DNA-synthetic period and then back again toward the center of the cell at the end of the S-phase doubling of the genetic material when the cells assume a spherical shape. The authors emphasize the interrelationship of cell cycle stage, membrane stress, and cell shape regulation during the process of cell specialization.

The lens maintains its ionic content at concentrations quite different from those of the aqueous humor. It is also known that the anterior lens epithelium can actively "pump" sodium ions out and potassium ions into the lens. It is unknown, however, whether or not the individual lens fibers participate in the active pumping of ions. James Rae[4,5], University of Texas, by means of electrophysiological techniques, found that apparently the lens fibers are able to "pump" ions under given experimental conditions and, even more important, that they are electrically coupled. This indicates that they are not independent of each other but integrated in the process of ion transport.

The work of Beryl J. Ortwerth and Orchid Chu-Der[6] at the University of Missouri is aimed at understanding lens protein synthesis. Ortwerth has studied the role of transfer tRNA in controlling the synthesis of lens-specific proteins in the lens cortex and has found significant differences between the tRNA populations of the lens cortex and capsule. Ortwerth also reported that lens tissue changes its tRNA population during differentiation, presumably resulting in a population that is optimum for the synthesis of the specialized proteins of these tissues.

John Chen et al.[7] of Columbia University have developed methods for isolating the messenger RNAs (mRNA) that code for the A and B chains of alpha crystallin. Thus, by using such messenger RNAs as templates, alpha crystallin polypeptide chains have been synthesized. Furthermore, the mRNAs have also served as templates for the synthesis of the alpha crystallin DNA utilizing a viral reverse transcriptase. This work will make it possible to study the control mechanisms involved in information transfer in the normal lens.

Basil Worgul and Howard Rothstein[8], University of Vermont, have studied the biological effect of ionizing radiation on the lens. Macroscopically, the lens reflects radiation damage by a gradual loss of transparency resulting in the development of cataract. Although radiation cataracts are in themselves of little clinical significance, histopathological studies are beginning to suggest that the cellular events associated with this pathology may in fact be the same as those found in the development of cataracts induced via other agencies. Therefore, it might serve well as a paradigm for studying cataractogenesis in general. According to Worgul and Rothstein, x-irradiation of amphibian eyes has shown that development of cataract is not only correlated with but depends upon the proliferative activity of the germinative zone of the lens epithelium. Rothstein's work suggests that lens cell damage from ionizing radiation is transduced by mitosis and expressed as an opacity by subsequent errant fiber genesis.

Genetically-induced cataracts in laboratory animals can provide an excellent tool to investigate the progressive morphological and biochemical changes in lens tissue which ultimately result in the opacification of the lens. Morphological changes in these hereditary cataractous lenses were investigated by Willis Gorthy and Yahya Abdelbaki[9] of Colorado State University; they found that the process is characterized by the formation of an anterior polar cataract followed by vacuolization in the posterior lens cortex. This leads to swelling and rupture of outer cortical fibers,

and eventual opacification of the entire lens. Larry Takemoto and Parviz Azari[10], Colorado State University, found also that the predominant chemical change observed in the lens proteins during formation of the hereditary rat cataract was the oxidation of cysteine sulfhydryl (-SH) groups to cystine disulfide (-S-S-) groups. The process was accompanied by the formation of high molecular weight protein aggregates composed of various species of lens proteins. The process could be simulated, to a large extent, by in vitro exposure of the rat normal lens to pure oxygen under pressure.

A few years ago it was observed that UV light can influence the state of the lens by converting tryptophan into cytotoxic photoproducts. Seymour Zigman et al.[11], University of Rochester, found that many proteins, especially the eye lens crystallins and aqueous humor serum proteins, have been chemically modified by exposure to near-UV light (320-390 nm) in the presence of tryptophan. Colored and fluorescent tryptophan photoproducts bind firmly to proteins, thereby altering their physico-chemical properties. Whether such a reaction would inhibit the action of various enzymes has been studied by Zigman et al. They reported that UV light interferes with catalase activities of bovine lens epithelia. UV light photoproducts of tryptophan are capable, according to Zigman, of deactivating catalase by altering its protein and possibly even its prosthetic groups.

In the lens, aldose reductase converts sugars to sugar alcohols which accumulate in the lens leading to formation of sugar cataracts. By understanding the physical, chemical and biological structure of this enzyme and its subunits it should be possible to better understand the formation of sugar cataracts in vivo. The physiological significance of this enzyme, which appears also in brain, liver, kidney and adrenal tissues, is not fully understood. Hence, understanding the mechanism of this enzyme may be helpful in an even wider sense in understanding disorders of carbohydrate metabolism. Clyde Doughty et al.[12,13] of the University of Illinois, have studied the properties of aldose reductase. The molecular weight of this enzyme, according to Dr. Doughty, is very high and composed of unequal catalytic and non-catalytic subunits. An aldose reductase from the lens and one from the brain have similarities in terms of some of their physical and chemical properties.

The principal source of biological energy in the lens is anaerobic glycolysis, and the enzyme hexokinase is the pacemaker of this metabolic pathway. The extremely important role of hexokinase in lens metabolism has been in part elucidated by Leo Chylack[14,15,16] at Harvard Medical School. Until recently hexokinase has been considered a single species; it is now clear that there are two quite distinct forms of hexokinase, type I and type II. Moreover, they are distributed differently in the lens. According to Chylack, senile cataract appears to be devoid of type II hexokinase. It was observed several years ago that in children there is a cataract formation in the presence of low sugar (hypoglycemia). Chylack has been able to recreate this condition in vitro using the rat lens, and has found that hexokinase has an unusual sensitivity to thermal inactivation that can be counteracted by glucose. Even at body temperature, when the glucose level is lowered, Chylack has shown that hexokinase is inactivated. This finding

once more suggests that an enzyme is responsible for the development of such types of cataract in children.

The structural lens proteins, the alpha-crystallins, are of considerable interest because of their possible involvement in the development of central senile opacities. Studies with bovine alpha-crystallins have clearly demonstrated in the past that this large class of proteins is not homogenous but composed of a number of populations of macromolecules. Abraham Spector et al. at Columbia University have demonstrated that, with aging of the lens, the proportion of macromolecules in the high molecular weight population increases substantially. Examination of a group of normal subjects as well as patients with nuclear sclerosis and senile cataract indicates, according to Spector, that scattering of light increases with aging of the in vivo lens.

How can this increase of back scattering with aging be explained? Recent work by Joseph Stauffer et al.[17] at Columbia University on the development of high molecular weight proteins in the human lens gives some insight into this problem. Examination of normal human lenses varying in age from 3 months to 89 years indicates that there is a gradual increase in the percent of high molecular weight protein (HMWP) in the soluble fraction commencing in the second decade of life, reaching approximately 12% to 15% in old lenses. Studies on isolated cortical and nuclear regions of the lens have shown that this process occurs almost entirely in the nuclear region. Little nuclear HMWP is detected before the age of 25; after then HMWP increases rapidly to reach levels of approximately 30% of the total soluble protein in the very old lens. Little HMWP can be detected in the cortex before the age of 40, and maximum levels of from 4% to 7% are found in lenses of approximately 80 to 90 years of age. Quantitative analyses of light back-scattering from lens suggests that in the nuclear region the increase in back scatter parallels the increase in HMWP, while in the cortex back scatter appears to be independent of HMWP. Spector suggested that cortical back scatter is due to intrinsic morphological characteristics, whereas nuclear back scatter is dependent upon the concentration of HMWP. Spector et al.[18] now have evidence that the transformation to high molecular weight aggregates is caused by interaction of modified polypeptide chains and calcium. Calcium has a pronounced effect on the formation of high molecular weight (HMW) alphacrystallin of the lens. They also demonstrated that there is much more calcium associated with HMW lens protein than with the low molecular weight (LMW) counterparts. Work now is in progress on the characteristics of the calcium-binding sites of protein in the search for a more effective calcium chelator.

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GLAUCOMA

Glaucoma is a debilitating group of diseases. It is one of the leading causes of blindness, accounting for approximately 12% of all blindness in the United States. Whereas its onset typically takes place in middle to older age groups, its occurrence is relatively unpredictable; and, due to the lack of obvious indications to the patient, it often results in considerable, nonreversible damage to the eye prior to diagnosis.

Damage to the optic nerve head (which is thought to be brought on by sustained increased intraocular pressure, following in turn from an imbalance in aqueous humor dynamics) initially causes loss of vision in various parts of the visual field. Over a period of time these areas of visual loss increase in size, visual acuity progressively worsens, objects appear broken or only partly seen, direct vision is lost, and the individual becomes unable to cope with the external world through the sensory modality upon which he has learned to be most dependent.

