ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL EYE INSTITUTE
FISCAL YEAR 1974

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

ANNUAL REPORT

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NATIONAL EYE INSTITUTE

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ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1973 - June 31, 1974

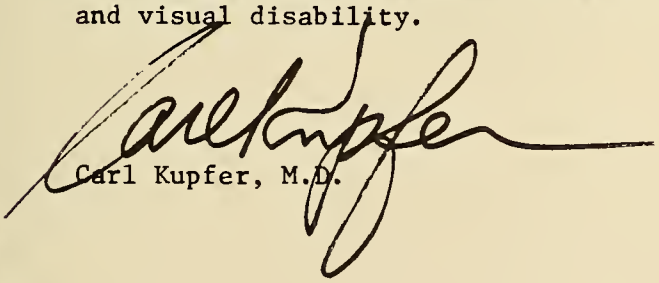
STATEMENT OF THE INSTITUTE DIRECTOR

Progress against leading causes of blindness and visual disability in the United States is highlighted in this report of research conducted and supported by the National Eye Institute during FY 1974.

Now in its fifth year, the NEI has formally begun to take stock of past accomplishments, assess the comprehensiveness and effectiveness of its current program, and document the requirements for the future development of vision research support. Preliminary analysis of three of its five major programs by a Program Planning Subcommittee of the National Advisory Eye Council has shown that although balance in these programs is generally good, there is need for greater emphasis in certain key areas of research in order to hasten the development of knowledge which can be applied to the improved prevention, diagnosis, and treatment of visual disorders.

In addition, the support of manpower training must be maintained and increased in order to sustain the high level of achievement that characterizes vision research in this country today. Without a consistent program of training support, the research momentum gained through the creation of the Eye Institute will be inevitably slowed.

Continued emphasis on the support of quality laboratory research, application of epidemiological techniques to eye disease problems, and stimulation of additional clinical research, particularly in retinal and choroidal disorders, will help provide the scientific base upon which more effective vision care and eye disease prevention programs can be built. In this manner, the National Eye Institute approaches its goal of reducing the enormous toll in human suffering and economic loss taken each year in our nation by blindness and visual disability.



Carl Kupfer, M.D.

INTRAMURAL RESEARCH

ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1973 - June 31, 1974

REPORT OF THE DIRECTOR OF INTRAMURAL RESEARCH

Carl Kupfer, M.D.

Although the National Eye Institute's Intramural Program will always constitute only a modest portion of the nation's total vision research effort, its continued development is crucial, not only for direct research activities, but for the evolution of NEI's total program. The expertise centered in the Clinical Branch and Laboratory of Vision Research, as well as the Office of Biometry and Epidemiology, serve as an invaluable resource for all Institute programs, providing guidance to extramural program management, program planning and analysis, and the Office of the Director. In general, the scientific direction of the Institute is greatly dependent upon this highly-regarded corps of professionals, many of whom are internationally known for their research contributions.

In a time of decreased Federal emphasis on research training, the intramural research facilities of the NEI can also serve the young investigator who desires experience in a stimulating intellectual atmosphere, as well as the senior vision research scientist who wishes to take advantage of the unique resources and opportunities for collaboration at the National Institutes of Health.

The Eye Institute has the only eye clinic in the United States where beds are allocated solely for the purpose of vision research and where there is such close and consistent collaboration between clinical and laboratory scientists. Its progress during the short time of Dr. Ballintine's administration has been noteworthy; yet, its needs remain critical. Because of limited staff there are no studies underway in two of the Institute's five major programs: cataract and corneal disease. Other programs are only minimally represented. In the coming year, major emphasis will continue to be placed on strengthening this vital program to further its development as a national resource and model for clinical vision research.

Clinical Branch

ANNUAL REPORT
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July 1, 1973 - June 30, 1974

REPORT OF THE CLINICAL DIRECTOR

Elmer J. Ballintine, M.D.

The Clinical Branch continued to develop its program for the study of ocular disease in patients by laboratory methods. Patients are admitted to the Clinical Branch only if they are appropriate for studies under one of the research plans. These research plans must meet the same standards of scientific validity that are imposed on non-human experiments and at the same time be ethically acceptable and incorporate appropriate safeguards for the patient's rights and welfare.

During the year a six member Protocol Review Committee, three of whose members were not physicians and three not part of the staff of the Clinical Branch, began a detailed review of all research protocols in the Clinical Branch for scientific merit and for ethical acceptability. Each investigator has had to revise his research plans to meet the requirements of the Committee. It is expected that additional review by Clinical Center agencies will be required in the near future.

The renovation of the outpatient service area was largely completed and we moved from our temporary quarters. The renovation of laboratories for tissue culture and for the study of retinal and choroidal biochemistry was completed. A completely renovated operating room, equipped for ocular microsurgery, vitreous surgery, and electroretinography was completed. Apparatus for performing B-scan ultrasonography of the eye and orbit was placed in operation. The equipping of a laboratory for neuro-ophthalmology was begun.

The staff of the Clinical Branch consists of four senior staff physicians, three physicians who are clinical associates, and one who is a staff associate. Two senior staff members are not physicians. Eight biologists and technicians and four secretaries support the staff. The commitment of the staff to research in the important categories of disabling and blinding eye disease is apparent in the individual research project reports.

In addition to the conduct of research on its research protocols, the Clinical Branch in collaboration with the Experimental Pathology Section of the National Eye Institute Laboratory of Vision Research examines histopathologically approximately 100 eyes per year, most of which come from the autopsy service of the Clinical Center. Consultations were furnished for 850 patients being cared for by other Institutes in the Clinical Center. There were 2,550 outpatient visits during the year, 150 admissions to the inpatient division, and 75 operations were performed.

The Clinical Branch continued to cooperate with other Institutes in the pursuit of unique research opportunities. The cooperative effort in the study of diabetic retinopathy as part of the study of diabetes among the Pima Indians by the Epidemiology and Field Studies Branch of the National Institute of Arthritis, Metabolism, and Digestive Disease (NIAMDD) was continued. A study of microangiopathy was undertaken among the group of patients with acromegaly being studied at the Clinical Center by NIAMDD. Other cooperative studies include the study of immunologic aspects of ocular malignant melanomas in cooperation with the Cellular and Tumor Immunology Section, Laboratory of Cell Biology, Division of Cancer Biology and Diagnosis of the National Cancer Institute, the study of Sjogren's syndrome among patients originally examined by the National Institute of Dental Research, and the planning of a study of the ocular effects of an anti-estrogen being used before treatment of breast cancer.

Serial No. NEI-73 CB 150(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ocular Hypertension Study

Previous Serial Number: None

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

Other Investigator: None

Cooperating Unit: None

Man Years:

Total:	0.7
Professional:	0.3
Others:	0.4

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 developed. Detailed procedures have 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 of the Project: It is expected that the project will continue for at least five years and we expect to enroll 100 subjects.

Honors and Awards: None

Publications: None

Serial No. NEI-73 CB 151(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Aqueous Humor Formation in Monkeys

Previous Serial Number: None

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
Others:	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 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: The methods for counting the isotopes, conducting the experiments, and the techniques for tapping the chambers of the eye have been developed and three monkeys have been tested.

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 of the Project: The project will continue according to the research planned.

Honors and Awards: None

Publications: None

Serial No. NEI-72 CB 038(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of Choroidal-Retinal Degenerative Diseases

Previous Serial Number: Same

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

Other Investigators: Muriel Kaiser, M.D.
Helga Kolb, Ph.D.

Cooperating Units:
1. NEI(I)-73 CB 142(c)
2. 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
3. NEI(I)-71 CB 006(c)
4. NEI(BE)-73-120

Man Years:
Total: 1.75
Professional: 1.0
Others: 0.75

Project Description:

Objectives: The objectives of this study are to properly classify, to further clinically define, and to search for new techniques which will elucidate the cause, prevention, or therapy of selected degenerative diseases. 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 is nearing completion. Surgical and electrophysiological studies in animals are continuing. Possible side effects of several drugs are being evaluated.

Major Findings: Approximately 300 patients were studied this year. At present the overwhelming majority of patients afflicted with choroidal and

retinal degenerative diseases are not curable. Nevertheless, most are helped by a combination of genetic counseling, 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 a year before ERG or fundus abnormalities are detectable. Cell-mediated immunity studies have demonstrated abnormal ability of lymphocytes from patients with pigmentary retinal degenerations to destroy retinoblastoma cell lines in tissue culture.

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 of the Project: Emphasis is being placed on earlier detection and standardization of technique to improve the accuracy of longitudinal studies and therapeutic trials. Animal and cell culture experiments are being undertaken to study the biological mechanisms which fail in degenerative diseases of the retina and choroid.

Honors and Awards: None

Publications:

Bergsma, D.R.: Ophthalmic aspects of Usher's Syndrome. Public Service Programs, Gallaudet College, Washington, D.C.: Symposium on Usher's Syndrome, 1973 (in press).

Char, D.H., Bergsma, D.R., Rabson, A.S., Albert, D.M., and Herberman, R.B.: Cell-mediated immunity to retinal antigens in patients with pigmentary retinal degenerations. Invest. Ophthal. 13: 189-203, 1974.

Serial No. NEI-72 CB 039(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of Ophthalmic Familial and Genetic Diseases

Previous Serial Number: Same

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

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

Cooperating Units: None

Man Years:

Total:	1.4
Professional:	1.0
Others:	0.4

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 intracranial 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 counseling. Cellular mechanisms were studied where possible. In the example above, lymphocytes from patients with primary open angle glaucoma and age matched controls are pre-incubated 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 one hundred and fifty 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 counseling, and advice regarding prognosis and rehabilitation.

Evidence for extreme variation of some inherited diseases within families has been documented.

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 counseling, etc. around the patient's problem. Discovery of biological markers and mechanisms at the cellular could provide means of prevention or therapy which cannot be obtained with clinical examination alone.

Proposed Course of Project: Clinical characterization of diseases and therapeutic trials will be continued. Tissue culture facilities are now available to study relevant cellular mechanisms.

Honors and Awards: None

Publications:

Bergsma, D.R. and Kaiser-Kupfer, M.: A new form of albinism. Amer. J. Ophthal., (in press).

Serial No. NEI-73 CB
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Development of Improved Instrumentation for Vitreous Surgery

Previous Serial Number: None

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

Others Investigators: Charles J. McCarthy

Cooperating Units: Biomedical Engineering Section, CC

Man Years:
Total: .5
Professional: .5
Others: 0

Project Description:

Objectives: The objectives are to study the methods by which the vitreous can be surgically manipulated and to develop safer and more efficacious instrumentation for vitreous surgery.

Methods Employed: As surgical problem areas are discovered in the clinical trial program, they are discussed with engineers in the biomedical engineering section of the NIH. From these discussions prototype instruments are fabricated and then evaluated in animals followed by clinical trial. Engineers have studied the surgical movies, observed in the operating room, and participated in animal surgery.

The vitreous instrumentation project can be divided into five principal areas: 1. development of a microscope positioning system, 2. development of ultrasonically driven vitreous cutters, 3. development of devices for mechanical control of suction force at the time of vitrectomy, 4. development of intravitreal treatment modalities, (light coagulation, cryothermy, and bipolar cautery) and 5. development of better devices to localize, apply cryopexy, and drain subretinal fluid at the time of retinal-vitreous surgery.

Major Findings: A three axis chin-operated switch has been designed, constructed, and clinically evaluated to permit control of the position of the operating microscope along three axes. This switch provides a simultaneous three-axis microscope movement without shifting the operator's

hand-body position, thereby greatly decreasing microscope positioning time, and spurious hand movement.

A hand-operated mechanical stringe drive has been designed, constructed, and clinically evaluated for providing the suction force to imbricate vitreous into the cutting port of a vitrectomy instrument. This offers better control, one hand operation, simplicity, and decreased operator fatigue over the present syringe suction method.

A head-wrist support system has been constructed and evaluated. This offers greater stability of the patient's head position and the surgeon's relative hand position.

Several prototypes of an ultrasonically driven vitrectomy device has been constructed and evaluated in animal eyes. None of these instruments offers any significant advantage over present vitrectomy machines, and all had the added problem of ultrasonically produced metal fatigue. This project is, therefore, suspended pending further developments.

Devices are also in the design stage which would permit drainage of subretinal fluid under ophthalmoscopic control, scleral marking under ophthalmoscopic control, and safer introduction of intraocular gases.

Significance to Biomedical Research and the Program of the Institute: Vitreous surgery is now being performed in many hospitals and centers throughout the United States. As surgical competence increases, surgeons are undertaking vitrectomies on eyes with ever increasing vision. It is possible that prophylactic vitrectomy may be evaluated in diabetes. The increasing use of this new technique especially in better vision eyes makes development of the safest possible instrumentation imperative. The combination of clinical trials with excellent biomedical engineering support is unique at the NIH and has resulted in several significant advances in the safety of vitreous surgery.

Proposed Course of the Project: The instruments that are in the design stage will be evaluated in animals and clinically when the prototypes are constructed. In addition, an attempt will be made to utilize direct pressure recording and high speed movies to further our understanding of the precise mechanisms in which vitreous may be cut and removed from the eye.

Honors and Awards: None

Publications: None

Serial No. NEI-73 CB 155
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

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

Previous Serial Number: None

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

Other Investigators: Ralph Helmsen, Ph.D.

Cooperating Units: None

Man Years:

Total	2.0
Professional:	2.0
Others:	0.0

Project Description:

Objectives: The objectives of this study are: 1. to search for and identify a vasoproliferative factor in the vitreous of patients with vasoproliferative diseases (e.g. diabetes mellitus, sickle cell disease, Ealse disease, central retinal vein occlusion, retrolental fibroplasia etc.), 2. search for factors involved with proliferaton of endogeneous cells in the condition of massive vitreous retraction and massive periretinal proliferation.

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. Vitreous from patients with retinal detachment and massive vitreous retraction is studied morphologically and with tissue culture techniques.

Major Findings: The major thrust of our protocol to date has been to search for vasoproliferative factors in the vitreous of patients with vasoproliferative disease. The nature of the project is that of a search mission to find a substance of unknown chemical characteristics with only one known biological activity--the production of endothelial mitosis and subsequent vasoproliferation. This task requires investigation of and modification of multiple bioassay systems. Only four months have passed since the first

vitrectomy was performed at the National Eye Institute in November 1973. In this brief time we have not yet achieved the primary goal of the vitrectomy project; i.e. to demonstrate the existence of an intraocular vasoproliferative factor. We are at the present time still evaluating several bioassay systems to determine which one is best suited to our purposes.

Attempts are underway to grow cells obtained from the vitreous of patients with preretinal proliferation and retinal detachment using existing tissue culture systems.

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.

Massive vitreous retraction and massive preretinal proliferation are responsible for most of the failures in retinal detachment surgery. The pathogenesis of this condition is not understood. It is believed by some that retinal pigment epithelial cells proliferate on the surface of the retina and cause contraction of the retina, stiffening of the folds, and inoperable redetachment of the retina. This again is an instance in which proliferation of tissues normally found in the eye can cause severe damage to the eye. It is obvious that understanding this serious intraocular disease is of prime importance in order to reduce the failure rate in retinal detachment surgery.

Proposed Course of the Project: It is intended to continue on the current protocol as outlined above in order to find factors causing vasoproliferation in the eye as well as the pathogenesis of massive preretinal and vitreous retraction. We plan to do this in the coming year. Some effort will also be made to understand the long-term effects of vitrectomy by studying this in rhesus monkeys.

Honors and Awards: None

Publications: None

Serial No. NEI-73 CB 152(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Inheritance of Susceptibility to Lymphocyte Transformation Inhibition

Principal Investigator: Kenneth Foon, M.D.

Other Investigator: Elmer J. Ballintine, M.D.
Carl Kupfer, M.D.

Cooperating Units: None

Man Years:

Total:	1.25
Professional:	1.25
Others:	0.00

Project Description:

Objectives: Other investigators have shown that blastic transformation of a patient's lymphocytes induced by photohemagglutinin can be inhibited by gluco-corticoids. The sensitivity to this inhibition may be related to whether the patient has glaucoma. It may also be inherited according to a simple genetic pattern and it is possible that the sensitivity may be determined by still other factors. In this study the sensitivity to inhibition of lymphocyte transformation is being determined in patients with a variety of ocular diseases: mainly diabetes and glaucoma, and in their relatives. Correlation with the patient's disease and with possible genetic inheritance patterns will be sought.

Methods Employed: Standard techniques for measuring lymphocyte transformation and inhibition in operation. Lymphocytes are obtained from patients admitted to the services of the Clinical Branch and from their relatives.

Major Findings: No simple correlation with the presence or absence of glaucoma has been found in the few patients so far examined. It has been demonstrated that it is practical to test adequate numbers of patients using methods so far developed.

Significance to Biomedical Research and the Program of the Institute: If a direct correlation with glaucoma can be established, this will serve as a clue to the cellular basis for simple glaucoma. A demonstration that some feature of the process of lymphocyte transformation is genetically

determined would be a point of departure for investigating immunologic processes in other ocular diseases which seem to have a familial distribution.

Proposed Course of the Project: Patients and their families will continue to be examined according to research plans.

Honors and Awards: None

Publications: None

Serial No. NEI-73 CB 140(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Argon Laser Photocoagulation of Retinal and Choroidal Diseases

Previous Serial Number: Same

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 intends to carry out a controlled trial of argon laser photocoagulation using specific treatment protocols for patients with several diseases of the retinal and choroidal vasculature and of the macula. The untreated controls will define the natural history of the disease processes, some of which are incompletely known. All patients undergo an exhaustive evaluation before treatment and during the followup period, through which it is hoped to gain considerably more clinical information about treated and untreated individuals with these diseases than is possible in the usual ophthalmic clinic setting. Finally, a number of ancillary clinical and laboratory studies are planned when a sufficient number of patients in various disease categories are enrolled in this program, which, it is hoped, will yield information on the pathogenesis of these conditions.

Methods Employed: Both treated and untreated patients have thorough initial eye examinations which are repeated at appropriate intervals in followup. Frequent and extensive stereoscopic fundus photographs and fluorescein angiograms are used for documentation, and special emphasis is placed on such physiological parameters as visual acuity, visual fields, and electrophysiological determinations (electroretinography, electrooculography). Patients in each disease category are assigned to treatment or control groups on a random basis. Patients with binocular disease which is considered treatable in each eye under the protocol are treated in one eye only, chosen at random.

Major Findings: Major conclusions in a study of this sort will of necessity not be available for some years, which is the time estimated to accumulate large enough numbers of patients in each disease group with sufficient followup of treated and untreated individuals to determine the effectiveness of the treatment. At the present time only about 50 patients have been treated, of whom the largest number (approximately 35) have diabetic retinopathy but were ineligible for the nationwide Cooperative Study. Preliminary impressions include the following: (1) Patients with symmetrical, proliferative diabetic retinopathy treated in one eye show marked reduction in neovascularization in the treated eye by comparison with the untreated control. Followup thus far has been too short to observe any marked differences in visual acuity in treated and control eyes, but thus far the only eyes to sustain major hemorrhages have been in the untreated group. (2) Patients who have lost one eye to proliferative diabetic retinopathy and have significant proliferation in the other eye are in a particularly high risk group for further, early devastating visual loss in the remaining eye. Although followup is still too brief for definitive conclusions, all treated patients in this group appear to have a regression in their proliferative retinopathy and a cessation of hemorrhagic activity. (3) Background diabetic retinopathy has shown a less dramatic response to treatment. Thus far, no marked difference has been observed between treated and untreated eyes in this group. (4) Patients with retinal neovascularization from any cause have frequently required retreatment, even when the initial, massive photocoagulation sessions have caused disappearance of all of the new vessels evident at that time. A preliminary impression from this work is that "peripheral ablation" of large areas of retina, as applied in the Cooperative Study, does not uniformly cause regression of new vessels at sites distant from the treated areas, and often further new vessels are seen to arise months after treatment at sites where they had not been apparent previously. The treatment protocol for retinal neovascularization in this study is, therefore, to combine direct treatment to sites of neovascularization with "peripheral ablation" on each patient. (5) Following the extensive treatment described above, no patient has lost central visual acuity. However, visual field changes have been observed in all treated eyes, ranging from mild to moderate contraction of the field to nerve fiber bundle defects and extensive field contraction with resultant "tunnel vision". Electroretinographic b-wave amplitudes have declined in these eyes following treatment, indicating extensive retinal destruction. (6) Treatment of macular diseases with subretinal neovascularization has thus far demonstrated the need for very heavy photocoagulation of these areas, since otherwise the vessels not only remain viable but their growth may actually be stimulated.

Significance to Biomedical Research and the Program of the Institute: This study continues to investigate a potentially useful treatment modality for a group of hitherto untreatable ocular diseases. In addition, it will provide extensive clinical information on these diseases and will gather a group of patients for a variety of planned investigations dealing with the pathogenesis of several forms of retinal and choroidal vascular disease.

Proposed Course of the Project: Continued enlargement of the number of patients enrolled in this study is highly important, and it is hoped to add other investigators and cooperating institutions, since such a project requires many patients and lengthy followup to reach meaningful conclusions. Several ancillary projects are being planned, including studies of platelet aggregation in relation to duration of clinical diabetes and presence of retinopathy and other vascular complications; capillary fragility in diabetes and its relation to capillary basement membrane abnormalities; anatomical studies of vascular basement membranes in diabetic and normal eyes, with the eventual hope of doing biochemical studies of ocular blood vessel basement membranes; and metabolic studies of various regions of the neural retina and pigment eptihelium with relation to their relative susceptibility to disease.

Honors and Awards: Lecturer in Retinal Physiology, The Wilmer Ophthalmological Institute, The Johns Hopkins University School of Medicine, November, 1973-January, 1974; visiting professor of ophthalmology, University of Tennessee College of Medicine, May, 1974, invited seminar lecturer, Retina Foundation, Boston, Mass., September, 1973 and the Jules Stein Eye Institute, Los Angeles, Calif., November, 1973; invited contributor to Ophthalmic Knowledge Assessment Program examination.

Publications:

Chumbley, L. C. and Frank, R. N.: Central serous retinopathy and pregnancy. Amer. J. Ophthal. 77: 158-160, 1974.

Frank, R. N.: Diabetic retinopathy. Ryan, S. J., Jr. (Ed.) The Eye and Systemic Disease. New York, Grune & Stratton, 1974 (in press).

Frank, R. N.: Argon laser photocoagulation and subretinal neovascularization. Ophthal. Surg. (in press)

Serial No. NEI-74 CB 141(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemistry of Vertebrate Retinal Receptor Outer Segments

Previous Serial Number: Same

Principal Investigator: Robert N. Frank, 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:	0.8
Professional:	0.8
Others:	0.0

Project Description:

Objectives: This project attempts to characterize the biochemical properties and function of the visual pigments in vertebrate visual cell outer segments, and to study the relationship of the visual pigments with other molecules also present in the outer segments. Of particular interest has been the finding, initially reported from this laboratory, that rhodopsin in vitro is phosphorylated by the terminal phosphate group of ATP and that this reaction is stimulated approximately twofold in the light. Studies are underway to determine if this reaction occurs in vivo, and if so to quantitate its extent and reversibility under various conditions of dark and light-adaptation, and of most importance, to determine its role in the physiology of vision.

Methods Employed: Techniques of isolating retinal photoreceptor outer segments, of radioactive labeling with $AT^{32}P$, and of polyacrylamide gel electrophoresis were described in last year's Annual Report. The most significant new technique added to this study has been the use of neutron activation analysis to measure quantitatively extremely small levels of phosphorus (in the tenth-nanomolar range) bound to rhodopsin protein. Samples of polyacrylamide gel containing rhodopsin protein are bombarded by a neutron beam in the National Bureau of Standards atomic pile. Inorganic phosphorus is converted to radioactive phosphorus-32 by this procedure, and

the isotope thus formed is measured by standard scintillation counting methods.

Major Findings: Light-sensitive phosphorylation of rhodopsin by ATP, described in last year's Annual Report, appears to be due to a specific enzyme (protein kinase) present in retinal photoreceptor outer segments. This enzyme apparently cannot be replaced by protein kinases from other tissues. The kinase is distinct from the visual pigment itself, and the light activation is apparently caused by conformational changes in the phosphate binding site on the rhodopsin molecule, rather than by light activation of the kinase itself.

Thus far, this reaction has been described only in vitro, and it will be important to learn if it takes place in intact animals. Studies on this point are now under way in collaboration with Dr. William Robinson. These will attempt to learn if rhodopsin phosphorylation occurs in vivo, and to quantitate precisely its extent in relation to the amount of rhodopsin which has absorbed light photons. Preliminary work has focused on finding precise quantitative measures of protein-bound phosphorus and of purified rhodopsin protein itself in the nanomolar range using polyacrylamide gel electrophoresis, which is the only method capable of separating rhodopsin from phospholipids and other membrane polypeptides, each of which, if present, could seriously hinder the interpretation of experimental results. Of the methods tested, neutron activation analysis is the best for phosphorus determination, and amino acid analysis of hydrolyzed gel slices is best for protein quantitation.

A detailed study of the properties of rhodopsin in polyacrylamide gel electrophoresis using the detergent sodium dodecyl sulfate (SDS) has recently been completed and is being prepared for publication. Experimental data accumulated in this study were analyzed using computer programs developed by Dr. David Rodbard of NICHD. Among the results of this study, it was shown that rhodopsin migrates "anomalously" in the gel electrophoretic system, and therefore that previous determinations of molecular weight and other molecular properties of this protein using this method must be interpreted with caution. In addition to the data on rhodopsin, this study yielded information on the behavior of a variety of proteins in SDS-polyacrylamide gel electrophoresis which should extend our knowledge of how this widely used technique works.

Significance to Biomedical Research and the Program of the Institute: These studies attempt to learn the properties and physiological function of a reaction (light-activated rhodopsin phosphorylation) that appears to have considerable significance for our understanding of the visual process. More generally, these studies should help to learn more about the biochemistry of the rhodopsin molecule, which has importance not only for visual scientists, but for all of those who are interested in the structure and function of membrane proteins. This basic biochemical and physiological knowledge is essential for our understanding of the normal visual process. without which the study of disease becomes enormously more difficult.

Proposed Course of the Project: Studies of in vivo phosphorylation in collaboration with Dr. Robinson will be continued.

Recent work has suggested a possible physiological function for the light-activated visual pigment phosphorylation, which has heretofore been puzzling because the reaction is too slow to play an important role in the initiation of the visual impulse. Experiments to test this hypothesis will be carried out in collaboration with Dr. Gerald Chader and his associates of the Laboratory of Vision Research, NEI.

Honors and Awards: None

Publications:

Frank, R. N. and Bensinger, R. E.: Rhodopsin and light-sensitive protein kinase activity of retinal outer segments. Exp. Eye Res. (in press).

Serial No. NEI-73 CB 143(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

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

Previous Serial Number: Same

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

Other Investigators: Barbara Rollings, R.N.
Rachael Thrasher, R.N.

Cooperating Units: Nuclear Medicine Department, CC
Radiopharmacy, CC

Man Years:

Total:	0.8
Professional:	0.5
Others:	0.3

Project Description:

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

Methods Employed: The patients included in this study are those referred for evaluation of pigmented lesions of the eye resembling malignant melanoma or patients in whom the ocular media is opaque. A thorough systemic evaluation is carried out for evidence of primary or metastatic disease. After the patient gives informed consent to receive an investigational, new drug,baseline gamma counting over the eyes, forehead, thyroid and liver are performed and the patient receives five hundred microcuries of radioiodinated (I-125) chloroquine analog orally. Gamma activity is monitored over the previously mentioned sites at one day, three days, seven days, and weekly thereafter until a total of 28 days has elapsed. Each site is counted for a minimum of three, one minute periods. The data from the ocular counts is computerized and a plot generated of the individual eyes,radioactivity versus time and days. The range of counts at the time of each assessment is displayed on the graph. Differences are considered of significance whenever the range of counting for the two eyes does not overlap.

Major Findings: To date studies have been completed in 26 patients. Nine of these have had verified malignant melanomas. Of seven verified malignant melanomas of the choroid, four have had a significantly higher amount of radioactivity in the eye bearing the melanoma than in the normal eye. Three eyes with subsequently verified malignant melanoma have had radioactivity equal to the normal eye in the same patient. The counts in an eye with a small iris melanoma were equal to the counts in the normal fellow eye. The counts over an eye with acquired melanosis with malignant degeneration were higher than the counts in the normal fellow eye of the same patient. Five patients with lesions suspicious of malignant melanoma have had counts equal in their two eyes. One patient with a lesion suspected of being a choroidal hemangioma has had counts equal in her two eyes. Two patients with monocular metastatic breast adenocarcinoma have had counts equal in their two eyes. Of eight patients in whom the lesions observed are thought to be benign pigmented lesions seven have had counts equal in the two eyes. One patient with a posterior pole densely pigmented lesion thought to be a nevus, but elevated, had counts higher in the lesion-bearing eye than in the normal eye. The patients with suspicious lesions and some of the patients with what are thought to be benign lesions continue under intermittant observation. In one patient with a suspicious lesion enucleation has been advised but refused. In another patient with a suspicious lesion the referring physician has reported that the advised enucleation will be performed in the near future.