Under normal conditions, the intraocular pressure is maintained by the combination of ciliary body production of aqueous humor and its outflow via the trabecular meshwork and Schlemm's canal to the venous circulation. This intraocular pressure as clinically measured normally is within the range of 14-20 mm Hg.

In angle-closure glaucoma, the aqueous outflow route is obstructed due to shallow anterior chambers and/or iris-lens positioning, e.g., by apposition of the iris with the openings in the trabecular meshwork. Intraocular pressure thus increases as the normal outflow passage is partially interrupted. In primary open-angle glaucoma, which is the most prevalent form of the disease, the outflow facility typically is reduced, apparently because of obstruction within the trabecular meshwork. However, the basic cause of primary open-angle glaucoma has yet to be established.

In the above and most all other categories of the glaucomas (e.g., congenital and various secondary or combined cases), the primary concern is neural damage caused by, or in conjunction with, sustained and abnormally high intraocular pressure. This damage often corresponds with increased optic disc depression or cupping. The pressure progressively damages nerve fibers in the disc, with subsequent degeneration and atrophy of the retina and optic nerve.

Since its inception as one of the National Institutes of Health, the National Eye Institute has supported research aimed at combating glaucoma. The principal efforts have been geared toward earlier detection, more reliable, accurate, and complete diagnosis, new and improved modes of treatment, prevention and cure, as well as support for that more basic research from which the above clinical applications ultimately develop.

EARLY DETECTION AND REFINED MEASUREMENTS

The present techniques used to identify glaucoma are successful in many cases only after the disease has progressed significantly and non-reversible

damage has occurred. Investigations oriented toward earlier detection and more refined measurement are beginning to yield results. These investigations are producing new procedures and instrumentation which ultimately should be invaluable in prevention and control of glaucoma.

One of the parameters which is currently employed in the diagnosis of glaucoma and in the evaluation of corresponding optic nerve damage is cupping of the optic disc. The extent of optic disc cupping may be estimated, e.g., from ophthalmoscopy or fundus photos; however, the reliability and validity of such techniques rests upon the skill of the individual examiners and are subject to human error and variation. Objective instrumentation and procedures for the measurement of optic disc cupping are being developed and refined by Michael S. Kottler, Ralph A. Rosenthal and David G. Falconer[1] , of the Stanford University Medical Center and the Electronics and Bioengineering Laboratory of the Stanford Research Institute. The topography of the optic nerve head is quantified through digital stereophotogrammetric procedures which yield cup depth, width, volume and profile area. The Zeiss Fundus Camera and the Allen Stereoseparator were used to produce stereoscopic pairs with 128 by 128 digitized points, each having one of 64 possible gray levels. These data were computer processed, yielding a depth sensitivity of 184 micra. The processing cost and equipment required are minimal and the clinical applicability is substantial. Continued studies by this and other groups are progressively refining the measurements.

The present methods which are normally used to measure intraocular pressure are inconvenient to the patient, particularly when they span extended periods of time, require concentrated effort and cooperation by the patient, and often yield data which are confounded by artifact. Lucien A. Couvillon, Jr., [2] of the Utah Biomedical Test Laboratory,and Michael E. Greene and Bobby Gilman[3] , of the Southern Research Institute,are developing devices which utilize hydrogel (soft contact lens) material and electronic and mechanical techniques to transmit and record the data. Devices such as these, when fully developed, will allow continuous monitoring of intraocular pressure for evaluation of diurnal variations, for determining the effects of various pharmacological agents used in the control of glaucoma, and for gaining better understanding of the mechanism of intraocular pressure regulation.

As part of a broader investigation of capillary function in eye diseases, James F. O'Rourke[4] , of the University of Connecticut Health Center,has employed radioactive tracer and scintillation detector techniques to study the anterior chambers in felines and humans. Experiments with various tracers such as xenon (whose clearance begins at once) and albumin (whose clearance takes as long as 30 minutes to become apparent) gave quantified data which are pertinent to physiological and pathological conditions associated with different diseases and lesions. O'Rourke believes that these tracer studies of anterior chamber functions will be helpful in providing: (1) new forms of data useful in improving ocular disease definitions; (2) data of continuous dynamic changes in the intact untouched eye; (3) specific information regarding tissue functions; (4) digitized objective data for analysis; and (5) informative data regarding various therapies and their responses.

MAINTENANCE OF INTRAOCULAR PRESSURE AND AQUEOUS HUMOR DYNAMICS

Due to the importance of maintaining intraocular pressure (IOP) within normal limits, one of the primary areas of investigation funded by the NEI glaucoma program is that concerned with the effects of various agents upon aqueous humor dynamics. A substantial amount of work has been carried out on the effects of prostaglandins on the eye in recent years, as described in the reviews by Arthur H. Neufeld, and Marvin L. Sears[5] , and Sears, Neufeld, and Lee M. Jampel[6] , of Yale University. Various species differences, dosage levels, potencies of different prostaglandins, and methods of administration have been shown, although agreement among investigators is not complete. Research has been oriented toward studying inhibitors of prostaglandin synthesis, and these inhibitory agents are being utilized in treatment therapies.

Targeir Vegge, Neufeld, and Sears followed the movement of plasma proteins in the aqueous humor with horseradish peroxidase tracer following topical administration of prostaglandin E_2. Electron microscopic analysis supports the argument that the proteins enter the posterior chamber from the stroma of the ciliary processes by way of the intercellular clefts of the nonpigmented epithelium.

Kenneth E. Eakins and his coworkers at Columbia University have studied prostaglandin synthetase inhibitors and their effects on ocular tissue and in ocular disease[8,9,10,11]. Dose response curves were established for the inhibitory effects of indomethacin on prostaglandin formation. Microsomal fractions of different tissues were compared. Straight line functions were found for percentage effect in organ tissues; the tissues studied, listed in order of decreasing potency of indomethacin on their prostaglandin forming mechanisms are spleen, kidney, medulla, conjunctiva, anterior uvea, and retina. These findings are of importance in studies of the aqueous humor, uveitis, and in the development of ocular anti-inflammatory agents. Dose response curves and regression lines also were determined and calculated for agents other than indomethacin. The order of relative effectiveness of the agents on prostaglandin formation of anterior uveal tissue is: indoxole pirprofen, naproxen, phenylbutazone, oxyphenbutazone, and indomethacin. The last 3 are not significantly different.

Intraocular pressure is dependent not only upon the production of aqueous humor and agents which may directly increase pressure, but also upon the outflow facility. The actual mechanism of the outflow system and aqueous humor drainage through the trabecular meshwork and Schlemm's canal and the associated resistance to drainage may provide the basis for most cases of open-angle glaucoma, as pointed out by Anders Bill[12] at the University of Uppsala.

Cells forming layers with large invaginations in the trabecular area may bulge into Schlemm's canal and become arrayed at the beginnings of openings into the canal. E. Michael Van Buskirk and W. Morton Grant[13] , of Howe Laboratories in Boston, have indicated that these invaginations may be dependent upon the pressure gradiant across the cells. Bill argues that the filtering process, wherever it takes place, may result in the accumulation of debris which clogs the meshwork and leads to increased resistance to outflow and thus to increased intraocular pressure (IOP) and, in some cases to open-angle

glaucoma. Adrenergic agents have been proposed as possibly indirectly influencing the cells which regulate outflow, and although no direct causal relationship to glaucoma has been established, these compounds are useful in glaucoma therapy, according to Sears and Neufeld[14] . Outflow resistance in enucleated eyes has been indicated by Jack Kayes[15] , of Washington University to be dependent upon intraocular pressure. Richard F. Brubaker[16] , of the Mayo Foundation, used enucleated human eyes and procedures designed to minimize the artifacts which had been purported to influence the previous authors' conclusions. These data show that increased resistance to outflow varies directly and linearly with intraocular pressure from 10-50 mm Hg. The calculated value indicates a 1 percent increase in outflow resistance for each millimeter of Hg rise in intraocular pressure.

Open-angle glaucoma patients have been studied by Stephen R. Waltman, and David Yarian[17] , and Herbert Cantrill, Harry Zink, Stephen Waltman and Bernard Becker[18] , of Washington University. In the first investigations, positive reactions to antinuclear antibody were found in 44 percent of the open-angle glaucoma patients as compared to 7.5 percent in a control group. The results raise the possibility of a connective tissue disorder as a causal factor in glaucoma.

In a second investigation lymphocyte cyclic AMP phosphodiesterase levels were found to be similar in patients with open-angle glaucoma, in patients who show elevation of IOP in response to topical corticosteroids, and in steroid nonresponders. In addition, the theophylline concentration required to inhibit the enzyme was also similar in all groups. The authors therefore believe these findings lend support to the conclusion that these agents have a general effect on cellular transport or metabolism in the glaucoma patients.