Significance to Biomedical Research and the Program of the Institute: Based upon the patients studied to date it appears that the I-125 chloroquine analog offers some aid as a useful test to differentiate patients with a diagnosis of intraocular melanoma from other patients with benign lesions or with metastatic disease. To date there have been no verified false positive tests. The use of this test is not the final word in the differential diagnosis of malignant melanoma inasmuch as there have been three false negative tests to date.

This test has the advantage, over other radioactive testing used in the diagnosis of intraocular melanoma, of employing a gamma radiation emitter as the tracer isotope. This allows radioactivity monitoring without surgical incision of the ocular tissue.

Proposed Course of the Project: Patients will continue to be enrolled in this study during the next year.

Honors and Awards: None

Publications: None

Serial No. NEI-73 CB 154(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Experimental Glaucoma in the Rhesus Monkey

Previous Serial Number: None

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
Others: 0.0

Project Description:

Objectives: 1. To assess the possibility of inducing in the eye of normal rhesus monkeys an experimental glaucoma by repeated circumferential photocoagulation of the recess of the anterior chamber angle. 2. Should the first objective prove successful, to study in detail retinal and optic nerve function in glaucoma eyes and to compare findings to observations in control eyes. 3. To study effects of chronic elevation of intraocular pressure upon formation of aqueous humor.

Methods Employed: The first objective has been under study. In one rhesus monkey a single circumferential treatment of the right anterior chamber angle structures was done with the argon laser photocoagulator. In five additional monkeys from 2-4 circumferential treatments of the anterior chamber angle structures were carried out. In these five monkeys both eyes were treated. The criteria used to determine when retreatment would be done were: 1. there was no clinical inflammation of the eye for a two week period of time, and 2. the intraocular pressure remained normal. In three of the ten eyes of the five monkeys retreatment was stopped after the second session despite no elevation of intraocular pressure being present. Each treatment session consists of application of approximately 200 confluent spots of argon laser energy with a 50 micron beam diameter, a 0.2-0.5 second duration, and a 0.4-0.8 watt energy level to the mid trabecular meshwork. The five animals which have received bilateral treatment and the one control animal with a singular monocular treatment have been followed for periods of time varying from four weeks up to six months after the time of their last

treatment. The control animal and two of the treated animals have been sacrificed for histopathologic studies. All six animals had in vivo anterior chamber perfusion, using a constant pressure method, at a period of time from four to 12 weeks after the end of the last treatment session.

An additional two monkeys have had treatment on two occasions to the right eye only. These monkeys have entered the treatment protocol and additional treatments will be carried out when the criteria for retreatment are met.

Major Findings: Two to four circumferential confluent treatments of the trabecular meshwork area of the anterior chamber angle were sufficient to induce an elevation of intraocular pressure within two weeks in seven of ten eyes. Of the remaining three eyes, within six months one has developed a sustained elevation of intraocular pressure. In all treated eyes the coefficient of aqueous outflow was greatly reduced. In the eye with one circumferential treatment the coefficient of outflow was normal, as it was in the untreated control eye. Six of the seven eyes with early onset sustained elevation of intraocular pressure developed clinical cupping of the optic nerve head. Histopathologic examination of four of these eyes and of the two eyes from the control animal demonstrated cupping of the optic nerve head with posterior bowing of the lamina cribrosa, selective loss of retinal ganglion cells in the perifoveal region, and obliteration of the canal of Schlemm, with localized scarring in the anterior chamber angle. Other structures of the eye are not primarily affected.

The time course of the elevated intraocular pressure is somewhat erratic. The pressure becomes elevated and will stay so for 4-6 weeks whereupon it is observed that in some animals it drops to normal for one to two weeks before again becoming elevated.

Significance to Biomedical Research and the Program of the Institute: There is available at present no experimental glaucoma in any animal wherein the elevation of intraocular pressure is sustained long enough to cause cupping of the optic nerve head and selective loss of retinal ganglion cells, and wherein no generalized damage is done to the eye in producing the glaucoma. Thus, none of the previously available experimental glaucomas allow meaningful, in depth, studies of the physiology and pharmacology of retina, optic nerve and ciliary body function in the presence of elevated intraocular pressure. It appears that this new experimental glaucoma will allow these studies to be conducted.

Proposed Course of the Project: Experimental glaucoma will be induced in one of the two eyes of additional experimental animals. These will then be used for controlled studies of the effects of elevated intraocular pressure upon the function of the eye.

Honors and Awards: None

Publications:

Gaasterland, D. and Kupfer, C.: Experimental glaucoma in the rhesus monkey. Invest. Ophthal. (in press).

Gaasterland, D. and Kupfer, C.: Experimental glaucoma in rhesus monkeys, presented at the 33rd Clinical Meeting of the Residents Association of the Wilmer Ophthalmological Institute, Johns Hopkins Hospital and University, April 19, 1974.

Serial No. NEI-71 CB 006(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

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

Previous Serial Number: Same

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
Others:	0.1

Project Description:

Objectives: Broad objectives include the application of current procedures for psychophysical tests, improving their form and scope and enhancing the usefulness of any ophthalmic instruments for clinical work. A primary aim is to replace or confirm purely subjective data by an approach to objectivity wherever possible. In an effort to standardize reporting we hope for general acceptance of any techniques which originate here and are found to be consistently useful and practical.

Methods Employed: Appropriate instruments are designed, constructed and standardized to test visual functions in various clinical and research projects. Psychophysical, electrophysical and other ophthalmic tests are conducted on appropriate patients. Findings are discussed, reported and entered in patient's medical records. For example, since the initiation of surgical vitrectomy in selected patients having no useful vision, the measurement of dark-adapted visual thresholds in four quadrants is being tried as an aid in evaluating visual ability, potential and improvement pre- and post-operatively.

An apparatus for performing electro-oculography has been designed and constructed, which when used with a commercial recording system has been found to be quite satisfactory.

Several variations in gonioprism design have been made for use in laser work with monkeys.

Major Findings: Psychophysical tests on 425 patients have been helpful in diagnostic or follow-up studies of degenerative and toxic retinopathies. The electro-oculogram (EOG) has been added to our battery of available diagnostic tests.

Eye-movement records have been used in a number of neurological and pharmacological studies.

Some decisions have been made regarding the preferred type of gonioprism for use with monkeys.

In summary, the data generated by the instruments and methods for visual examination contribute to the other research projects described for the Clinical Branch, NEI.

Significance to Biomedical Research and the Program of the Institute: Functional testing of patients and the improvement of procedures and instrumentation contributes not only to the ongoing clinical program, but occasionally suggests or stimulates research projects.

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

Honors and Awards: None

Publications: None

Serial No. NEI-73 CB 142(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Anatomy and Pathology of the Mammalian Retina

Previous Serial Number: Same

Principal Investigator: Helga Kolb, Ph.D.

Other Investigators: Donald R. Bergsma, M.D.
Peter Gouras, M.D.
David Huxoll, D.V.M.*
Edward V. Famiglietti, Ph.D., M.D.
Ralph Nelson, Ph.D.
Ralph Rosenthal, M.D.**

Cooperating Units: Walter Reed Army Medical Hospital, Washington, D.C.
Stanford Medical School

Man Years:

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives: To understand the neural circuitry of the mammalian retina.

Methods Employed: Light and electron microscopy. Golgi silver impregnation methods. In addition ophthalmoscopy, fluorescein angiography, and electroretinography for chloroquine study.

Major Findings: Chloroquine retinopathy: Rhesus monkeys have been injected intramuscularly with chloroquine hydrochloride daily for periods up to three years. No further degeneration has been observed than that reported after one year of chloroquine, i.e. the retinal neurons are accumulating membranous cytoplasmic bodies (MCBs).

Ophthalmoscopy, fluorescein angiography and electroretinography are still within normal limits for animals having undergone 2 1/2 years of chloroquine therapy.

Electronmicroscopy of the inner plexiform layer of the cat retina: Improved ultrastructural preservation and serial section techniques have

allowed positive identification of nerve processes in the inner plexiform layer. Cone bipolars synapse directly on ganglion cells whereas rod bipolars do not. The rod system apparently uses amacrine pathways to get information to ganglion cells. One of these pathways has been identified as being via a small, diffuse, functionally bistratified amacrine cell.

Significance to Biomedical Research and the Program of the Institute: Detailed studies on the "wiring" of the neurons within the retina are essential for our understanding of the visual system.

Proposed Course of the Project: The projects have been terminated.

Honors and Awards: None

Publications:

Famiglietti, E.V. and Kolb, H.: Gap junctions and two varieties of amacrine cells in the retina of the cat. Anat. Res. 178: 353, 1974.

Famiglietti, E.V. and Kolb, H.: Synapses in the retina of the cat. Soc. for Neuroscience, 3rd Annual Meeting.

Kolb, H.: The connections between horizontal cells and photoreceptors in the retina of the cat: electronmicroscopy of Golgi preparations. J. Comp. Neurol. (in press).

Kolb, H. and Gouras, P.: Electronmicroscopic observations of human retinitis pigmentosa; dominantly inherited. Invest. Ophthal. (in press).

Kolb, H. and Famiglietti, E.V.: Rod and Cone bipolar cell connections in the inner plexiform layer of the cat retina. Science (in press).

Serial No. NEI-71 CB 030(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of Parameters of Intraocular Pressure

Previous Serial Number: Same

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

Other Investigators: Karyn Ross
Lessie McCain

Cooperating Units: Normal Volunteer Office, CC
Pharmaceutical Development Services, NIH
Biomedical Engineering and Instrumentation Branch, DRS, NIH

Man Years:

Total:	2.3
Professional:	1.3
Others:	1.0

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 effects upon the parameters of both acute and chronic administration of medications which might be used in the treatment of glaucoma.

Methods Employed: Eight parameters--intraocular pressure, episcleral venous pressure, total facility of outflow, true facility, pseudofacility, aqueous humor flow, P_k of Goldmann, and the ocular rigidity are determined before and after medication is given topically to one eye. This allows assessment of acute effects of medication. Replicate measurements on sophisticated subjects are made. In addition, chronic studies of the effects of topical dexamethosone 0.1% given four times a day to one eye with the other eye being an untreated control have been initiated. Also, attention has been directed at expanding the volume of baseline data available regarding the values of parameters in older normal subjects.

Major Findings: The intraocular pressure in older normal subjects is essentially the same as in younger normal subjects. A significantly lower true facility of outflow in older subjects is counterbalanced by a significantly lower calculated rate of aqueous flow. Pseudofacility, the amount of pressure dependence of aqueous inflow, apparently does not change with aging in normal subjects.

Topical dexamethasone in most subjects causes a rise of aqueous inflow of small magnitude. This declines in conjunction with a decline of the true facility of outflow. In some subjects, particularly those who are high responders, there is the early onset of a significantly higher inflow of aqueous humor which is persistent. In association, the true facility of outflow slowly declines and the intraocular pressure rises. Whether this observation will hold for all subjects who have a high response of the intraocular pressure to topical steroids remains to be tested.

Dose response studies of the effects of monocular topical epinephrine in young normal subjects have been undertaken. In a preliminary assessment it appears that low doses of epinephrine cause a reduction of the aqueous inflow without affecting pseudofacility. This is similar to the effects of topical isoproterenol, a beta adrenergic agonist. At higher dosage levels topical epinephrine causes a reduction in pseudofacility, as previously reported.

Expansion of studies in glaucoma patients has been hampered by a technical difficulty. First, in patients receiving medications chronically the acute affects of the same or different medications are hard to assess. Second, in glaucoma patients off medications, the intraocular pressure is often at a high level. A frequent observation is that in these patients it is possible to obtain a measurement of intraocular pressure and episcleral venous pressure, but when a pediatric blood pressure cuff is inflated around their neck, the intraocular pressure either does not change, or falls, with the rise of episcleral venous pressure that develops. The explanation of this observation awaits further study.

A study of the effects of chronic atropinization upon the acute response to topical antiglaucoma medications has been completed. The method employed was atropinization of one eye chronically, and study of both eyes before and after topical pilocarpine, isoproterenol, and systemic actazolamide. The results indicate that in young subjects chronic atropinization does not affect any parameter of intraocular pressure. However, chronic atropinization completely inhibits changes of parameters of intraocular pressure induced by pilocarpine, partially inhibits the effects of isoproterenol upon aqueous flow, and has no affect upon the changes induced in parameters of intraocular pressure by systemic acetazolamide.

Significance to Biomedical Research and the Program of the Institute: Study of the patterns of alteration of the parameters of intraocular pressure allows a clearer interpretation of the nature of the mechanisms of action of

the pharmacologic agents used to treat glaucoma. This allows identification of desirable and undesirable properties of various pharmacologic agents, and will hopefully allow development of agents having only desirable properties.

Proposed Course of Project: This project will continue with extension of several of the outlined studies and with continued emphasis upon studies in glaucomatous patients and in subjects with normal eyes.

Honors and Awards: None

Publications:

Gaasterland, D., Kupfer, C., and Ross, K.: Pilocarpine induced changes and parameters of intraocular pressure. Presented at the Association for Research in Vision and Ophthalmology meeting, Sarasota, Florida, May 5, 1973.

Serial No. NEI-71 CB 013(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

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

Previous Serial Number: Same

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

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.0
Others:	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 to possibly 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.

Major Findings: Using pharmacologic and physiologic methods, a schema has been developed to explain some mechanisms by which aqueous humor is formed. The mechanism of drug inhibition of aqueous humor formation has also been clarified.

Pre-ganglionic stimulation of the ciliary ganglion produces a marked increase in the rate of aqueous humor formation. The post-ganglionic fibers of this extra-ocular ganglion synapse, intraocularly, at sites which pharmacologically

also behave as ganglia and have been labelled as "E-2" sites. The neuronal end of this neurogenic pathway is adrenergic in nature, i.e. it produces the release of norepinephrine for the end response. The data accumulated to date indicates that electrical stimulation of the ciliary ganglion or pharmacologic stimulation of "E-2" sites produces a vasoconstriction of efferent blood vessels of the ciliary processes to decrease blood flow and to increase ultrafiltration.

L-epinephrine, d,l-isoproterenol, pilocarpine, acetazolamide and ouabain have all been demonstrated to decrease the rate of aqueous humor formation by an agonistic action on a second set of intraocular ganglion-like receptors identified as "E-1" sites. The end response is adrenergic in nature. Blood and aqueous humor flow data would indicate that the inhibitory responses of these agents on aqueous humor formations is via a vasoconstriction of afferent blood vessels of the ciliary processes, to cause a decrease in the rate of ultrafiltration. The data appears quite conclusive that the actions of ouabain and acetazolamide are independent of their known enzymic activity.