Two investigations conducted at the University of Uppsala by Bill[19] and his co-worker Bjorn Svendbergh[20] , have sought to determine inflow and outflow facility. Increments in the oncotic and osmotic pressure in the anterior chamber failed to result in significant increases in inflow. Thus aqueous humor formation and drainage do not seem to depend upon movement through the small pores in the iris vessels.

In a second study monkeys were anaesthetized, placed prone with the head positioned horizontally, and their eyes perfused for 3-7 hours. In one eye the intraocular pressure was maintained within a few mm. Hg. of the spontaneous level. Intraocular pressure in the fellow eye was maintained at 33-48 mm Hg. The artificial elevation of the high pressure eye resulted in measured increase in outflow facility of from 160-630 percent (mean - 350%) with the maximum values reached in 2-3 hours. Ultra-structure changes were also observed in the high pressure eye, i.e. swollen endothelial cells in the trabecular meshwork and cell debris.

NEW MODES OF TREATMENT

Over the past few years, the use of lasers in the treatment of glaucoma has become of interest[21] . Relatively short duration beams from gas lasers are attained by shutters or strobe flash pulsing, and the more recent solid state lasers are able to produce very high levels of energy in nanosecond

durations. Based on the Q-switched technique, the solid state lasers maximize vaporizing effects and minimize thermal effects. Laser procedures relevant to possible glaucoma treatment can be performed rapidly, simply and with minimum patient discomfort; these procedures include peripheral iridotomy, alteration of the trabecular meshwork, and destruction of the ciliary body. Whereas the efficacy of these procedures has not yet been fully established, the "punching" or puncture effect has been estimated to decrease intraocular pressure for periods of time up to several months[22] . Establishing the clinical role of these procedures should prove to be a fruitful line of investigation.

A microsurgical procedure, referred to as the aqueous-venous shunt, has been described by Pei-Fei Lee and Wing-Tze Wong[23] , as potentially valuable in reducing intraocular pressure. The procedure was performed on 15 eyes of 15 patients with uncontrolled glaucoma. During the post-operative period of 6-9 months, 9 of the 15 eyes displayed at least partial success.

The reported usefulness of delta-9-tetrahydrocannabinol, a marijuana derivative, in the potential treatment of glaucoma has been debated[24] ; and while a therapy program is far from being established, initial findings indicate that the agent is effective in reducing intraocular pressure. Keith Greene, of the Medical College of Georgia, speculates that since THC is as effective as most currently used agents, a THC agent is quite likely to be made available for clinical use by the ophthalmologist once appropriate controlled clinical trials have been conducted.

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SENSORY AND MOTOR DISORDERS OF VISION

INTRODUCTION

The interactions between eye and brain are critical components in the normal and abnormal functioning of the visual system. The central nervous system uses a complex array of interlocked processes to translate light energy striking the retina into the experience of sight. In addition, a complex neural control system exists to effect rapid changes in the eye's position and/or the refractive power of the lens and thereby determine the image that is presented to the retina. Better understanding of these processes in both health and disease is the concern of the Sensory and Motor Disorders of Vision program.

When the retina receives an appropriate stimulus, electrical activity can be recorded almost instantaneously in the visual cortex (area 17 or striate area) and in the adjacent cortex, areas 18 & 19. Although the exact function of the visual cortex is unknown, the reconstruction of the perceived image probably occurs at this level. If the image is to be retained within the visual field and remain in sharp focus, several actions must occur under controlled and coordinated conditions. The eyes move to the right or left, up or down in a tracking movement so that the image of the target falls on the retina. These movements result from contraction and relaxation of the extraocular muscles, which are controlled by impulses from the III, IV and VI cranial nerves. Sharp focusing of the image on the retina is maintained by increasing or decreasing the refractive power of the crystalline lens. This process is likewise regulated by a portion of the nervous system (the antonomic nervous system) through its control of the intraocular muscles. Other portions of the central nervous system (CNS) also contribute to maintenance of the visual image Abnormalities in any of these structures or interruption of the pathways connecting the various structures could result in permanent visual disorders.

CONGENITAL, DEVELOPMENTAL AND DEGENERATIVE ABNORMALITIES

Functional amblyopia is a disorder of vision in which there is poor vision in one eye that appears to have no organic disease. This condition usually arises from failure to use the eye during the first few years of life, and central visual acuity is usually arrested at the level of development attained when interference with visual development occurred.

Strabismus is a condition in which the images falling on the retina of the two eyes are not aligned. Gunter K. von Noorden[1], Baylor College of Medicine, in a discussion of amblyopia and strabismic amblyopia, proposed that the primary factor in the production of amblyopia is a modification of normal visual experience during a period when the visual system is susceptible to such alterations. In spite of the etiological difference in the nature of the underlying clinical conditions, common amblyopiogenic factors appear to be operative in strabismic, anisometropic (conditions in which there is a significant difference in the refractive error of the two eyes) and form vision deprivation amblyopia. These causative mechanisms are stimulus deprivation by inadequate image formation on the fovea, abnormal binocular interaction by incongruity of the visual input received by the two eyes or perhaps a combination of both factors. It must be assumed that experimental condi-

tions that produce amblyopia in animals such as the monkey must be similar to anomalies that occur in the human visual system which result in amblyopia. Continuing experimentation and production of experimental amblyopia in an animal model such as the monkey is of fundamental importance in advancing our understanding of the site and mechanism of amblyopia in humans.

In earlier work von Noorden et al.[2,3] reported that amblyopia could be induced in rhesus monkeys by unilateral lid suture and artificial esotropia during visual immaturity. The critical age at which such procedures will cause loss of visual acuity has been determined to lie between birth and the third month of life. In the current series of studies, von Noorden[4] was able to determine visual acuity at a level of 20/140 which precluded the diagnosis of milder degrees of amblyopia that may have been present in animals in which the eye was occluded after the age of three months. As a result of this study, he reported that the effect of unilateral lid closure on visual acuity shows that the age of susceptibility to unilateral deprivation in rhesus monkeys lies between birth and the ninth week of life. Lid closure at the age of twelve weeks did not produce amblyopia, and visual function in the formerly deprived eye returned rapidly and completely after closure of the sound eye. Animals were retested after a period of six to twenty-four months and not a single animal recovered visual acuity in the previously sutured eye. Short term unilateral lid closures were also studied, and results indicated that deprivation during visual immaturity will cause severe amblyopia in eyes in which the lid is sutured closed for a brief period of two to four weeks. Lid closure of four weeks during the end of the sensitive period (8 to 12 weeks) had no effect on the development of normal visual acuity. Strabismic amblyopia developed only when esotropia (one eye fixed on a target, the other deviates inward) was induced within the first three months of life, which suggests that the period of sensitivity of the visual system to abnormal visual stimulation is similar for both forms of amblyopia. Von Noorden expressed the opinion that the similarity between the organization of the visual system in monkeys and humans, and correlation of the results of this experiment with clinical observation in humans, are of special interest. Visual deprivation in human infants, if present at birth or developed during early childhood, can result in irreversible amblyopia which persists despite surgical correction. The human visual system appears to be sensitive to the effects of unilateral deprivation for a considerably longer period of time than that of the macaque monkey. The question, therefore, arises as to whether the human visual system responds similarly to that of the monkey to brief periods of occlusion. Clinical observation also suggests that, as in monkeys, unilateral occlusion in humans for only brief periods during infancy may cause irreversible amblyopia. In addition to amblyopia and strabismus, in unilateral lid closures in monkeys one can also expect to find equivalent structural anomalies in the visual system. Such anomalies have been described in the retina or optic nerve, in the lateral geniculate nucleus, and in the visual cortex of lower animals. Von Noorden has prepared and studied sections from the retinas, lateral geniculate nuclei, and areas 17 and 18 of the visual cortex from monkeys with behaviorally demonstrated amblyopia and cortical neurological and neurophysiological changes. The only change noted was a significant reduction in cell areas in all sections of the lateral geniculate body or nucleus that received input from the deprived esotropic eye. He has postulated, however, that since similarities of visual input from the two eyes

caused by unilateral lid sutures had been shown to effect geniculate cell sizes in visually immature kittens, that binocular competition at the geniculate or cortical level is operative in both unilateral lid closures and strabismus and causes similar changes in the primate visual system.

Continued work in this area is essential to determine if histological changes occur in the visual cortex in monkeys as has been recorded in lower forms of animals, and to correlate these histological changes with disturbances of the visual system. Such studies should lead to a better understanding of visual deprivation and amblyopia in humans, especially children, during the "sensitive" period.

Kurt Simons and Robert D. Reinecke[5] , Albany Medical School, recently reported on the results of screening tests to determine the effectiveness of these tests for amblyopia screening. In one experiment, the Titmus Stereo Fly, Circle Test and the Animal Test were administered to 70 patients with known visual dysfunctions. Seventy-nine percent of the patients tested on the Stereo Fly passed. Results indicate that the Stereo Fly test is ineffective at discriminating subjects with either significant amblyopia, or anisometropia or heterotropia, from normals. Passing the Circles test at the Number 4 or lower target, or any of the Animal tests, appeared to be as nondiscriminating a measure as the Stereo Fly test, because patients with as much as two lines or more difference in acuity between the eyes and significant strabismus, including vertical deviations, passed at this level. Passing the Circle Test at the Number 5 circle or better provides a more reliable test. They concluded that results of this study indicate that with the exception of the relatively fine threshold of the Number 5 to Number 9 Circle tests, these Stereo tests are unreliable in discriminating patients with amblyopia from normals, but may indicate an artifactual stereoscopic ability.

In a second series of screening tests, Reinecke and Simons[6] used the random-dot stereogram method styled after the "tumbling E." The patient is asked to distinguish between a simultaneously presented random dot "E" (RDE) and a "stereoblank" stereogram containing no three dimensional figure. The RDE was administered to 68 of the patients tested in the earlier tests plus two additional patients with anisometropia. All patients had refractive corrections, if any, in place during test administration. The second series of patients, 121 pre-school children, were tested to obtain normative data on stereoscopic capability of the RDE in the age range of interest as well as to assess the testability under realistic screening conditions. Thirty-nine percent of the 70 patients tested on the RDE passed. All patients passing the RDE fell into one or two clearly delineated categories: (1) passing at either a card-to-patient distance of 50 cm or less or (2) at a distance of 1 meter or more. All the patients passing in the upper category (1 meter or better) were near normal. Tested in normal children, the RDE had a 1.6% overreferral rate on the initial and no overreferral with retesting. From a screening standpoint, the RDE's reliability is complemented by the ease of use with children as young as 3 years of age.

A second feature, noted in the findings reported, is derived from the RDE's apparent ability to detect microtropia reliably (at least in esotropic form). Reinecke and Simons concluded that the clinical version of the Julesz

random-dot stereogram, the random-dot 'E', appears to be a reliable screening test for amblyopia as well as for a variety of potentially amblyopia-related visual dysfunctions.

VISUAL CORTICAL AREAS

In testing normal animals Frank H. Baker of Johns Hopkins University, Peter Grigg of the University of Massachusetts, and Gunter von Noorden[7] of Baylor College of Medicine, demonstrated that a relatively small percentage of neurons were driven from only one eye, and most cells of the prestriate cortex and many cells of the striate cortex had strong input from both eyes. They divided the neurons into two categories on the basis of their response to binocular stimuli. In the first category are cells which give a binocular response which is roughly equivalent to the response to stimulus presented to the dominant eye alone. The response of the second class of neurons, the binocular depth cell, definitely depends on the retinal disparity of the stimuli. The investigators also demonstrated in both the striate and pre-striate cortex a number of neurons that they were unable to drive with visual stimuli. The number of these neurons is important because visual deprivation might increase the number of neurons which lack visual input. The investigators indicate that the number of such cells in a healthy brain would not be greater than 10%.

The eyelids of one eye were sutured closed in two monkeys at age 7 days and in a third at 35 days. At the end of the experimental period the two animals which had been deprived of light from age 7 days had only ghost-like perception in the deprived eye, the third animal had very gross form perception. Microelectrode recordings in these animals from the primary visual cortex appeared normal in their response. Compared to normal animals, however, there was a remarkable paucity of cells which could be stimulated through deprived eyes. In monkeys in which the eyelid had been sutured at 7 days of age, no cells could be driven by stimuli to the deprived eye, and only a few neurons could be driven in the animal whose eye was sutured at 35 days. In no instance did binocular stimulation activate a cell in the pre-striate cortex which was unresponsive to monocular stimuli, nor was any neuron influenced by binocular stimuli when monocularly presented stimuli could drive it only from one eye.

Fundoscopic examination of the experimental animals revealed a normal-appearing retina and optic papilla. Electroretinograms in one animal revealed no differences between normal and deprived eyes in the response to flashes. Histological changes were noted, however, in the lateral geniculate nucleus of the animals. These studies seemed to indicate that the earliest changes in histology of the visual pathways in deprived eyes appear in the lateral geniculate bodies.

Edward L. Keller[8] , University of California, Berkeley, has been studying the motor-neuron firing rate to determine, if possible, the level of integration which governs eye movement. A detailed statistical analysis of motor-firing rates suggests that at least for the vergence component the combination must take place prior to motor-neuron innervation. In previous studies Keller and Robinson[9] showed that all abducens neurons participate in vergence movements by decreasing their firing rate for convergence and increasing

their discharge rate for divergence. These earlier studies led this group to the conclusion that vergence movements are not produced by separate set of motor neurons. Their data, however, were not sufficient to prove that the firing range of a motor-neuron was statistically identical for a given position reached by version or vergence.

Unit discharge rate during the dynamic phase of vergence movement was also examined. There was an abrupt change of discharge rate from one tonic level to another for both convergence and divergence and all medial rectus units were found to participate dynamically in the same manner with the opposite direction of the rate change from ipsilateral abducens units. Since the simple step change in motor neuron firing rate occurred for all units studied, the implication is that vergence movements are the result of step change in net extraocular muscle force. This report represents a continuing effort of individuals working in this field to ascertain how the eye is able to function in tracking objects in fixation and in normal visual function.

Rainer W. Guillery and John H. Kaas[10] , University of Wisconsin School of Medicine, have been interested in the visual pathways and strabismus as found in albino animals. They have previously reported abnormalities in Siamese cats, even those that are not cross-eyed. In carnivores, the abnormalities are seen as irregularities in the cell layers of the lateral geniculate nucleus. It is, therefore, possible to look at the histology of laminar structures of the lateral geniculate nuclei in a carnivore brain and determine whether it shows the albino abnormalities. Guillery and Kaas reported on an histological study of the brain from an albino tiger whose eyes were normally aligned, however, microscopic examination of the lateral geniculate nucleus showed the "albino" abnormality. Guillery proposed that in man some form of strabismus may be produced by similar deviation of the retinal-geniculate fibers associated with a reduced amount of pigment formation either in the body in general, but more probably in the eye itself. This area of research appears to be promising and it is important that investigators continue research in this area with emphasis on those animals more closely related to man.

Injury to the occipital cortex may result in loss of visual sensation to all or a portion of the visual field. Classically, patients have not reported seeing light presented to the visual field represented by the area of loss. In cases of severe damage to the occipital cortex, perception is limited to sudden changes in illumination of the visual field. Whitman Richards[11] , Massachusetts Institute of Technology, working with nine patients with brain injury of the visual area,utilized standard techniques to determine visual losses. In addition, for each participant, forced-choice stereo discriminations were made in a portion of their blind field and in a symmetrical portion of the intact field. A third "straddle" condition was also presented, i.e. one of the stimulus lines went to the blind field and the second member of the pair went to the intact field. The results of these studies were: (1) there is no outstanding stereoscopic discrimination when light flashes are presented within the scotomota, (2) there is a possibility that monocular stimuli may be distinguished from binocular stimuli even when they are presented within the scotomota, and (3) approximately half of the patients were capable of identifying the monocular from the binocular

(disparate) stimuli in the straddle condition.

This study suggests that at least one midbrain structure (the pretectum) might be more responsive to dark than to light objects. Research in this area should help to define the visual function of the various portions of the visual system.

VISUAL SENSORY AND PERCEPTUAL DISORDERS

The eyes move constantly, even though attempts are made to fixate a stationary target. Fixation of an image on the retina requires the elimination of eye movements. Although several theories have been advanced as to the function or purpose of this eye movement, none have been established definitively, some have been refuted, and research continues. Attempts have been made to develop apparatus that would eliminate eye movement to permit study of the visual system under conditions of non-movements.

Robert Jones, John G. Webster, and Ulker Tulunay-Keesey[12], University of Wisconsin, reviewed the literature in this area and reported on a new device that appears to eliminate some of the less desirable properties of existing equipment.

The apparatus developed by this group consists of a simple feedback system which detects eye motion and rotates a mirror through which the target is viewed, thereby compensating for eye motion. The need for such apparatus in the study of vision is readily apparent, because it is necessary to eliminate eye movement in order to study the effect of stationary gaze.