It is yet to be shown that E-1 sites are an integral part of a physiological nervous pathway as has been demonstrated with the E-2 sites.

In a background study for the possible future use of anesthetized monkeys in this research, the effects of the anesthetic agents on aqueous formation was investigated. It was found that pentobarbital Na decreased the rate of aqueous humor formation by 40% when compared to phencyclidine alone or with the combination of phencyclidine plus urethane.

Significance to Biomedical Research and the Program of the Institute: It is very important that secondary sites for drug action have been uncovered. Further studies regarding the sensitivity of these sites to various pharmacologic agents could well lead to more effective treatment of glaucoma or ocular hypotony.

The findings also clarify the apparent paradoxes of unlike pharmacologic agents (sympathetic and p-sympathetic) producing similar responses on aqueous humor formation and outflow.

The results obtained in this study also strongly suggest a central mechanism for the control of intraocular pressure.

Proposed Course of Project: Further studies will be made using ouabain and acetazolamide to determine with greater certainty the concept presented here for their mechanism of action.

Retrobulbar sympathetic nerves will be studied to determine if E-1 receptor sites represent a link in the nervous pathway.

It is planned to study the role of E-1 and E-2 receptor stimulation on the facility of aqueous humor outflow, using the method of Sears.

In a modest way, it is planned to screen prototypes of various autonomic drugs for their ability to stimulate "E-1" and "E-2" receptors.

Honors and Awards: None

Publications:

Macri, F.J. and Cevario, S.J.: The induction of aqueous humor formation by the use of Ach + Eserine. Invest. Ophthal. 12: 910-916, 1973.

Macri, F.J. and Cevario, S.J.: The arterial pressure dependency of the increased aqueous humor formation induced by Ach + Eserine. Invest. Ophthal. 13: 153-155, 1974.

Macri, F.J. and Cevario, S.J.: A pharmacodynamic study of the inhibitory effects of l-norepinephrine, l-epinephrine and d, l-isoproterenol on aqueous humor formation in the enucleated, arterially perfused cat eye. Invest. Ophthal. (in press).

Cevario, S.J. and Macri, F.J.: The inhibitory effect of pentobarbital Na on aqueous humor formation. Invest. Ophthal. (in press).

Serial No. NEI-73 CB 153(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

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

Previous Serial Number: None

Principal Investigator: Karyn Ross

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

Cooperating Units: None

Man Years:

Total:	0.4
Professional:	0.4
Others:	0.0

Project Description:

Objectives: To determine the effect of unilateral common carotid artery ligation upon the flow of blood to the ciliary body and upon formation of aqueous.

Methods Employed: Aqueous humor and serum concentrations of ascorbic acid are being determined by titration with 2,6-dichlorophenol indophenol dye. The ability of the transport mechanism for ascorbic acid into the eye to become saturated is being studied by increasing the blood level of ascorbic acid. Determinations of aqueous humor formation are being made using the serum albumin I^{125} dilution-turnover technique.

Major Findings: The normal concentration of ascorbic acid in the aqueous humor of the rhesus monkey is the same for both eyes and is approximately 25 times higher than that in the blood.

When the serum concentration of ascorbic acid is elevated, the aqueous humor ascorbic acid concentration increases, indicating the presence of saturation kinetics.

Unilateral common carotid artery ligation causes a reduction of ciliary body plasma flow. The reduction of plasma flow is associated with decreased aqueous humor formation. This is consistent with the view that aqueous humor is produced by ultrafiltration.

Significance to Biomedical Research and the Program of the Institute: The relationship between blood flow in the ciliary body and aqueous humor formation has not previously been demonstrated in vivo. The definition of this relationship allows a better understanding of the mechanism of aqueous humor production and is important in the management of glaucoma patients.

Proposed Course of the Project: This project is currently ongoing and with continue through next year.

Honors and Awards: None

Publications: None

Serial No. NEI-73 CB 139(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Comparative Treatment of Bullous Keratopathy with the Bausch and Lomb "Soflens" and the American Optical "Bandage" Soft Contact Lenses

Previous Serial Number: Same

Principal Investigators: William R. Sullivan, M.D.
Carl Kupfer, M.D.

Other Investigators: Fred Ederer

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Others:	0.0

Project Description:

Objectives: This study is designed to determine whether either the Bausch and Lomb "Soflens" or the American Optical "Bandage Lens" are effective in the treatment of bullous keratopathy. In addition, the relative efficacy of the two lenses will be compared.

Methods Employed: Patients with bullous keratopathy are screened for the study and given a three month therapeutic trial with each of the soft contact lenses. Parameters which are measured and evaluated are symptom questionnaires, visual acuity, corneal thickness, slit lamp examination, and stereo photographs.

Major Findings: Analysis of the data indicates that corneal vascularization occurs with the use of both lenses with a much greater frequency than has been previously reported. No difference could be demonstrated between the two lenses. Study of the other parameters has failed to yield significant conclusions.

Significance to Biomedical Research and the Program of the Institute: Soft contact lenses are currently being advocated for therapeutic use in a variety of pathologic conditions of the cornea. One of the lenses studied in this project, the American Optical "Bandage" lens (now known as the Warner-Lambert "Softcon" lens), has received FDA approval for use in the treatment

of bullous keratopathy.

Carefully controlled, objective research on the therapeutic uses of the hydrophilic lenses will provide data needed for the critical evaluation of this form of treatment in corneal disease.

Proposed Course of Project: The project has been terminated; and the results are being prepared for publication.

Honors and Awards: None

Publications: None

Serial No. NEI-73 CB 144(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of the Effect of Histocompatibility (HL-A) Testing and Corneal Transplantation

Previous Serial Number: Same

Principal Investigator: William R. Sullivan, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Others:	0.0

Project Description:

Objectives: The objectives of this study are to determine the roles of histocompatibility testing in cases with an unfavorable prognosis for penetrating keratoplasty.

Although corneal transplantation is performed on patients with densely vascularized corneas, the likelihood of immunologic graft rejection is greater than in the non-vascularized cornea. In kidney transplants the improved success has been attributed to histocompatibility (HL-A) matching of donor and recipients. It has been shown in kidney transplants that the HL-A antigen system is the major transplantation antigen system in man, and at the time of kidney graft rejection antibodies formation in the recipient against the HL-A antigens of the donor can frequently be found. These antibodies are called lymphocytotoxic antibodies.

Methods Employed: Patients undergoing penetrating keratoplasty at the National Eye Institute are evaluated pre-operatively for the presence of lymphocytotoxic antibodies. Attempts were made to obtain lymphocyte typing of all donors. Post-operatively the patients were followed with serial serum testing for the formation of lymphocytotoxic antibodies.

Major Findings: Patients have received corneal transplants and undergone collection of pre- and post-operative serum samples or testing for lymphocytotoxic antibodies. Studies to date suggest a correlation between the

development of anti HLA antibodies and immune graft rejection. In addition, patients in whom the presence of lymphocytotoxic antibodies was detected pre-operatively appeared to have an increased chance of graft failure following the development of immune graft reaction.

Significance to Biomedical Research and the Program of the Institute: The mechanisms involved in the immune graft rejection process have not previously been well worked out. It is hoped that better understanding of these mechanisms will make it possible to better control immune rejection as a cause of graft failure, both in corneal transplantation and in other transplantation systems.

Proposed Course of the Project: This project is to be terminated pending evaluation of the serum data from the most recent group of patients.

Honors and Awards: None

Publications:

Stark, W.J., Opelz, G., Newsome, D., Brown, R.S., Yankee, R., and Terasaki, P.: Sensitization to human lymphocyte antigens by corneal transplantation. Invest. Ophthal. 12-9; 639-645, 1973.

Serial No. NEI-73 CB 145(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Treatment of Keratoconjunctivitis Sicca with N-acetyl-l-cysteine

Previous Serial Number: Same

Principal Investigators: William R. Sullivan, M.D.

Other Investigators: Carl Kupfer, M.D.

Cooperating Units: Pharmaceutical Development Service, CC, NIH
Mead Johnson Research Laboratories

Man Years:

Total:	0.3
Professional:	0.3
Others:	0.0

Project Description:

Objectives: The study is designed to evaluate the efficacy and safety of n-acetyl-l-cysteine, a mucolytic agent in the treatment of the signs and symptoms of keratoconjunctivitis sicca, the most prevalent of the various dry eye conditions. The effect of this treatment on conjunctival goblet cells, the source of mucus in the normal tear film, will be studied.

Methods Employed: Patients with keratoconjunctivitis sicca were treated for four weeks with n-acetyl-l-cysteine and with a placebo in a double blind, crossover fashion. Parameters which were measured and evaluated are symptom questionnaries, visual acuity, slit lamp examination, tear film breakup time, rose bengal staining, intraocular pressure, Schirmer test, photography, and conjunctival biopsy.

Major Findings: Patients with keratoconjunctivitis sicca are enrolled in the study, and data is being gathered.

Significance to Biomedical Research and the Program of the Institute: This study will provide needed information on the treatment of patients with dry eye conditions. Evaluation of conjunctival goblet cells will allow a clearer understanding of the pathologic mechanisms involved in keratoconjunctivitis sicca.

Proposed Course of Project: The project will continue.

Honors and Awards: None

Publications: None

Laboratory of Vision Research

ANNUAL REPORT
LABORATORY OF VISION RESEARCH
July 1, 1973 - June 30, 1974

by Jin H. Kinoshita
Chief, Laboratory of Vision Research

During the past year no major changes occurred in the personnel of Laboratory of Vision Research. The only changes to occur were in the temporary catagories of research associates and visiting scientists. Undoubtedly this situation will continue since no increase in the number of positions is anticipated in the immediate near future. Even though no new investigators were added to the Laboratory, several new areas of research were initiated during the past year. For example, Dr. Gouras is now collaborating with Dr. Chader and Dr. Marshall Nirenberg, NHLI, studying the electrical responses of cells on tissue culture. These studies eventually will be extended to the retinoblastoma cells and pigment epithelial cells of genetically abnornal retinas. In Dr. Kuwabara's Section, (Experimental Pathology) Dr. Robison has made significant advances in the study of retinal pigment epithelium of an animal model for patients with the Chediak-Higashi disease. In the study of the herediatry mouse cataract, Dr. Kinoshita and his associates have demonstrated that the initiating factor in the cataractous process appears to be a defect in the Na-K ATPase. It appears likely that this apparent enzyme deficiency is caused by the presence of an ATPase inhibitor.

Another fact that becomes readily apparent reading the individual Annual Reports is the numerous collaborative projects that have developed between the members of the Laboratory of Vision Research and investigators of other Institutes. Every section of the Laboratory is involved in this type of collaboration, taking advantage of the rich research environment that exists at NIH.

A regular event that should be mentioned as an indication of the further development of the Laboratory is our "Brown Bag Seminars". These occur informally at noon time lunches and continue to be a popular forum to facilitate the exchange of ideas. They also serve as an effective means for each investigator to keep abreast of new advances in the broad area of vision research. Such a forum is essential for developing cohesion and unity in a research laboratory composed of several multidisciplinary units.

Serial No. NEI-73 LVR 136
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Chemistry and Metabolism of the Lens

Previous Serial Number: Same

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: In last year's report our findings indicated that as the bovine lens ages a new form of gamma crystallin emerges. The newly-emerging γ-crystallin in the older lens has a molecular weight of 24,000, while the γ in the young calf lens has a molecular weight of 20,000. The γ with the higher molecular weight appears in the lens cortex where it is probably synthesized, while the lower molecular weight form is concentrated in the lens nucleus. In the human lens the same process appears to occur. The γ crystallin of larger molecular dimensions predominates over the lower molecular species in the older human lens, while in the younger lens the lighter form is found in greater abundance. Apparently, with aging, as new cortical fibers are laid down the γ crystallin that is synthesized is the

one with the higher molecular weight. This age-related phenomenon has now been demonstrated in both the bovine and human lenses.

Conditions to culture disassociated epithelial cells of lenses from normal and cataractous adult mice have now been established. These cultures show rapid growth and form spherical bodies which appear to be composed of lens fibers. During tissue culture of these single cells three stages are discernible. In the first stage the isolated cells proliferate and multiply. In the second stage these cells become confluent so that sheets of epithelial cells are formed. In the third stage these cells elongate and form spherical bodies. These lentoid bodies are made up of lens fiber-like material. We are now in the process of examining these fibers with an electron microscope and of demonstrating the presence of lens crystallins.

Up to this time investigators have had difficulty in the tissue culture of lens epithelial cells. They were able to obtain growth, but the cells were unable to retain their characteristics as lens cells. In the hands of other investigators the tissue culture of lens epithelium was not successful in that the cells did not retain the differentiative traits and dedifferentiation was the usual outcome. In our case, the conditions have been optimized so that the cultured cells are able to differentiate into lens fibers.

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 δ crystallins is one of the first examples of the effect of aging on the lens proteins.

Development of the tissue culture of isolated, disassociated cells of lens epithelium provide us with another means of studying cataracts, particularly the congenital cataracts. Now that it is possible to take single lens epithelial cells and grow sheets of epithelium, a thorough study into the nature of the defect in cataracts should be possible. Currently, we are extending this study to the congenital mouse cataract. It is conceivable that this procedure may also be helpful in the study of human congenital cataracts as well.

Proposed Course of Project: With the organ culture procedure we hope to determine the optimal conditions which will maintain the transparency of the lens for a protracted period. We are also attempting to grow isolated cells of the lens epithelium in tissue culture to determine if they can maintain their differentiative properties of the lens cells.

Honors and Awards: None

Publications:

Jedziniak, J., Yates, C. and Kinoshita, J. H.: Lens polyol dehydrogenase. Exp. Eye Res. 16: 95-104, 1973.

Kabasawa, I. and Kinoshita, J. H.: Carbohydrate associated with gamma crystallin of the calf lens. Exp. Eye Res. 16: 143-150, 1973.

Kinoshita, J. H. and Merola, L. O.: Oxidation of thiol groups of the human lens. In Ciba Foundation Symposium 19: The human Lens in Relation to Cataract. Amsterdam ASP, 1973, pp. 173-184.

Holt, W. S. and Kinoshita, J. H.: The soluble proteins of bovine cornea. Invest. Ophthalmol. 12: 114-126, 1973.

Kinoshita, J. H. and Merola, L. O.: Cyanate effects on the lens in vitro. Invest. Ophthalmol. 12: 544-547, 1973.

Kabasawa, I., Lou, M. F., Merola, L. O. and Kinoshita, J. H.: Inositol-1-phosphatase in the lens. Ophthal. Res. (in press).

Serial No. NEI-73 LVR 137
1. Laboratory of Vision Research
2. Section of Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cataracts

Previous Serial Number: None

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

Other Investigators: Lorenzo O. Merola

Cooperating Units: None

Man Years:

Total:	2.5
Professional:	1.5
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 these cataracts is to employ the lens organ culture technique. This can be done by exposing the isolated lens to elevated levels of either glucose, galactose or xylose in the incubating medium.