Douglas Anderson, Bascom Palmer Eye Institute, and Anita Hendrickson[13], University of Washington, have been investigating the effect of intraocular pressure on the biological phenomenon of axoplasmic transport in the nerve fibers of retinal ganglion cells, especially where they transfer to the optic nerve-head and exit from the eye. Axoplasmic flow is a well established phenomenon in the visual system. In the rhesus monkey, for example, radioactively labeled protein will cover the distance from the retina to the lateral geniculate nucleus within six hours. This study dealt with the most rapid component of axoplasmic transport along with the slightly less rapid component (phase 2) in the owl monkey (Aotes trivirgatus). Checking the exoplasmic transport against increases in intraocular pressure, Anderson and Hendrickson found that under normal conditions the incorporated label had reached the lateral geniculate body by eight hours.

When the intraocular pressure was elevated to achieve a perfusion pressure of 60 mm of mercury, there was partial obstruction of axoplasmic transport. When intraocular pressure was elevated to the point that it was only 25 mm of mercury below mean femoral blood pressure, the injected radioactive material accumulated in the nerve fiber bundles in the region of the lamina cribrosa with little or none having reached the orbital portion of the optic nerve or lateral geniculate body. The researchers were unable to determine if the obstruction was mechanical or whether it was secondary to a reduced blood flow. Research in this area is important because of its possible relationship to the pathogenic mechanism of glaucomatous cupping and to determine the importance of axoplasmic flow through the optic nerve up to the

lateral geniculate body.

The superior colliculus of the cat has been shown to receive visual input from the retina of both eyes and from the visual cortical areas of the ipsilateral visual cortex, and possible other cortical areas. Inputs from all of these areas terminate in the superior gray and optic layers of the colliculus.

It has been demonstrated by a number of workers that the cells in the most superficial collicular layers respond to moving stimuli and a large proportion of the cells are selectively responsive to stimuli moving in a particular direction within well-defined receptive fields. Most of these units can be driven equally well by stimuli from either eye. It is thought that direction selectivity and the effectiveness of the ipsilateral eye in driving collicular units are both dependent on the influence descending from cells within area 17 of the visual cortex.

Larry A. Palmer and Alan C. Rosenquist[14] , University of Pennsylvania, explored the striate cortex of the cat to determine which cells in area 17 project to the superior colliculus and what visual information is contributed to the colliculus from area 17. Once a cortical tectal unit was identified and isolated, its minimal response field was plotted for the dominant eye and the properties of the cell studied quantitatively with moving and flashing stimuli projected onto a screen with hand-held projections.

Histological sectioning of the striate cortex revealed three main groups of cells or units, based on their responses to electrical stimulation of the superior colliculus. At least 95% of the units isolated in the cortex were not affected in any way by collicular stimulation. The remaining 5% of the units isolated in the striate cortex were driven either antidromically (impulses travelling in the opposite direction along the neuron from the direction in which impulses normally travel) or orthodromically (impulses passing in the normal direction) by stimulation of the colliculus.

Most of the cortical tectal units had large receptive fields which could not be divided into mutually antagonistic components. They were spontaneously active with mean rates seldom exceeding five spikes per second. The cortical tectal units responded with sustained discharge, usually at rates exceeding 80 spikes per second as an appropriate slit or edge was moved slowly across the visual field. The receptive fields of the cortical tectal units were located well within the binocular overlap zone of the cat and most were driven about equally by the two eyes when tested one at a time.

Palmer and Rosenquist identified those cells in the cat striate cortex whose axones terminate in the superior colliculus. These neurons lie in layer V and consist largely of binocular, direction and orientation selective complex cells with large receptive fields. Most of these units lack the property of summation with stimulus length along the receptive fields axis which appears to be characteristic of most cells in the striate cortex. Several outstanding properties of the cortical tectal cells are precisely those which are lost in the colliculus following removal of area 17. Thus, as in the superior colliculus of the intact animal, most cortical tectal units

are direction selective and binocular. These data reinforce the idea, based upon ablation studies, that directions of activity and effectiveness of the ipsilateral eye in driving collicular units are dependent on binocular, direction selective input from the striate cortex. This study provides evidence further identifying the interconnections of the superior colliculus and area 17 of the visual cortex. Information of this type is essential in constructing the visual pathways which carry impulses from the retina to the visual cortex, and in understanding the influence of interconnecting cortical areas on the incoming impulses from the retina.

Barry E. Stein, Elemer Stein and Lawrence Kruger[15], University of California, Los Angeles, studied the optical system in kittens to determine the properties of neurons in the superior colliculus in the period preceding eyelid opening, and to follow the sequence of development of responsiveness to light, the appearance of input from both eyes, and the maturation of several physiological properties including sensitivity to movement and appearance of directional "preference" and velocity selectivity.

This study shows that information processing in the superior colliculus advances with age and that a distinct maturational sequence can be demonstrated with some neuronal response characteristics, although each property did not appear simultaneously in a given neuron. During the period preceding eyelid opening, all neurons were activated only from the contralateral eye. It seems that the ipsilateral pathway matures more gradually, thus accounting for the progressive increase in binocular activation with age. The earliest response to a moving stimulus was detected in one neuron in a seven day old kitten. By the ninth day many neurons responded to both diffuse light and moving stimuli, and on the thirteenth day, neurons excited only by moving stimuli were detected. The proportion of neurons responding uniquely to moving stimuli progressed steadily with increasing age. In adult cats the majority of neurons responded most vigorously to moving stimuli. This study has shown a progressive step by step maturation of the neurons within the superior colliculus, and the gradual change from primarily a contralateral eye response to a binocular response with an increasing number of neurons responding to movement.

Maturational changes in neuronal specialization paralleled the sequence of events and development of visually guided behavior may reflect the maturation of the cortical tectal pathway.

It can be demonstrated through the use of microelectrodes that neurons in the primary visual cortex of the brain of various animals respond to certain types of visual stimuli. In the cat, for example, these cells can be classified as simple, complex or hypercomplex, in terms of the selectivity of their response. Histological preparation of the same area has shown that most cortical neurons are either stellate or pyramidal in shape. The question then arises as to whether there is some correlation between the structural and functional properties of these cells. Various researchers have demonstrated that the distribution of simple cells in different cortical layers tend to parallel the distribution of stellate cells, and that the distribution of complex cells parallels the distribution of pyramidal cells. David L. Van Essen and Donald H. Kelly[16], Harvard Medical School, reported on a correlation

study in cats using a fluorescent dye, Procion yellow, and microelectrode recordings.

The receptive field of each cell was mapped using spots and slits of light projected onto a tangent screen upon which the cat eyes were focused. When a cell was satisfactorily classified, it was injected with Procion yellow. Because of the unusual technical difficulties involved, only a few cells were stained in each experiment. Most of the simple and complex cells that were injected conformed to a general pattern. Five injected cells had well-defined complex receptive fields; four of these cells were pyramidal and one was stellate. The three probably complex units were all pyramidal cells. These experiments demonstrate that it is technically possible not only to record electrically from cortical cells but also to tag these cells by suitable markers for later histological examinations. Studies of this type will permit researchers to determine both structure and function of cells within all parts of the visual cortex that can be successfully impaled with microelectrodes.

A larger sample of cells is obviously needed to determine more precisely the relationship between structure and function in each cortical layer. The authors expressed hope that this approach will eventually lead to a more complete understanding of the different stages of information processing within the cortex.

Robert Sekuler[17] , Northwestern University, reviewed the literature of importance to an understanding of spatial vision that was published between approximately 1961 and 1974. In the introductory statement, he lists three major developments during the period covered by this review: (1) an increased awareness of the complexity of the anatomy and physiology that supports spatial vision, (2) a growing cross-fertilization between clinicians and non-clinicians who share a common interest in spatial vision and (3) the successful application of linear systems analysis to various problems in spatial vision. This publication is a useful source of literature reference in the field of spatial vision.

CEREBELLUM

The role of the cerebellum in eye movement has long been an area of controversy and has not been thoroughly researched. It is known, however, that stimulation of the vermis lobes VI & VII evoke ipsilateral, horizontal eye movement. Stimulation of the hemisphere has resulted in reports that include ipsilateral horizontal movement, nystagmus, contralateral or up movements, and rotary up movements. To date, not a single cerebellar structure has been reported in which stimulation did not evoke eye movement.

The study of cerebellar stimulation as undertaken by Samuel Ron and David A. Robinson[18], Johns Hopkins, was done to study quantitatively the direction and type of eye movement evoked by stimulation of each sub-division of the entire cerebellum in the alert intact monkey. Eye movements were recorded and measured accurately for each sub-division explored. Stimulation of much of the cerebellum, such as the lobes 1 & 1V, the paramedian lobes, and the paraflocculus produced no eye movement. Saccades were evoked from

the vermis, lobes V-VII, saccades and smooth movement were produced from stimulation of the hemispheres, crus 1 & 11 and lobulus simplex. Nystagmus was produced from stimulation of the flocullus, nodulus and uvula. All movements were conjugate. Stimulation of all of the cerebellar nuclei evoked eye movement with ipsilateral horizontal components.