Major Findings: We are continuing to obtain evidence supporting the concept that the enzyme aldose reductase is involved in initiating sugar cataracts. This enzyme seems to be the common mechanism by which the sequence of events leading to the development of all sugar cataracts are initiated. There is substantial evidence that aldose reductase triggers off the process that leads to the diabetic, galactosemic and xylosemic cataracts. When sugars are elevated, as in hyperglycemia or hypergalactosemia, aldose reductase in the lens converts sugars to sugar alcohols. The accumulation of abnormally high levels of polyols in the lens fibers causes a hypertonicity leading to osmotic swelling. This represents the initial stage in sugar

cataract formation. An active inhibitor of aldose reductase (A.R.) has recently been shown to prevent cataractous changes in the cultured lenses exposed to high concentrations of galactose. When given orally, the A.R. inhibitor markedly decreased the accumulation of polyols in the lenses and sciatic nerves of galactosemic rats and of streptozotocin-diabetic rats. In addition oral administration of this inhibitor to rats fed galactose led to an effective delay in the formation of cataracts. The topical application of this inhibitor to eyes of galactosemic rats consistently delayed the onset of cataracts. However improved method of delivery of the compound by topical application must be achieved before this procedure of controlling cataract formation can be considered effective.

According to the polyol theory of cataractogenesis, hyperglycemia is an important factor in the initiation of cataract development. The existence of mutant strains of mice with hyperglycemia that do not develop cataracts appears as a contradiction to the sugar alcohol theory. However, it was found that the lenses of these hyperglycemic mice contain very little sorbitol. Therefore, the reason for cataracts not developing in the hyperglycemic mice is that significant amounts of polyol do not accumulate in the lens.

These findings stimulated interest in more detailed studies of lens aldose reductase activity in various strains of normal mice. Some mice, when fed a galactose diet accumulated dulcitol in their lenses, but not anywhere near the level found in lenses of galactose-fed rats. Consistent with this is that mouse lens aldose reductase activity is extremely low, about one-tenth of the rat lens. Thus, the conversion of sugar to polyol is not significant even when the lens is subjected to a hyperglycemic or hypergalactosemic state. The absence of cataracts in the hyperglycemic mouse strain therefore appears related to the low aldose reductase activity in the lens and lends further support to the poly theory of sugar cataracts.

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 of Project: This project will be continued.

Honors and Awards:

Proctor Award by the Association for Research in Vision and Ophthalmology, to Dr. Jin Kinoshita, April 28, 1974.

Publications:

Dvornik, D., Simard-Duquesne, N., Kraml, M., Sestanj, K., Gabbay, K., Kinoshita, J. H., Varma, S. D. and Merola, L. O.: Inhibition of aldose reductase in vivo. Science 182: 1146-1147, 1973.

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

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

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

Kinoshita, J. H.: Cataractogenic effects of lactose and galactose. In Sipple, (Ed.): Sugars in Nutrition, New York, Academic Press.

Serial No. NEI-73 LVR 134
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

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

Previous Serial Number: Same

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

Other Investigators: Lawrence R. Herz
R. Theodore Fletcher
Barbara O. Wiggert, Ph.D. (Guest Worker)
W. Gerald Robison, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.4
Professional:	0.9
Other:	1.5

Project Description:

Objectives: The aim of this project is to study factors which determine the normal development and function of the retina and pigmented epithelium. In particular, (1) the influence of hormones on normal and abnormal development and (2) the uptake and binding of vitamin A in the retina were investigated during embryonic development.

Methods Employed: Biochemical analyses of enzyme activities were performed to assess the effects of hormones on the retina after in vivo application or incubation with retinas in organ culture. Hormonal effects on pigmented epithelium cells maintained in monolayer culture were also assessed biochemically through enzyme assay and morphologically through the inverted tissue culture microscope. The technique of sucrose gradient centrifugation was used to determine the properties of the specific vitamin A and glucocorticoid receptors in the retina.

Major Findings: 1) The hormone cortisol affects normal enzyme development in the embryonic retina while progesterone appear to retard normal biochemical development. 2) A specific, high affinity receptor that binds vitamin A has been found in the embryonic retina. 3) Pigmented

epithelium cells respond in culture to hormones such as thyroxine and cyclic AMP with marked changes in pigmentation, morphology and enzyme activity.

Significance to Biomedical Research and the Program of the Institute: Factors involved in retinal dystrophy and degeneration are poorly understood. The present work attempts to pinpoint early signals (hormones, vitamin A uptake, etc.) that are critical to normal retinal development and contrast them with those found in the diseased state. It is hoped that such studies will ultimately uncover compounds (e.g. hormones) which can correct abnormal retinal development in the embryo.

Proposed Course of Project: 1) The role of progesterone in retarding the normal biochemical development of the embryonic retina will be further investigated. 2) The vitamin A receptor will be characterized to help determine its function in normal or abnormal retinal development. 3) Factors involved in the development of pigmentation in cultured pigmented epithelium cells will be studied with emphasis on their relation to retinitis pigmentosa.

Honors and Awards: None

Publications:

Chader, G. J.: Some factors affecting the uptake, binding, and retention of (^{3}H) cortisol by the chick embryo retina as related to enzyme induction. J. Neurochem. 21: 1525-1532, 1973.

Wiggert, B. O. and Chader, G. J.: Studies on the specific glucocorticoid-hormone receptor in the developing chick retina. Exp. Eye Res. (in press).

Newsome, D. A., Fletcher, R. T., Robison, W. G., Jr., Kenyon, K. R. and Chader, G. J.: Effects of cyclic AMP and Sephadex fraction on cloned retinal pigmented epithelium in tissue culture. J. Cell Biol. (in press).

Gouras, P. and Chader, G. J.: Retinitis pigmentosa and retinol-binding protein. Invest. Ophthalmol. (in press).

Serial No. NEI-73 LVR 148
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cyclic Nucleotides and Vision

Previous Serial Number: None

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

Other Investigators: R. Theodore Fletcher

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

Man Years:

Total:	1.2
Professional:	0.7
Other:	0.5

Project Description:

Objectives: The concentrations of cyclic nucleotides in retinal photoreceptors fall dramatically when a dark-adapted retina is exposed to light. These intracellular hormones may thus act as intermediates in translating the initial photic stimulus on the retina into the neural response which is transmitted to the brain. This project was designed to study the enzymes of cyclic nucleotide synthesis and degradation to determine the critical point at which light exerts its influence.

Methods Employed: Retinal photoreceptor units from dark-adapted bovine retinas were isolated by sucrose density gradient centrifugation and the activities of the enzymes of cyclic nucleotide synthesis (cyclase) and degradation (phosphodiesterase) were assayed biochemically in the dark-adapted and in light-bleached preparations. The effects of various inhibitors and activators of these enzymes were tested in in vitro incubations. Cyclic AMP and cyclic GMP concentrations were determined by protein binding and radioimmuno-assay techniques, respectively.

Major Findings: Extremely high concentrations of cyclic GMP were found in retinal photoreceptors. The levels of both cyclic GMP and cyclic AMP were found to be dependent on the percentage of light-bleach of the retina. Phosphodiesterase, the enzyme of cyclic nucleotide metabolism, was found to be activated by light and thus controls cyclic nucleotide concentrations in

the photoreceptor. This is the only well-defined enzymic activity known to be regulated by photic energy.

Significance to Biomedical Research and the Program of the Institute: It is presently not known how the initial photic stimulation of the retinal photoreceptor is converted into a neural response. The present study will, we hope, lead to a better understanding of the biochemical basis of the process in normal vision and lay the groundwork for an understanding of the underlying problems of retinal degeneration and blindness.

Proposed Course of Project: Efforts will be concentrated on the identification of the physiological and pharmacological factors which control the levels of cyclic nucleotides in retinal photoreceptors.

Honors and Awards: None

Publications:

Chader, G. J., Bensinger, R. E., Johnson, M. and Fletcher, R. T.: Phosphodiesterase: an important role in cyclic nucleotide regulation in the retina. Exp. Eye Res. 17: 483-486, 1973.

Bensinger, R. E., Fletcher, R. T. and Chader, G. J.: Guanylate cyclases inhibition by light in retinal photoreceptors. Science 183: 86-87, 1974.

Chader, G. J., Johnson, M., Fletcher, R. T., and Bensinger, R. E.: Cyclic nucleotide phosphodiesterase of the bovine retina: activity, subcellular distribution and kinetic parameters. J. Neurochem. 22: 93-99, 1974.

Bensinger, R. E., Fletcher, R. T. and Chader, G. J.: "Piggyback", chromatography: assay for guanylate cyclase in retina and other neural tissue. J. Neurochem. (in press).

Chader, G. J., Fletcher, R. T., Johnson, M. and Bensinger, R. E.: Rod outer segment phosphodiesterase: factors affecting the hydrolysis of cyclic GMP. Exp. Eye Res. (in press).

Chader, G. J., Herz, L. R. and Fletcher, R. T.: Cyclic nucleotide hydrolysis: some possible natural regulators of phosphodiesterase activity in retina and rod outer segments. J. Neurochem. (in press).

Chader, G. J., Herz, L. R. and Fletcher, R. T.: Light activation of phosphodiesterase activity in retinal rod outer segments. Biochim. Biophys. Acta. (in press).

Serial No. NEI-71 LVR-027
1. Laboratory of Vision Research
2. Section of Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

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

Previous Serial Number: Same

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

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

Cooperating Units: Department of Poultry Science, University of Maryland

Man Years:

Total:	0.1
Professional:	0.05
Other:	0.05

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 well 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: Day-old chicks were raised for a period of 16-18 weeks on a corn-soybean practical diet supplemented with 7% glycine. These animals, in contrast to those in previous experiments with a purified dietary regimen, exhibited a growth depression after 7 weeks of feeding as compared to their controls. This difference in weight between the two groups however was not seen at the end of the experimental period.

An enlargement of the eyeball (buphthalmia) was still observed in the experimental group when chickens were paired between the two flocks according to body weight at 18 weeks of age just prior to enucleation. These observations lead to the conclusion that buphthalmos can be produced in older birds and is not affected by the rate of body growth.

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 to be useful for studying biochemical changes which take place in developing vitreous but in maturing nervous system as well.

Proposed Course of Project: Because glycine has been postulated to function as an activator of the first enzymatic step in hyaluronate biosynthesis in vitreous hyalocytes, amino acid profiles will be performed on dialysates of control and experimental vitreous respectively to determine if the neutral amino acid 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.

Honors and Awards: None

Publications:

Rubin, M., Helmsen, R.J., and Gaasterland, D.E.: The buphthalmos syndrome in chicks fed an excess of glycine. Poultry Science 52: 2079, 1973 (Abst.).

Serial No. NEI-72 LVR-111
1. Laboratory of Vision Research
2. Section of Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Chemistry of the Cornea

Previous Serial Number: Same

Principal Investigator: Ralph Helmsen, Ph.D.

Other Investigators: Rosanne Hess, M.S.
Merrill Lynn, Ph.D., (Corning Glass Works)

Cooperating Units: Technical Staff Division, Corning Glass Works

Man Years:

Total:	0.8
Professional:	0.3
Other:	0.5

Project Description:

Objectives: To isolate tissue-specific soluble and membrane proteins from the epithelium and 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 use of pressure chromatography on columns of glass beads coupled with affinity chromatography 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: Individual antiserums have successfully been prepared in sheep against dialyzed soluble proteins derived from pooled samples of fresh calf corneal epithelium and frozen whole rabbit cornea. Each immune serum obtained after prolonged immunization exhibits only one precipitin line when tested against an extract of its respective antigen by the Ouchterlony double diffusion technique. No cross reaction between the two antigens was observed in experiments which involved the use of either antiserum. The antigen detected in calf corneal epithelium was found to be precipitable at pH 5.75 and appeared to be tissue-specific since its precipitin line was not absorbed out on immunodiffusion plates when either calf plasma or serum was

included in the agarose medium. Sheep antibody against calf epithelial proteins has been extensively purified by ammonium sulfate precipitation with almost full retention of activity.

Significance to Biomedical Research and the Program of the Institute: Successful isolation of a tissue-specific protein from a corneal layer in large amounts would provide source material for immunological studies to determine whether the macromolecule functions as a transplantation antigen and/or receptor site for viruses in experimental animals. If such studies were successful, further investigations with a chemically modified protein might prolong the survival time of corneal grafts and/or reduce the extent of viral invasion of the tissue in humans.

Proposed Course of Project: Because the antigen from calf cornea has been reported to aggregate at neutral pH, isolation of this protein is being approached through the method of absorbing a crude extract of the calf antigen onto various insoluble derivatives of its purified antibody and then releasing the antigen from the resulting immune complex at an acidic pH. If this procedure is successful, a similar approach will be pursued with the corresponding immune protein derived from rabbit cornea. Glass bead chromatography will be used in conjunction with the above method to partially purify the antigen before reaction with the immunoabsorbent if necessary.

Honors and Awards: None

Publications: None

Serial No. NEI-74 LVR-147
1. Laboratory of Vision Research
2. Section of Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

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

Previous Serial Number: None

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

Other Investigators: Rosanne Hess, M.S.
Margery Sullivan
Duran Harris
Rachel Levinson

Cooperating Units: Laboratory of Pathology, NCI

Man Years:

Total:	2.4
Professional:	0.7
Other:	1.7

Project Description:

Objectives: To isolate 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.

Methods Employed: Sucrose density gradient centrifugation will be employed to isolate the plasma membranes from disrupted herpes infected cells in addition to methods outlined under "Chemistry of the Cornea."

Major Findings: Herpes simplex virus (HSV-I)-induced antigens were demonstrated on membranes of infected rabbit corneal cells (SIRC line) by specific immunolabeling. After infection these cells were treated with anti-HSV human IgG, washed and then exposed to sheep anti-human IgG anti-horse ferritin hybrid. The preparation was subsequently treated with ferritin and fixed for electron microscopy. Ferritin was found to be localized on cytoplasmic membranes often concentrated in focal patches over areas of membrane thickening, along internal membranes and at nuclear membranes of disrupted cells and around extracellular virus. Control studies performed on both infected and non-infected cells reveal that the binding of ferritin was specific for HSV-induced antigens.

Significance to Biomedical Research and the Program of the Institute: The use of the hybrid antibody method for localization of antigenic changes on 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 antigens which form on cellular membranes during herpes infection may be recognized as foreign by the host in a manner analogous to the recognition of foreign HL-A antigens in an incompatible tissue graft may prove useful to those investigators involved in formulation of treatment for this ocular disease.

Proposed Course of Project: Ghosts of plasma membranes from infected and uninfected corneal cells will be separated following cell disruption from cytoplasmic and nuclear debris by density centrifugation and brought in solution for electrophoresis on sodium dodecyl sulphate (SDS) polyacrylamide gels. An attempt will be made to isolate and characterize those protein bands only present in gels from infected cells for use as immunological tools in the study of the pathogenesis of herpes simplex keratitis.

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 neoantigens on infected corneal cells using sheep anti-human IgG anti-ferritin hybrid antibodies. Lab. Invest. 30: 376, 1974, (Abst.).

Serial No. NEI-73 LVR 135
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

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

Previous Serial Number: Same

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

Other Investigators: David R. Whikehart, Ph.D. (NIH Special Research Fellow)
Julia E. Derr, B.A.

Cooperating Units: None

Man Years:

Total:	3.0
Professional:	2.0
Other:	1.0

Project Description:

Objectives: The broad aims of the project are (1) to determine the characteristic biochemical composition of photoreceptor, neuronal and glial membranes (with emphasis on rod outer segment membranes); (2) to identify and assess the usefulness of biochemical marker substances (or ratios of substances); (3) to use indices in conjunction with other chemical constitutents and enzymes to study retina (and vision-related regions of brain) in normal, experimentally-induced, and heritable pathological conditions; and (4) to study properties of artificial membranes (liposomes) similar in composition to retinal rod outer segment membranes, normal and pathological.

Methods Employed: Analyses are carried out on whole retinas (frog and rat) and on intact retinal rod outer segments of frogs. Methods in use include spectrophotometry; fluorometry; atomic absorption spectroscopy with graphite furnace; microelectrophoresis; gas chromatography; thin layer chromatography (TLC); and light microscopy (ordinary and polarizing).

Major Findings: I. Studies of glycosphingolipids of frog retina and rod outer segments, and of rat retina: Glycosphingolipid fractions of retina have not been well studied in the way that the phospholipid fraction has been. Glycosphingolipid (as well as glycoprotein) synthesis and turnover may be

involved in cell recognition and adhesion, as suggested by marked reductions in activity of the respective glycosyltransferases in transformed cells; these compounds also may be important in membrane permeability and ion transport. Our aim is to study the amounts, types, and histological localizations of the sphingoglycolipids in the retina, and their possible physiological role. To obtain direct evidence on the chemical structure of these compounds, we have been developing a more sensitive and quantitative assay for sphingosine that can be combined with our highly sensitive fluorometric method for sialic acid to establish the sphingosine/sialic acid ratio in water-soluble sphingolipids; in addition, the galactose/glucose ratio and the fatty acid present will be determined by gas chromatography. We have found the water-soluble glycolipids (gangliosides) to be 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). Water insoluble glycolipids or ceramide hexosides (lacking sialic acid) are present in much lower concentrations than previously indicated by analyses on total lipid extracts by orcinol- or anthrone-sulfuric acid methods. After interfering substances were removed from whole retina total lipid extract, the remaining carbohydrate containing compounds represented about 1% of the retina dry weight, in accord with our finding of only 2 small spots on TLC. We have collected rat retinas to prepare large enough amounts of these lipids to study their composition. In addition, we found that some of the carbohydrate appears to be associated with the proteolipid protein that neutral chloroform: methanol extracts from retina. This protein may be a small fraction of rhodopsin (or opsin), or on the other hand may be a different glycoprotein.

II. Liposomes as model membranes for the rod outer segment: Model systems serve as simplified vehicles for understanding complex prototypes. The liposome represents such a system and has been used by Dr. Whikehart to simulate and study the chemical and physical properties of the rod outer segments of photoreceptor cells. Liposomes are composed of concentric spherical bilipid layers (separated by aqueous compartments) and are so formed by swelling lipid in aqueous salt. Sonication can convert these structures to single bilipid spheres with an aqueous interior. As such they are ideal for investigation of membrane function.

In this study, liposomes were formed that had a lipid composition similar to that of the rod outer segments of photoreceptors. They were compared with liposomes of simpler composition. The properties investigated were: birefringence, electrophoretic mobility, ion leakage and protein binding. The influences of certain proteins (lysozyme, a basic water soluble protein; proteolipid, a membrane protein from myelin; and opsin, the bleached protein product from rhodopsin) were carefully monitored after interaction with the liposomes. It was found that although varying the lipid composition of the liposomes has little or no effect on their physical appearance, subtle changes are produced by protein interactions. These changes are manifested by alterations in electrophoretic mobility and ion leakage and are dependent on both the type and concentration of protein used. The findings imply a marked specificity of influence of proteins upon lipid membranes and their

concomitant biological function. In the case of rod outer segment membranes, they may provide clues to the exact role of rhodopsin as an electrical transducer of light.

Significance to Biomedical Research and the Program of the Institute: Data on the biochemical composition of normal photoreceptor, neuronal and glial membranes will contribute to an understanding of their function in retina and brain. Glycosphingolipids may be important in the cell surface interrelationships of rod outer segment and pigment epithelium or in the permeability of the rod 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 of Project: Normal biochemical architecture and pathology of retina will be emphasized. With completion of our 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 rats).

Honors and Awards: None

Publications:

Embree, L.J., Hess, H.H. and Shein, H.M.: Microchemical studies of lipids, proteins and nucleic acids in polyoma virus-transformed hamster astroglia. J. Neuropath. Exp. Neurol. 32: 542-551, 1973.

Bass, N.H., Hess, H.H. and Pope, A.: Microchemical studies of altered cell membranes in Creutzfeldt-Jakob disease. Arch. Neurol. 30, (in press) 1974.

Serial No. NEI-71 LVR 008

1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Chemistry of Rhodopsin

Previous Serial Number: Same

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

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.7
Other:	0.1

Project Description:

Objectives: To study the structural and functional aspects of the bovine rhodopsin molecule.

Methods Employed: The molecular weight of rhodopsin, the self-association of the detergent Triton X-100, and the association of the detergent with rhodopsin was studied by analytical ultracentrifugation. The self-association of Triton X-100 and its membrane diffusibility was also studied by determining the kinetics of its dialysis against water. The extractability of rhodopsin as a function of detergent concentration was determined by mixing aliquots of purified rod outer segment suspensions with detergent solutions of known concentrations, rapidly ultrafiltering after an appropriate time interval, and then determining the rhodopsin concentration by measuring the optical density at 500 nm.

Major Findings: A molecular weight of 35,000 was found for delipidated opsin, which may be considered as the apoprotein form of rhodopsin. Binding studies with Triton X-100 indicated that there was a maximum of 362 binding sites available per rhodopsin molecule and that the intrinsic association constant gave a free energy of binding of -4.52 kcal per mole of Triton bound, as compared with a free energy for detergent self-association of -3.52 kcal per mole of monomer. These free energy changes are consistent with the values expected for the formation of hydrophobic bonds. The computer techniques developed for these analyses clearly demonstrated

the advantages of using non-linear least squares curve fitting procedures for determining the requisite parameters as compared to the earlier procedures of Klotz and Scatchard which required transformation of the variables in order to permit linear fitting techniques. Studies on the detergent concentration dependence of rhodopsin extraction showed that the extraction increased as the concentration was increased, reaching a maximum at half the critical micelle concentration, then decreasing slightly and leveling out at higher concentrations. By calculating the total free energy change for detergent binding at the extrapolated concentration where rhodopsin solubilization was initiated, it was possible to estimate that the maximum total free energy of membrane lipid binding to rhodopsin was -406 kcal per mole of rhodopsin. Knowledge of the maximum number of binding sites and the molecular dimensions of the detergent made possible an estimate of the molecular surface area, and this parameter, combined with the molecular volume which was calculated from the molecular weight and the partial specific volume, permitted the calculation of the molecular dimensions for various models. A prolate ellipsoid of revolution would have a length of 111 A and a diameter of 27 A, while an oblate ellipsoid would have a diameter of 64 A and a thickness of 20 A. These, and a number of other models satisfy the requirements for the proper surface to volume relationship, but the techniques used here do not permit determining which model is most appropriate.

The mathematical model which was developed for the analysis of the detergent diffusion data permitted the calculation of the micellar size and association constant as well as the diffusion coefficient. In these studies a micellar size of 100 monomers was found, as compared with 105 for the ultracentrifugal studies, and a free energy of self-association of -3.66 kcal per mole of monomer was obtained, compared to a value of -3.52 kcal per mole of monomer found previously. Considering the marked difference in the two techniques, this must be considered quite good agreement. A diffusion coefficient of .000143 cm/hr was obtained. This now permits the investigator using Triton X-100 or similar non-ionic detergents to estimate how long a period of dialysis is required in order to remove the detergent following its use for the solubilization of rhodopsin or other membrane proteins. The mathematical model also indicates that it should be possible to measure detergent-protein interactions by comparing the kinetics of detergent membrane diffusion for equal detergent concentrations in the presence and absence of protein.

Significance to Biomedical Research and the Program of the Institute: It has been postulated that hydrophobic bonding between rhodopsin and the lipids of the rod outer segment membrane is of major importance for the maintainance of the structure of the membrane and for the functional role of rhodopsin in that membrane. The thermodynamic values which have been obtained here support at least the first of these concepts, and we now have a clearer concept of the magnitude of the forces which are involved in the rhodopsin-lipid interactions in the membrane. Additionally, we now have the ability to predict molecular dimensions for various models for the structure of rhodopsin and consider these for consistency with other

evidence concerning the behavior of rhodopsin during the visual process. In furnishing information concerning the structural and functional role of rhodopsin in the rod outer segment, these studies are intended to contribute to an understanding of the basic biochemical mechanisms which are involved in both the normal and pathological aspects of scotopic vision.

Proposed Course of Project: Studies are continuing toward further elucidation of the thermodynamic aspects of detergent and phospholipid binding to rhodopsin and to the behavior of the rhodopsin in a non-aqueous milieu, and to the significance of these to the function of rhodopsin in the rod outer segment membrane.

Honors and Awards: None

Publications:

Lewis, Marc S., Krieg, Laura C., and Kirk, William D.: The molecular weight and detergent binding of bovine rhodopsin. Exp. Eye Res. 18: 29-40, 1974

Serial No. NEI-71 LVR-009
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Physical Chemistry of Model Gel Systems

Previous Serial Number: Same

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

Other Investigators: Soo Il Chung, Ph.D., LB, NIDR
John E. Folk, Ph.D., LB, NIDR
Jules Gladner, Ph.D., LBC, NIAMDD

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.3
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 may in any way 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 data reduction and systems analysis as being the most effective means for studying systems of interacting macromolecules.

Major Findings: The subunit structure of the transglutaminases (activated blood coagulation factor 13) from human plasma and platelets have been studied by gel filtration, analytical ultracentrifugation, and chemical cross-linking, both in the presence and absence of catalytically essential calcium ion. The plasma enzyme in the absence of calcium ion is composed of four subunits and has a tetrameric structure similar to that of the zymogen except that the two A chains have been proteolytically modified by the activating enzyme, thrombin. Addition of calcium ion causes a reversible dissociation of the plasma enzyme molecule into a catalytic dimer (AA) and a non-catalytic dimer (BB). The platelet enzyme with or without calcium exists as a catalytic

dimer derived by thrombin activation from the zymogen dimer. Isolated non-catalytic dimer from the plasma enzyme may be combined with platelet enzyme in the absence of calcium to give a tetrameric molecule which is not distinguishable from the plasma enzyme, supporting earlier evidence that the catalytic chains of the two zymogens are either identical or very similar. The ultracentrifugal studies demonstrated that while the plasma zymogen and the activated enzyme were homogeneous in the absence of calcium, with molecular weights of 296,700 and 289,500 respectively, the presence of calcium resulted in what appeared to be a calcium concentration-dependent dimer-tetramer equilibrium of the plasma enzyme subunits.

Significance to Biomedical Research and the Program of the Institute: Studies on calcium dependent macromolecular association or dissociation offer the possibility of yielding information which may be significant in interpreting the role of calcium in the changes which occur in the rod outer segment membrane following exposure to light, and thus contributing to an understanding of the basic biochemical mechanisms which are involved in both the normal and pathological aspects of scotopic vision. Additionally, since a deficiency or absence of coagulation factor 13 cometimes results in impaired blood clotting and wound healing, these studies are relevant to those aspects of glaucoma and corneal diseases which require surgical intervention.

Proposed Course of Project: The studies on the calcium ion dependence of the dissociation of the plasma transglutaminase will be continued in order to attempt to elucidate the mechanism of the reaction from a thermodynamic as well as a structural point of view. The studies on the effects of chemical modification such as succinylation on proteins such as fibrinogen and actin, which undergo polymerization to form gels, will be continued in order to determine the effects of these modifications on the cross-linking reactions, with the intentions of extrapolating these results to the interpretation of the effects of these modifications on proteins of particular interest in visual biochemistry.

Honors and Awards: None

Publications:

Chung, Soo Il, Lewis, Marc S., and Folk, J.E: Relationships of the catalytic properties of human plasma and platelet transglutaminases (activated blood coagulation factor XIII) to their subunit structures. J. Biol. Chem. 249: 940-950, 1974.

Serial No. NEI-71 LVR 015

1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Synthesis of Sugar-Containing Polymers in Retina

Previous Serial Number: Same

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

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.3

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 bovine 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 found to be incorporated into rhodopsin using isolated retinas from either the cow or the frog. The radioactive sugars were found in both visual pigment and an opsin-like protein fraction suspected of being a precursor to rhodopsin. The latter was the predominant labeled component in outer segments from the cow, but was far less abundant in frog outer segments. Using bovine outer segments, it was found that the suspected precursor would be converted to visual pigment by the addition of 9-cis retinal. Consequently, it appears that no further additions of sugar residues are necessary to allow the pre-rhodopsin to accept a vitamin A chromophore to produce light-sensitive visual pigment.

Significance to Biomedical Research and the Program of the Institute:

The similarities between frog and cow rhodopsin synthesis justify direct comparison of this work with work from other laboratories using the frog system and serve to encourage coordinated efforts. The differences suggest that the bovine system may be more useful in studying modifications that occur to opsin after it has arrived in the outer segment. The growth of the carbohydrate component of opsin is a possible mechanism for its transport from the inner segment and could also be esential for its proper insertion and orientation in the new disc membrane. Failure of the sugar transfer steps in either the inner or outer segment could be responsible for impaired outer segment renewal or defective membrane assembly leading to abnormal retinal function.

Proposed Course of Project: Attempts will be made to confirm the identity of radioactive mannose incorporated into rhodopsin and to determine whether any sugars are transferred to opsin in the outer segments. Efforts will also be directed toward a resolution of differences between biochemical evidence obtained in this laboratory and autoradiographic evidence from another laboratory, both apparently based on the presence of a large sugar nucleotide pool which dilutes radioactive sugar precursors. Attempts will be made to determine the location of pre-rhodopsin in the outer segment and relate it to observable structural features.