Stimulation was given to 71 sites in cerebellar nuclei and the adjacent white matter. Stimulations of the fastigial nuclei evoked saccades with up or down components in all but the most inferior part, where slow stages of nystagmus were elicited. Stimulation of the interpositus nuclei evoked saccades and smooth movements with down components. Stimulations of the dentate nuclei principally evoked saccades and smooth movements with up components. Stimulation of the caudal part of the fastigial nuclei and the adjacent white matter evoked nystagmus, or only its slow phase, similar to those obtained by stimulation of the vermis lobes IX and X. Three regions of the cerebellum participated in oculomotor control.

Within each region, the type of eye movement was the same, but the direction varied with stimulation location so that all eye movement directions were represented in each region. All structures other than the three regions were considered unrelated to the oculomotor system because no eye movement could be evoked by a stimulus current of 1 ma or the threshold for limb movement. The importance of this study was in the careful methodical stimulation of the cerebellar cortex and its nuclei in the alert unanesthesized preparation. The delineation of areas in which stimulation evoked eye movement will serve as a basis for continuing studies by other workers interested in this area and will help determine the interrelationship of the cerebellum and the oculomotor components in the control of eye movements in normal and abnormal conditions.

When a human is asked to look at an object or to fix on a target, he or she rotates the eyes until the image falls within the fovea. The image or target, therefore, falls on the portion of the retina where the target is seen in the sharpest focus. If the subject is asked to maintain fixation on the target, the eyes make a series of continuous but irregular patterns of slow and fast miniature eye movements. The pattern is composed of three kinds of separate movements of the eye: saccade, drift, and physiological systagmus. Saccades are small and very fast changes in eye position which occur one to three times every second. The eyes also drift slowly back and forth in the interval between saccades. Physiological nystagmus is a high frequency tremor that is superimposed upon the saccades and drifts. Robert M. Steinman et al.[19] , University of Maryland, have been studying the pattern of movement (saccades, drift and physiological nystagmus) in normal vision. It has been shown by others that drifts appear to be necessary as well as sufficient to keep a target from fading when it is stabilized on the retina.

It is also reasonably certain that target visibility does not depend upon saccades. Steinman has postulated that the pattern of eye movement during fixation that are recorded in the laboratory are almost confined to the laboratory. During normal vision or fixation the eye appears to rest on a target for several seconds at most, which is sufficient time to take in all relative information without any of the fixation saccades. It is only

when the subject is asked to fix or pay special close attention to a target that the tiny saccades come into play. When the individual is searching the visual world, large saccades may be present. In a series of experiments, this group has found that subjects could make voluntary saccades as small as those made during maintained fixation and that the subjects were aware of having made the saccades. Fixation saccades are thought to be an overlearned motor activity so that when an individual is asked to maintain fixed vision the pattern (saccade) occurs without the subject making any conscious effort or being aware of individual saccades occurring.

Normal humans are usually unaware of large saccades that are made during normal search of their visual world, nor are they aware of the highly overlearned but initially conscious eye movement patterns used whenever they read. If they are asked to pay attention to large voluntary saccades they become aware that their eyes are jumping around, the overlearned pattern no longer runs itself off in exactly the same way. The tiny saccades that occur during maintained fixation show similar characteristics. This group concluded that the characteristics and probable function of miniature saccades made during maintained fixation are not different from the characteristics and probable function of large saccades made during visual exploration. If this hypothesis is true, then the task of understanding human oculomotor performance is simplified because the distinction between large voluntary saccades and miniature involuntary saccades can be discarded. In studying the miniature saccades in their maintenance of fixation patterns, this group found that both man and the rhesus monkey have an effective slow control system that keeps a target image relatively stationary on the retina when saccades are not made.

The ability to suppress saccades in the human will permit studies of slow control characteristics free from contamination by periodic high-velocity eye movements that alter the characteristic of any saccadic drift. It is also thought that miniature saccades are busy work and that they accomplish nothing. They are not required to prevent target fading and they are not required to keep the eye in place for long periods of time.

Gerald Westheimer and Sydney M. Blair[20] , University of California, Berkeley, report that stimulation of certain regions of the brain stem of alert monkeys was found to inhibit saccadic eye movements. This report on saccades and other types of eye movements shows that it is possible to elicit, artificially and controllably, a state highly reminiscent of the clinical syndrome of gaze paralysis found with certain lesions of the pons. There was no interference with smooth eye movements and with convergence eye movements. There was no change in amplitude or phase of the smooth component of vestibular eye movement and accommodation was normal. There was likewise no interference with the menace of corneal blink reflexes. This study reconfirms works of others that the saccadic movement is independent of other eye movements.

Evidence has accumulated over the years from various workers in the field to indicate that the mammalian superior colliculus participates in visual orientation and localizing. The situation in primates appears somewhat more complex and different in that lesions of the superior colliculus

do not abolish visual orientation in monkeys, and it seems probable that the primate colliculus is only incidentally involved in this function.

The problem of characterizing more accurately how the primate superior colliculus participates in vision was the object of the research work reported be Bruce V. Updyke[21] , University of Oregon. Recordings were obtained from the superior colliculus of six cebus monkeys from implanted stainless steel electrodes.

All of the neurons encountered in the superior layer were visually responsive and 72% were spontaneously active. Moving stimuli elicited more consistent responses than stationary stimuli for most of the units studied. While units responded over a range of movement velocities without obvious tuning for particular velocities within that range, there were wide variations in the maximum effective velocities.

Only 2% of the neurons examined were clearly directionally selective, exhibiting selectivity that was independent of stimulus contrast and stimulus position in the response field.

Over half of the neurons also responded to stationary spots of the appropriate size flashed on or off in the response field. Only 16% of the units responded as consistently to stationary as to moving stimuli. In a determination of response to stimulus size, 19% of the units lacked size preference and gave equivalent responses to large or small stimuli presented anywhere in the response field. About a third of the units exhibited spatial summation for stimuli within the response field, and another third exhibited spatial suppression for stimuli in the response field. Over half of the units responded equally well to light or dark edges entering the response field. Approximately 17% of the superficial layer units examined showed sensitivity to stimulus orientation. The defining characteristics of these units were the presence of spatial summation along one axis of the response field and suppression along the perpendicular axis. A total of 60 neurons was examined in the intermediate and deep layers. Of the units studied, 90% were visually responsive and 88% exhibited spontaneous activity.

Updyke concluded from this work that neurons of the cebus monkey colliculus are heterogeneous population with respect to visual response characteristics. One of the striking features of the colliculus was the presence of units which responded selectively to objects presented in their response field. These units did not constitute a homogeneous group, nor did they always respond exclusively to visual objects. All attempts to determine the trigger feature for the object-selective responses were unsuccessful. The presence of object-responsive neurons suggests that the primate colliculus integrates information about the behavioral importance of stimuli. This activity is compatible with a role in mediating visual attention and in facilitating visual orientation.

Barbara Gordon[22] , University of Oregon and Linda J. Gummow have completed preliminary studies on the effect of artificial squint on the superior colliculus to determine its role in the control of eye movements. In animals in which the right medial and right lateral rectus muscles were sectioned during the second year of life, they found that the artificially

produced squint had a definite effect on the cellular activity within the superior colliculi. In the colliculus contralateral to the normal eye 90% of the cells were dominated by the normal eye. In the colliculus contralateral to the squint eye, 50% of the cells were driven equally well by both eyes. It appears that the colliculus is therefore dominated by the eye that is able to make fixation movements. It is hoped that the studies on the effect of artificial squints will contribute to an understanding of the neurological abnormalities accompanying squint in humans.

The past year saw the development of formal psychophysical (behavioral) techniques for testing the visual capacities of human and monkey infants. It is now possible to test both human and monkey infants, by a new preferential looking technique, between birth and six months of age. In addition, operant techniques can now be used to test visual processes in monkey infants of five weeks and older.

Davida Teller et al.[23,24,25], University of Washington, have used these techniques to show that infant monkeys at five weeks already have trichromatic color vision; that human infants are at least dichromatic (have at least some color vision) at the age of two months; and that grating acuity in human infants improves with age between two and six months but does not appear to vary with the orientation of the acuity grating.

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CONTRACT NARRATIVE

WASHINGTON UNIVERSITY (NIH-NEI-71-2514)

Title: Evaluation of the Effectiveness of Diphenylhydantoin (DPH) in Reversal of Recent Glaucomatous Field Defects

Principal Investigator: Dr. Bernard Becker

Current Fund Allocation: $271,094 for the period June 24, 1971 through June 23, 1975

Objective: This project is a clinical trial to evaluate the effect of DPH in early glaucomatous visual field loss. Patients with primary open angle glaucoma are randomly assigned to a treatment or control group. The research protocol includes the normal testing and management of ocular hypertension.