Honors and Awards: None

Publications: None

Serial No. NEI-71 LVR 016
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Protein Synthesis in the Retina

Previous Serial Number: Same

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

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.3

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 transport of opsin to the outer segment and any further modifications of opsin necessary to produce light-sensitive disc membranes.

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: Several highly labeled proteins are found in rod outer segments after incubation of bovine retinas with radioactive leucine. One component is rhodopsin but the remainder is composed chiefly of a protein having the electrophoretic and chromatographic properties of opsin and is suspected of being a precursor to rhodopsin. Treatment of labeled rod outer segments with either 9- *cis*- or 11- *cis* retinal causes the opsin-like protein to be converted to visual pigment. All-*trans* retinal is ineffective. The same pattern of labeling is seen when isolated bovine retinas or vitrectomized bovine eye cups are used for the incubations. However, much less of the opsin-like protein is seen with isolated frog

retinas. The differences appear to be related to species and not to the presence or absence of protective pigment epithelium.

Significance to Biomedical Research and the Program of the Institute: The indication is that opsin is transported to the rod outer segment before the vitamin A chromophore is attached to produce a light-sensitive visual pigment. The presence of pigmented epithelium does not appear to influence the rate at which the chromophore is added to newly-synthesized bovine opsin which accumulates in the outer segment. Isolated frog retinas, on the other hand, show little accumulation of opsin and seem to add chromophore shortly after opsin is transported to the outer segment. It appears that the final steps in outer segment renewal are regulated largely by the photoreceptors, not by the pigment epithelium. This fact has an important bearing on the identification of the cell types responsible for the defects in any of the various retinal dystrophies.

Proposed Course of Project: Attempts will be made to demonstrate the location of rhodopsin precursors in the rod outer segments and to correlate the amount of precursor seen in frog or cow rods with specific differences in the structure of the rods.

Honors and Awards: None

Publications:

O'Brien, P. J. and Muellenberg, C. G.: The biosynthesis of rhodopsin in vitro. Exp. Eye Res. 18: 241-252, 1974

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemistry of Visual Pigments

Previous Serial Number: Same

Principal Investigator: Hitoshi Shichi, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

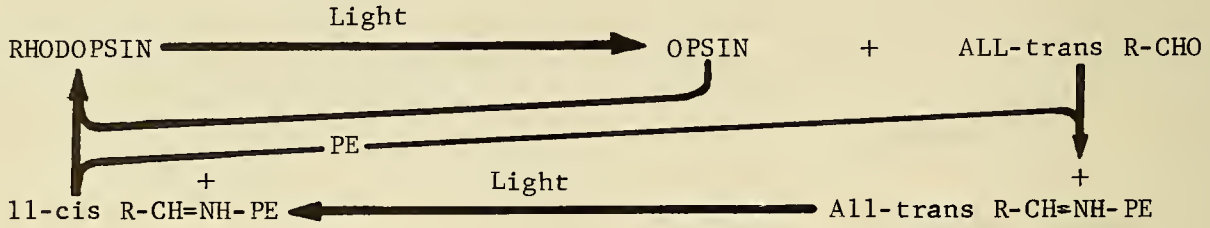
Project Description:

Objectives: (1) To investigate phospholipid requirements for the conformational stability of the visual pigment protein. (2) To elucidate a molecular mechanism by which phospholipid stimulates photoregeneration of rhodopsin from all-trans retinal and opsin.

Methods Employed: Biochemical methods such as centrifugation, column and thin-layer chromatography and spectroscopic analysis.

Major Findings: We have previously found that there are two types of phospholipid which are required for the structure and function of the visual pigment rhodopsin. The distinct roles of these phospholipids were separately investigated. (1) One type of phospholipid is associated with opsin protein and stabilizes a native (preferred) conformation of the protein. No specificity is observed in regard to the type of phospholipid required for this function. The native conformation (a conformation found when associated with disc membranes) of opsin thus stabilized is essential for binding 9-cis retinal or 11-cis retinal to form visual pigments; the retinal binding reaction per se does not require additional phospholipids. (2) The other type of phospholipid is directly involved in the photoisomerization of all-trans retinal to 11-cis retinal, a reaction essential for rhodopsin synthesis. Experimental results may be summarized as follows: Phosphatidylethanolamine (PE) forms a complex with all-trans retinal in the dark. When this complex (a protonated Schiff base with λ_{max}= 458 nm) is irradiated with 460 nm light,

11-cis retinal is preferentially formed. However, even after photoisomerization, PE remains complexed with the isomerized retinal; it releases retinal when opsin becomes available for rhodopsin synthesis. As much as 30% of the PE molecules (i.e. about 13 moles PE per mole of rhodopsin) associated with rod outer segment membranes are capable of forming a complex with retinal for photoisomerization. Since the concentration of the retinal-PE complex extractable from dark-adapted retinal rods is less than one mole per mole of rhodopsin, the number of PE molecules actually involved in photoisomerization in the in vivo conditions may be small. From these results, we have advanced the following arguments: According to the so-called "visual cycle", the all-trans retinal released from the visual pigment during light adaptation migrates from the retina to the pigment epithelium and the process is reversed during dark adaptation. Recent studies, however, indicate that rhodopsin regeneration takes place without the pigment epithelium. If this is true, there must be an alternative mechanism of retinal mobilization for rhodopsin synthesis. As a possible mechanism for the in situ regeneration of rhodopsin in the rod outer segments, we propose a reaction cycle shown below:



According to this scheme, the all-trans retinal formed in the light is immediately complexed with PE and stored in the form of protonated retinal-PE. If additional photons are reaching the retina, some of the complexed retinal will be isomerized to the 11-cis form. During dark adaptation, the 11-cis retinal thus stored is mobilized for rhodopsin synthesis. Since the whole process takes place in the retina, the pigment epithelium is not essential for rhodopsin synthesis.

Significance to Biomedical Research and the Program of the Institute: (1) Phospholipid is an essential component of various biological membranes including rod disc membranes and is known to be important for activity of membrane enzymes (e.g. ATPases, mitochondrial and microsomal oxidoreductases). The present finding that phosphatidylethanolamine is directly involved in photic isomerization of all-trans retinal to 11-cis retinal is probably the first instance in which a "pseudo-enzymic" function of membrane phospholipid is demonstrated and could provide a model reaction for phospholipid functions in other membrane systems. The modified visual cycle proposed above, though open to critical evaluation and testing, should attract increased attention of vision researchers of different disciplines to the molecular problems of rhodopsin synthesis and dark-light adaptation of the eye. (2) Abnormal rod function observed under certain pathological conditions, e.g. retinal dystrophy, may be related to degeneration of phospholipids associated with rod disc membranes. For example, phospholipid peroxidation by light has been suggested to be responsible for Turkey Blindness Syndrome, a veterinary disorder characterized by a degenerative endophthalmitis with detachment of the retina.

Proposed Course of Project: (1) The quantitative aspects of the photoisomerization reaction mediated by PE, including quantum efficiency and detailed reaction kinetics, will be investigated. (2) Rod disc membranes are unique in that most of the PE molecules associated with the membrane contain a highly unsaturated fatty acid, docosahexaenoic acid (C_{22} Δ_6 ω_3), at the β position of the glycerol moiety. Since the fatty acid may be important for retinal-PE complex formation, requirements for specific fatty acid side chains in PE for the photoisomerization reaction of retinal will be studied. (3) Specific requirements of opsin structure and conformation for retinal binding activity, i.e. for visual pigment synthesis, will be investigated by chemical modification of various amino acid residues of opsin and by reacting cis isomers of retinal with modified opsin preparations.

Honors and Awards: None

Publications:

Shichi,H.: Conformational aspects of rhodopsin associated with disc membranes. Exp. Eye Res. 17: 533-543, 1973.

Shichi, H., and Shelton, E.: Assessment of physiological integrity of sonicated retinal rod membranes. J. Supramol. Struct. (in press).

Serial No. NEI-73 LVR-138
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Photoexcitatory Processes in Visual Cells

Previous Serial Number: None

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
Professional	2
Others	0

Project Description:

Objectives: Two basic phenomena which unify many different areas of life science are how ions and other substances move across cellular membranes and how the cell governs these movements. These questions are particularly central in the study of the visual system, whether the investigation is concerned with the control of incoming radiation or its detection and interpretation.

In the visual receptor cell, the movement of ions 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 of 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 ions across the visual cell membrane are the focal points of our investigations.

Methods Employed: The ionic currents of the visual cells are being studied by electrical techniques in conjunction with very rapid pertubations of the milieu of the retina.

To determine the ionic contents of the visual cell in response to light, metabolic inhibitors, and rapid changes in ionic environment, the electron microprobe analytical method is being adapted and developed for our purpose.

The ionic fluxes of visual cells are being studied with aid of radionuclides and fluorescent probes on isolated receptor cells.

Major Findings: 1) Nature of the excitatory transmitter: We are continuing to test our hypothesis that calcium ion is the excitatory transmitter in the photoexcitation process in rod and cone cells. If the calcium concept is correct, increasing or decreasing the A_{Ca}^{2+} within these cells should respectively decrease or increase the dark current. The calcium ionophore X-537A, an agent known to increase the permeability of biological membranes to calcium, permits us to make this test. The addition of X-537A to the plasma membrane of visual cells should cause the intracellular A_{Ca}^{2+} of these cells to reflect more closely than normal the A_{Ca}^{2+} of the extracellular space. Consequently the dark current of visual cells treated with X-537A should become more sensitive to changes in extracellular A_{Ca}^{2+}. We have found that this is indeed the case. The calcium ionophore X-537A increases the sensitivity of the dark current to external calcium by about 2000 fold.

2) The metabolism of the visual cell: We find that the large visual cell ionic current is tightly coupled to the metabolic energy production of the cell and hence they influence one another strongly. Studies are being conducted on the relation between the biochemical mechanisms of the visual cell and the production and regulation of the ionic currents.

3) Ionic flux analysis: Keystone to the calcium hypothesis is the demonstration of light-induced calcium flux changes of the receptor cell. Experiments are currently underway to examine this.

4) Ionic analysis of the visual cell: We have been able to obtain qualitative results on the ionic content of the visual cell and are currently developing methods to obtain quantitative information by use of the electron microprobe.

5) Topology of photoreceptor membranes: We have discovered several nontoxic fluorescent probes which permit us to distinguish in the living retina the topology of its photoreceptor membranes and measure the intactness of these membranes before and after isolation of visual cells. These probes clearly allow one to differentiate visually the membrane topology of rod type from that of cone in the live retina. The significance of this observation is that it supports the evidence that an excitatory transmitter is present in the excitatory process of the rod visual cell. These probes also serve to report whether or not the plasma membrane of the rod is intact or disrupted. This latter measurement is needed to interpret correctly some of the studies outlined above. Moreover, these fluorescent membrane probes will be useful in the investigation of the biochemistry of isolated visual cells or rod outer segments.

Significance to Biomedical Research and the Program of the Institute: The understanding of how membrane-bound proteins control the passage of materials through membranes is of foremost importance whether these materials be ions in the photoexcitatory process or ions and substrates of various sorts in the

processes of development and maintenance of ocular and nervous tissues. Revalations of how visual pigments, probably the best characterized membrane protein, control movement of materials across membranes will certainly be a major contribution to biomedical research.

The successful adaptation of the electron microprobe for the quantitative study of biological cells will have impact in many areas of biomedical science for it will fulfill the demand for a rapid and sensitive method for determining the ionic compositions of cells as small as one micron in diameter.

Our finding that calcium plays a very dramatic and central role in the excitatory process in vision suggests that abnormalities in calcium metabolism by ocular tissues may lead to impairment of vision. Furthermore, it may be highly significant if any pathology of vision could be understood on this basis.

The fluorescent probes we discovered which report the nature of the isolated visual receptor plasma membrane may be of use to others studying membranes, in particular to those who are investigating the biochemistry of isolated rod outer segments.

Proposed Course of Project: A clear understanding of the photoexcitatory process of the visual cell is dependent on our knowledge of its ionic and biochemical contents, the ionic fluxes across its plasma membrane, and how the visual pigment participates in the control of the ionic currents. Investigations in these areas on the visual cell will be continued.

Honors and Awards: None

Publications:

Yoshikami, S. and Hagins, W.A.: Control of the dark current in vertebrate rods and cones. In Langer, H. (ed.): Biochemistry and Physiology of Visual Pigments, p. 245, Springer-Verlag, Berlin, 1973.

Serial No. NEI-71 LVR 001

1. Laboratory of Vision Research
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ocular Morphogenesis

Previous Serial Number: Same

Principal Investigator: A. J. Coulombre, Ph.D.

Other Investigators: Jane L. Coulombre, B.S.
David Reese, Ph.D.
Yasuhiko Tsunematsu, M.S (Guest Worker)

Cooperating Units: Clinical Branch, National Eye Institute; Intramural Research, National Institute of Child Health and Human Development; Department of Surgery, School of Medicine, University of California, Los Angeles

Man Years:

Total:	3.375
Professional:	2.000
Other:	1.375

Project Description:

Objectives: The project seeks to identify and to characterize the 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 for 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 wide variety of teratological influences.

Methods Employed: Routine chemical, histochemical, experimental-embryological, light-microscopical, electron-microscopical and tissue-cultural techniques are used to analyze the development of the eyes and visual systems in: newts; in embryos of frogs and domestic fowl; and in embryos, fetuses or adults of the mouse and man.

Major Findings: During FY74 information was published under this project bearing upon three of the current programs of the Institute.

1. Retinal and choroidal diseases: A. Production of collagen (a fibrous protein) by pigmented epithelium (PE): Recent work under this project, and in other laboratories, has demonstrated that several sheets of cells (epithelia) of the developing eye synthesize, and deposit outside the cells, layers containing fibrils of collagen. These findings correct the long-standing misconception that only cells of mesenchymal origin synthesize fibrillar collagen. This year we reported success in cloning PE cells from the retinas of embryos of the domestic fowl. Experiments using these cultures added the retinal PE to the list of epithelia known to produce collagen and yielded several other findings. 1. Cells retained their cellular polarity in tissue culture. 2. Populations derived from single PE cells aggregated in polar register, became joined at their apices by junctional complexes and formed extensive epithelial sheets. 3. Such sheets deposited collagen in two forms (basement lamina and fibrils) at their basal surfaces. The fibrils: a. formed only where basement lamina was already present; b. had a 550-600A macroperiod; c. had diameters which correlated positively with donor age and, less markedly, with time in culture. B. These results have been, or are being applied in several ways. In FY73 we reported their use to demonstrate that the collagen-bearing layer produced by the PE can induce the sclera, a finding that carries a step forward our understanding or normal development of the eye wall. Additionally, the ability to grow relatively large, continuous sheets of PE in isolation on millipore filters is being exploited by others for studies of transport, and of other physiological functions of this layer of the retina, which could not be carried out in vivo.