Progress to Date: Fifty (50) patients have been enrolled in the study as required by the research protocol. A final analysis of data from these patients has been undertaken by a biostatistician. Until this analysis is complete, results will be masked from investigators participating in the study.

Significance to NEI Programs and Biomedical Research: The successful development of more effective drug therapy for glaucoma patients would represent a major breakthrough in the treatment of this serious disorder which is one of the major causes of blindness and visual disability.

Proposed Course: The study will be completed in June 1975. It is anticipated that a final report will be available in July 1975.

Keyword Descriptors: double-blind drug trial, reversal of glaucomatous field loss

NIH Research Program: Glaucoma--Primary Glaucoma (Open-Angle Glaucoma)

Experimental Subject or Tissue Source: Human

Research Objective: Treatment

CONTRACT NARRATIVE

GEORGE WASHINGTON UNIVERSITY (NIH-NEI-72-2114)

Title: Study and Improvement of Surgery on the Outflow Channels in Glaucoma Eyes

Principal Investigator: Dr. Mansour F. Armaly

Current Fund Allocation: $479,796 for the period June 30, 1972 through June 29, 1975

Objective: The objectives of this contract are three: (1) To evaluate in normal monkey eyes *in vitro* and *in vivo* the outflow facility; (2) To develop and implement mechanical and chemical methods to increase the outflow in normal monkeys; and (3) Techniques defined in the first and second parts introduced to human use.

Progress to Date: A number of important studies have been completed. Among these are: (1) Development of a reliable, sensitive fluorophotometer capable of determining fluorescence in aqueous as well as total fluorescence with a sensitivity of 10^{-9}; (2) Development of a method to demonstrate mucopolysaccaride electron microscopically; (3) Development and testing of a servo perfusion system and studies of its reliability in the monkey and at the operating table in man.

Significance to NEI Programs and Biomedical Research: The project is part of a major research program for the development of new techniques in the diagnosis and treatment of glaucoma.

Proposed Course: It is expected that the project will be continued for an additional year to complete ongoing studies.

Keyword Descriptors: intraocular pressure regulation

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: Human/Monkey

Research Objective: Treatment, Diagnosis

CONTRACT NARRATIVE

THE UNIVERSITY OF UTAH (NIH-NEI-73-2115)

Title: Development of a Non-Invasive Sensing System to Measure Intraocular Pressure Continuously

Principal Investigator: Mr. Lucien A. Couvillon

Current Fund Allocation: $95,927 for the period June 1, 1973 through June 15, 1975

Objective: This project is designed to meet the need for a more thorough understanding of the pharmacologic changes in the anterior and posterior segments of the eye in glaucomatous patients. More specifically, this entails the development of a non-invasive system for the continuous monitoring of intraocular pressure to evaluate more precisely the diurnal variations in pressure of those glaucomatous patients who have progressive field loss despite apparent adequate control of the intraocular pressure. In this manner, more accurate and more acute diagnosis can be made and more timely pharmacologic therapy administered.

Progress to Date: The contractor has developed and tested in dogs a prototype system. The system involves a small transducer which is held against the conjunctiva over the sclera by the pressure of the lower eyelid tissue, and no surgical incisions are required. The output of the transducer is sent via a small and flexible wire to a small transmitter, mounted on the patient's temporal region. The transmitter has a range of several meters, and data is recorded and displayed in a nearby receiver console. Data from initial tests of the system in dogs has been presented for Institute evaluation.

Significance to NEI Programs and Biomedical Research: This project is a significant component of the Institute's program in glaucoma research. Current means for the measurement of intraocular pressure require considerable patient participation, cooperation, and are inconvenient (factors which in some cases causes artifactual alteration of the record). This condition impedes accurate and adequate diagnosis and hinders administration of timely pharmacologic therapy. The successful development of a non-invasive system for continuous monitoring of intraocular pressure will largely overcome these obstacles, and will help to elucidate the physiological and anatomical changes that occur in glaucomatous patients. These changes, together with the role of blood pressure and blood flow associated with the optic nerves and nerve fiber layers of the retina in nerve damage and field loss, are among the least understood aspects of glaucoma.

Proposed Course: The next phase of this project will involve a clinical evaluation of the system to determine whether it can be worn comfortably by man. It is anticipated that this phase of the study will require approximately three (3) months.

THE UNIVERSITY OF UTAH (NIH-NEI-73-2115)

Keyword Descriptors: intraocular pressure measurement, non-invasive technique, continuous monitoring

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: Human

Research Objective: Treatment, Diagnosis

CONTRACT NARRATIVE

JOHNS HOPKINS UNIVERSITY (NIH-NEI-73-2127)

Title: Evaluation of Indicator Substances for Use in the Study of the Retinal and Choroidal Circulation

Principal Investigator: Dr. Arnall Patz

Current Fund Allocation: $413,507 for the period June 30, 1973 through June 29, 1976

Objective: The objective of this project is to conduct a systematic and comparative evaluation of indicator substances in the study of the retinal and choroidal circulation.

Progress to Date: The contractor has continued to identify and obtain dyes, pigments, and stains for testing and evaluation as indicator substances for the study of retinal and choroidal circulations. Over 500 of these substances have been ordered and received. The dyes are tested for solubility in various solvents, such as in acidic and basic water solutions, in ethanol, in ethelene glycol, and in dimethyl sulfoxide. Those substances are subjected to absorption and fluorescence spectral analyses. A total of 138 of the most promising dyes have been tested for gross toxicological effects in rodents.

Significance to NEI Programs and Biomedical Research: The morphologic abnormalities of various disease states of the retina, choroid, and vitreous have been previously investigated and documented. However, the interrelationships between blood flow, vascular characteristics, and tissue metabolic requirements in the vasculature of normal and diseases eyes have not been adequately quantitated. Although fluorescein angiography has contributed signficnatly to the understanding of several retinal and choroidal diseases, there still remains major limitations in the study of these two circulations, particularly the choroidal circulation. More specifically, sodium fluorescein has inefficient transmission qualities through the pigment epithelium and xanthaphil pigment in the macula. This study is designed to overcome these obstacles.

Proposed Course: Additional new dyes will be tested for solubility, fluorescence, and gross toxicity during the immediate next phase of the study.

Keyword Descriptors: indicator substances, retinal and choroidal circulation

NEI Research Program: Retinal and Choroidal Diseases--Vascular and Circulatory Abnormalities

Experimental Subject or Tissue Source: Rodent

Research Objective: Diagnosis

CONTRACT NARRATIVE

GEORGE WASHINGTON UNIVERSITY (NIH-NEI-73-2131)

Title: Development of a Clinically Useful, Objective, Photographic Technique for Fluorescein Angiography

Principal Investigator: Dr. Mansour F. Armaly

Current Fund Allocation: $85,591 for the period June 18, 1973 through August 31, 1975

Objective: The objective of this project is to develop an automatic focusing system for a fundus camera to improve the quality and consistency of fluorescein angiograms.

Progress to Date: The development of the automatic focusing device and its coupling to the Zeiss fundus camera has been completed. A clinical evaluation and final refinement of the system is now underway.

Significance to NEI Programs and Biomedical Research: Differences in fluorescein angiographic findings in glaucoma are controversial, with partial agreement limited to the very advanced stage of the disease. The main reason for this lack of uniformity is technical. The quality of fluorescein angiograms varies markedly with the expertise of the photographer, and even then it is not of predictable quality. The major variables in this regard have been accuracy of focus and contrast quality. The development of techniques to overcome these limitations would represent a significant advance in eye research.

Proposed Course: It is expected that the project will be completed within the next six (6) months.

Keyword Descriptors: fluorescein angiography

NEI Research Program: Glaucoma / Retinal and Choroidal Diseases

Experimental Subject or Tissue Source: Human

Research Objective: Diagnosis

CONTRACT NARRATIVE

THE WHITTAKER CORPORATION (NIH-NEI-73-2132)

Title: Development of a Clinically Useful, Objective, Photographic Technique for Fluorescein Angiography

Principal Investigator: Dr. David Sheena

Current Fund Allocation: $90,967 for the period June 30, 1973 through August 31, 1975

Objective: The objective of this project is the development of a modified fundus camera for the taking of improved fluorescein angiograms. The modifications are expected to result in new techniques for obtaining optimal focus and optimal contrast photographs and maintaining quality throughout each angiography series.

Progress to Date: The contractor has completed development of an automatic focusing system for the fundus camera. A sensitive silicon vidicon television camera has been adapted to the fundus camera and has obtained good quality pictures of a model eye. Clinical evaluation of this focusing device is now underway.

Significance to NEI Programs and Biomedical Research: Differences in fluorescein angiographic findings in glaucoma are controversial, with partial agreement limited to the very advanced stage of the disease when the optic nervehead and the peripapillary region are virtually devoid of a visible capillary bed. The main reason for this lack of uniformity is technical. The quality of fluorescein angiograms varies markedly with the expertise of the photographer, and even then it is not of predictable quality. The major variables in this regard have been accuracy of focus and contrast quality. The development of techniques to overcome these limitations would represent a significant advance in eye research.

Proposed Course: It is expected that the project will be completed within the next six (6) months.

Keyword Descriptors: fluorescein angiography

NEI Research Program: Retinal and Choroidal Diseases / Glaucoma

Experimental Subject or Tissue Source: Human

Research Objective: Diagnosis

CONTRACT NARRATIVE

THE CHILDREN'S HOSPITAL MEDICAL CENTER (NIH-NEI-73-2133)

Title: Development of the RCS Rat as a Model of Hereditary Degenerative Diseases of the Retina

Principal Investigator: Dr. Mathew LaVail

Current Fund Allocation: $235,639 for the period June 30, 1973 through June 29, 1976

Objective: This project is for the development of genetically stable breeding stocks of inbred strains of Royal College of Surgeons (RCS) rats with retinal degeneration. These stocks are provided as a research resource to interested investigators for study of retinal structure and function as well as the pathogenesis of hereditary retinal degenerative diseases.

Progress to Date: The contractor has produced RCS animals for use in different breeding schemes and has distributed several dozen breeding pairs to interested investigators throughout the country.

Significance to NEI Programs and Biomedical Research: Animal models are urgently needed for research on retinal degenerations, a serious cause of human blindness. Hereditary retinal degenerations are widely distributed among dogs, but these have had limited study due to the difficulties involved in obtaining relatively pure genetic conditions in the animals, and the expense and difficulty of breeding and maintaining them. Recent advances had led to the uncovering of genetic loci which, when manipulated by selective breeding techniques, have led to the development of a new substrain of RCS rat. These animals present a great opportunity for studying the genetics of degenerative retinal diseases since relatively pure genetic conditions can be obtained, and these animals are less expensive and less difficult to breed and maintain.

Proposed Course: Under this contract, genetically stable breeding stocks of six (6) cogenic inbred strains of RCS rats with retinal degeneration will be developed. Approximately fifty (50) breeding pairs of each substrain will be available for distribution at different times in the contract period.

Keyword Descriptors: animal model, retinal degenerative diseases

NEI Research Program: Retinal and Choroidal Diseases--Development, Structure, Function, and Degeneration

Experimental Subject or Tissue Source: Rat

Research Objective: Etiology

CONTRACT NARRATIVE

WESTAT, INCORPORATED (NIH-NEI-75-2108)

Title: Critique of Available Data Concerning the Prevalence and Costs of Visual Disorders and Disabilities

Principal Investigator: Mr. John Cahill

Current Fund Allocation: $68,489 for the period November 4, 1974 through November 3, 1975

Objective: The objective of this project is to assemble from secondary sources the most recent data available on the incidence, prevalence and economic cost of visual disorders.

Progress to Date: The contractor has conducted an extensive literature search to identify data sources on morbidity, treatment, and socio-economic costs of visual disorders. Questionnaires have been developed to gather information from local, state, federal, and private agencies dealing with statistics on visual disorders.

Significance to NEI Programs and Biomedical Research: Reliable, up-to-date and well-documented morbidity and blindness statistics for the United States are required by the National Eye Institute for purposes of program planning and budget justification. In addition, information on the socio-economic costs of blindness, visual disability, and eye disease in the United States is essential for determining research priorities and the proper allocation of resources in the support of vision research.

Proposed Course: The contractor will prepare a single document which critically evaluates and presents the best available data in a systematic and consistent manner.

Keyword Descriptors: prevalence and cost of visual disabilities, data collection and analyses

NEI Research Program: All Research Programs

Experimental Subject or Tissue Source: None

Research Objective: Etiology, Treatment, Diagnosis

CONTRACT NARRATIVE

THE UNIVERSITY OF VERMONT (NIH-NEI-75-2105)

Title: Development of a Pedigreed Strain of Octodon Degus for Cataract Research

Principal Investigator: Dr. David Boraker

Current Fund Allocation: $53,298 for the period October 15, 1974 through October 14, 1975

Objective: The objective of this project is to develop through selective breeding a colony of degus with mild hyperglycemia and a high incidence of cataracts. The animals will be utilized to study the synergistic effects of multi-cataractogenic factors such as radiation, steroids, nutritional deficiencies, and diabetes.

Progress to Date: The contractor has established a breeding colony of degus consisting of 77 adult males and females. The colony will be expanded over time and carefully monitored for the presence of cataractous animals. Several shipments of animals have been received by the Institute for use in the cataract research program.

Significance to NEI Programs and Biomedical Research: Research on the etiology, diagnosis, treatment and prevention of cataracts is one of the major program interests of the National Eye Institute. Animal models for the study of cataracts are urgently needed to advance our understanding of this disease. Degus, a species of rodent, native to the Andes Mountains of South America, have unique biological features that may provide new leads in the study of cataracts associated with diabetes.

Proposed Course: The breeding colony of degus will be expanded to provide for the availability of 36 hyperglycemic and 18 cataractous animals each month for two years.

Keyword Descriptors: animal model, diabetes cataract

NEI Research Program: Cataract

Experimental Subject or Tissue Source: Octodon Degus

Research Objective: Etiology

CONTRACT NARRATIVE

GEORGE WASHINGTON UNIVERSITY (NIH-NEI-74-2167)

Title: Biostatistical Analysis of Collaborative Glaucoma Study

Principal Investigator: Dr. Mansour F. Armaly

Current Fund Allocation: $94,481 for the period June 17, 1974 through June 16, 1975

Objective: The objectives of the project are to evaluate the hypothesis that one or more tests of intraocular pressure and its dynamics before and after water loading are predictive of the future development of the visual field loss of glaucoma. Additionally, the hypothesis will be tested that ocular and other patient characteristics, whether singly or in combination, are predictive of glaucomatous field loss. Data obtained from the Collaborative Glaucoma Study will be verified and assessed to accomplish these objectives.

Progress to Date: The contractor has initiated intensive efforts to evaluate the quality and coverage of data from the Collaborative Glaucoma Study. Computer programs have been written to identify errors and verify the completeness of study data. A detailed study of the coding procedure of the Collaborative Glaucoma Study was made coupled with review of patient charts coded as having specific defects in the visual field.

Significance to NEI Programs and Biomedical Research: The project is part of the Institute's research program on the etiology, diagnosis, treatment and prevention of glaucoma. Knowledge is fragmentary concerning: (1) the significance of moderate elevations of intraocular pressure and disturbances of aqueous humor dynamics; (2) the frequency and rapidity of field defects developed in eyes with various degrees of pressure elevation and disturbed aqueous humor dynamics. The information collected from the Collaborative Glaucoma Study may be valuable in determining when to begin medical treatment to lower the intraocular pressure and restore the aqueous humor dynamics to normal. In addition, the collection of and subsequent analysis of large amounts of data over many years may reveal signficiant clues to pathogenesis.

Proposed Course: It is expected that two years will be required for completion of this study.

Keyword Descriptors: collaborative glaucoma study, data analysis

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: None

Research Objective: Etiology

CONTRACT NARRATIVE

BAYLOR COLLEGE OF MEDICINE (NIH-NEI-74-2162)

Title: Development of a Method for the Large Scale Preparation of Rod Outer Segments

Principal Investigator: Dr. Robert Anderson

Current Fund Allocation: $26,175 for the period May 6, 1974 through May 5, 1975

Objective: The objective of this project is to develop an inexpensive method for the preparation of large quantities of dark-adapted bovine rod outer segments of acceptable purity. The method sought shall have the capacity to produce 3 grams of lyophilized rod outer segments in each of three consecutive months.

Progress to Date: A rapid and simple procedure has been developed for the preparation of crude ROS. Efforts are now directed to the preparation of ultrapure ROS by zonal centrifugation procedures.

Significance to NEI Programs and Biomedical Research: Research on the visual pigment and outer segments would be greatly facilitated if an inexpensive, large scale method were available for the preparation of rod outer segments. This resource, once developed, would be made available to investigators involved in various studies dealing with this segment of the retina.

Proposed Course: It is expected that the project will be completed by June 1975.

Keyword Descriptors: research resource, rod outer segments

NEI Research Program: Retinal and Choroidal Diseases--Development, Structure, Function, and Degeneration (Visual Pigments, Photoreceptors and Visual Transduction Disorders)

Experimental Subject or Tissue Source: Bovine

Research Objective: Etiology