II. Cataract: The following observations extend previous work on the initiation in organ cultures of the early molecular events associated with the metaplastic transformation of iris into lens: A. the highest rate of DNA replication occurs at pH 8.0, the same pH requirement observed previously for RNA synthesis; B. the initiation of DNA replication can be blocked by hydrocortisone, whereas RNA synthesis does not appear to be affected; C. epinephrine seems to have a slight stimulating effect on both DNA and RNA synthesis; D. there is a dialyzable factor (or factors) in fetal calf serum which causes a two-fold reduction in the uptake and/or the incorporation of pyrimidines into RNA, while purines do not appear to be affected; E. one purine (adenosine), however, shows a two- to three-fold increased uptake into RNA if bovine serum albumin is substituted for fetal calf serum.

III. Corneal Disease: Clinically recognizable immunologic graft rejection occurs with significant frequency following penetrating keratoplasty, particularly in patients with vascularized corneal beds. As steps in evaluating the role of HL-A histocompatibility antigens in such rejections of corneal allografts, the project (in collaboration with members of the Clinical Branch, NEI; Intramural Research, NICHHD; and the Department of Surgery, School of Medicine, University of California, Los Angeles):

A. established the stability of morphological and cytological characteristics of serially-propogated, pure cultures of human corneal epithelial cells, endothelial cells and keratocytes; B. for the first time, demonstrated, by cytotoxic methods, the presence of HL-A antigens in these cells.

Significance to Biomedical Research and the Program of the Institute:
The project makes contributions, over any period of several years, to all five of the NEI programs. In FY74 the contributions of the project related to three NEI programs: Retinal and Choroidal Diseases; Cataract; Corneal Diseases. A. The demonstration that the PE cells of the embryonic retina can synthesize fibrillar collagen lends substance to the suspicion, long entertained, that these cells may produce the abnormal deposits of collagen seen in such disorders as senile maculopathy and Drüsen formation. B. The discovery that collagen-bearing layers produced by retinal PE can induce the sclera, focuses attention on the possible involvement of this epithelium in conditions such as blue sclerae and some colobomata. C. Currently used strategies for dealing with lens cataracts include preventive measures and lens extraction. Two very remote possibilities require extensive basic work before even their promise as potential options can be assessed: the development of specifics for the arrest or reversal of cataractous processes; and the biological replacement of the lens. This project has used two experimental approaches to the latter possibility. In the past we have identified and characterized some of the factors governing reconstitution of the lens from surgically implanted lens epithelium. This year emphasis was placed on in vitro studies extending the list of previously-identified factors governing the early steps in the metaplastic regeneration of lens from the dorsal iris of newts, animals with a natural ability to regenerate a normal lens. These findings define more rigorously the in vitro conditions that optimize early steps in Wolffian lens regeneration. D. The localization of HL-A antigens in corneal cells: 1. lays a basis for the analysis of immune sensitization of the host by the donor graft in cases of penetrating keratoplasty; 2. should facilitate studies of the role of the major histocompatibility factors in the rejection of corneal allografts; and 3. indicates the desirability of exploring further the use of HL-A matching procedures to imporove prognosis following this operation. E. During FY74 the project submitted for publication, for ophthalmologists, a summary of the relationships between disruption of specific tissue interactions during ocular morphogenesis and the subsequent development of congenital anomalies of the eye. The list of anomalies analyzed in this way, and in part on the basis of findings made over the years by this project, included: anophthalmia, cyclopia, congenitally cystic eye, aphakia, partial ocular agenesis, metaplastic production of neural retina from the outer wall of the eye cup, pure microphthalmia (nanophthalmia). persistent corneal-lenticular stalk, dysplasia of the lens, microphthalmia, aniridia, aplasia or hypoplasia of the optic disc, blue sclera, congenital absence of the cornea, cornea plana, microcornea and coloboma.

Proposed Course of Project: The project continues to focus on the tissue interactions involved in ocular embryogenesis with current emphasis on the roles played in morphogenetic foldings and induction by epithelially-produced extracellular matrices. During FY 1975 we will use embryos of domestic fowl to investigate: 1. the role of proteoglycans in the establishment of the collagenous architecture of the corneal stroma; 2. the roles of collagen and proteoglycans in mediating the induction of scleral ossicles by conjunctival papillae; 3. the development of permeability barriers in the epithelium and endothelium of the cornea; and 4. the involvement of embryonic basement membranes in the invaginations of the lens and optic cup.

Honors and Awards: None

Publications:

Coulombre, A. and Coulombre, J.: The skeleton of the eye. II. Overlap of the scleral ossicles of the domestic fowl. Dev. Biol. 33: 257-267, 1973.

Coulombre, A. and Coulombre, J.: Morphogenesis of the eye. In Zinn, K. (Ed.): International Ophthalmology Clinics, Boston, Little, Brown & Co. (in press).

Coulombre, A., Johnston, M. and Weston, J.: Conference on neural crest in normal and abnormal embryogenesis. Dev. Biol. 36: f1-f5, 1974.

Goldberg, S.: Studies on the mechanics of development of the visual pathways in the chick embryo. Dev. Biol. 36: 24-43, 1974.

Kenyon, K.: Ocular ultrastructure of inherited metabolic diseases. In Goldberg, M. (Ed.): Genetic and Metabolic Eye Disease. Boston, Little, Brown & Co., 1974, pp. 139-185.

Kenyon, K., Sensenbrenner, A. and Wyllie, R.: Hepatic ultrastructure and histochemistry in mucolipidosis II (I-cell disease). Pediatr. Res. 7: 560-568, 1973.

Newsome, D. and Kenyon, K.: Collagen produced in vitro by the retinal pigmented epithelium of the chick embryo. Dev. Biol. 32: 387-400, 1973.

Newsome, D., Takasugi, M., Kenyon, K., Stark, W. and Opelez, G.: Human corneal cells in vitro: morphology and histocompatibility (HL-A) antigens of pure cell populations. Invest. Ophthalmol. 13: 23-32, 1974.

Serial No. NEI-73 LVR 129
1. Laboratory of Vision Research
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Anatomic and Pathologic Studies of Ocular Tissue

Previous Serial Number: Same

Principal Investigator: Toichiro Kuwabara, M.D.

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

Cooperating Units: None

Man Years:

Total:	3.7
Professional:	2.8
Other:	0.9

Project Description:

Objectives: A great number of unexpected but valuable eye specimens are sent to this laboratory from the NIH Clinical Center and various sources. Detailed studies of these materials have been performed effectively by advanced techniques and knowledge. Results from these studies lead into direct understanding of various diseases of the eye.

Methods Employed: Normal and pathologic eyes are studied by light microscopy, histochemistry, transmission electron microscopy and scanning electron microscopy. Several animal experiments are pursued for the control study of the corresponding pathologic materials.

Major Findings: Microcystic dystrophy of the cornea epithelium is studied electron microscopically (Dr. Cogan). The study reveals that this disease begins with formation of an abberant basement membrane within the epithelium. The epithelial cells which are trapped beneath the abnormal basement membrane appear to undergo degenerative change and form the cyst. The epithelial layer over the basement membrane is free from the cystic change.

Congenitally cataractous mouse lens has been studied electron microscopically (Dr. Hamai). The maturation of the lens cell of the mutant animal appears to occur slower than that of the normal cell. Nucleated cells are found to be accumulating abundantly in the bow zone. Also, these lens cells persist their microorganelles. The earliest degenerative change, swelling of lens fibers, is found in the posterior cortical zone.

Attachment of the retina to the pigment epithelium is studied by scanning electron microscopy (Dr. Sakuragawa). The outer tips of the photoreceptor cells and the microvilli of the pigment epithelium are demonstrated clearly. This study reveals that it is almost impossible to separate the retina from the pigment epithelium without breaking the fine microvilli of the latter cells. A gentle lifting of the fresh retina always results in breaking of the microvilli. The attachment of the retina to the pigment epithelium appears to be firmer than generally thought. This information is significantly important for understanding the pathophysiology of retinal detachment.

Dr. Avins (NICHD) has demonstrated the electron microscopic view of the optic neuropathy which occurs following the administration of para-chlorophenylalanine and phenylalanine to the developing rat. The drugs are found to cause a slowly progressive necrotic change in the optic nerve fiber. Myelin formation by the oligodendroglia cell appears to be inhibited by the toxication. The findings explain the pathogenesis of the neuropathology of human phenylketonuria.

The denucleating mechanism of the lens cell is studied in developing mice (Dr. Kuwabara). The appearance of the nucleus of the maturing lens cell becomes indistinguishable from that of the surrounding cytoplasm. Then the nuclear membrane disappears through vesicular changes. No degeneration or extrusion of the nucleus is demonstrated. However, the nucleoli are fragmented and remain within the denucleated lens cells for a long period of time. These materials have generally been called degenerating nuclei.

Significance to Biomedical Research and the Program of the Institute: Detailed study of pathologic materials is one of the most basic and important tasks in biomedical research. This laboratory is one of a few laboratories which is capable of pursuing this type of research on pathologic eye materials.

Proposed Course of Project: A similar project will be continued in the next fiscal year.

Honors and Awards: None

Publications:

Sanderson, P. O., Kuwabara, T., Stark, W. J., Wong, V. G., Collins, E. M.: Cystinosis, A Clinical, Histopathologic, and Ultrastructural Study. Arch. Ophthalmol. Vol. 91: 270-274, 1974.

Robb, R. M. and Kuwabara, T.: The Ocular Pathology of Type A Niemann-Pick Disease. Invest. Ophthalmol. Vol. 12, No. 5, pp. 366-377, 1973.

Hamai, Y., Fukui, H. N. and Kuwabara, T.: Morphology of Hereditary Mouse Cataract. Exp. Eye Res. Vol. 18, 1974.

Serial No. NEI-73 LVR 130
1. Laboratory of Vision Research
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effect of Laser on the Retina

Previous Serial Number: Same

Principal Investigator: Toichiro Kuwabara, M.D.

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

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	0.7
Other:	0.8

Project Description:

Objectives: Although laser photocoagulation is one of the most widely used therapeutic techniques for diabetic retinopathy, basic studies concerning the cellular damage to the retina from use of this modality have been reported only sparsely. Placement of multiple laser burns in the posterior retina is believed to be effective not only in the treatment but in prevention of further development of angiopathy. A systematic study of these drastically treated retinas has not been documented.

Methods Employed: Retinas of normal rhesus monkeys are photocoagulated by ruby laser following the technique identical to that of the clinical application. Animals are killed at several time intervals and the retinas are studied histologically, electron microscopically and by trypsin digestion technique for retinal blood vessels.

Major Findings: Although necrotic changes in the photoreceptor cells and the pigment epithelium in each burn lesion are extensive, the retinal tissue appears to tolerate the treatment quite nicely. Each lesion is sharply circumscribed and the repair process seems to be taking place within the damaged area. The structure of the retinal tissue between the burns is not disturbed at all. No appreciable swelling or cell infiltration is seen in this normal area. This treatment appears to remove a great number of photoreceptor cells from the retina. Also blood capillaries diminish secondarily.

The simplification of the tissue may be the reason for the clinically beneficial effect of this treatment. Small numbers of surviving retinal cells seem to be sufficient to maintain the normal function of the retina.

Significance to Biomedical Research and the Program of the Institute: The findings of the present study favorably support the use of photocoagulation therapy. The multiple laser burn appears to be safe to the retinal tissue.

Proposed Course of Project: Effect of the newly developed YAG laser is under investigation. Ultrashort pulses of radiation of the YAG laser have been given to several monkey retinas.

Honors and Awards: None

Publications: None

Serial No. NEI-73 LVR 131
1. Laboratory of Vision Research
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Light Effect on the Retina

Previous Serial Number: Same

Principal Investigator: Toichiro Kuwabara, M.D.

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

Cooperating Units: None

Man Years:

Total:	1.7
Professional:	0.8
Other:	0.9

Project Description:

Objectives: Among several important contributions in the recent retinal studies, demonstrations of the morphologic changes of the outer segment membranes by light and of the renewal mechanism of the photoreceptor cells appear to be most significant. The basic change of the photoreceptors by non-thermal light exposure has not been demonstrated in the monkey retina. Electron microscopic details of minute changes of the photoreceptor membranes are to be demonstrated.

Methods Employed: Retinas of the rhesus monkeys are exposed to continuous (10 flashes per second) flash light by an electronic strobe light installed in the lamp house of an ophthalmoscope. Animals are divided into two groups. One group is exposed for one hour and the other is exposed by three daily 30-minute exposures. Animals are examined electron microscopically at various time intervals after the exposure.

Major Findings: All exposed retinas begin to show pathologic changes in the outer segments starting on the second day. Two days after the one-hour exposure, the plasma membrane of the outer segment is fragmented and vesiculated. The stack of the disc membranes becomes irregular. After the three 30-minute exposures, the outer segments form knots a few microns away from the ciliary junction. The outer segments close to the inner segment, which are believed to be newly formed portions, are found to be larger than the distal halves. This suggests that mild repeated stimulation resulted in hypertrophy of the outer segments.

These changes are found to be staying in the retina for a long period of time. Irregular arrangement of the outer segments is found one year after the exposure. Also a small number of photoreceptor cells in the exposed area begin to degenerate in the later period.

Significance to Biomedical Research and the Program of the Institute: It is common to find pathologic changes which are identical to those of the present experiment in the outer segments of the "normal" human retinas, particularly at the macular zone. These findings indicate that the human retina may accumulate photic changes and that along with certain other factors, photic damage may induce the macula degeneration.

Proposed Course of Project: Similar experiments on the rhesus monkey with different conditions in the energy and duration are under way. Also using albino rats, chronic effects of low dose exposure (100 foot-candles) are sought for. Other experiments which have been pursued actively are on the effect of cyclic illumination of the retina.

Honors and Awards: None

Publications: None

Serial No. NEI-73 LVR 132
1. Laboratory of Vision Research
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effect of Intraocular Pressure on the Ciliary Epithelium

Previous Serial Number: Same

Principal Investigator: Shigekuni Okisaka, M.D.

Other Investigators: Toichiro Kuwabara, M.D.
Masakazu Funahashi, M.D.

Cooperating Units: None

Man Years:

Total:	1.9
Professional:	1.1
Other:	0.8

Project Description:

Objectives: The mechanism and site of aqueous formation remain major questions in eye physiology and are important to understanding the pathogenesis of glaucoma. The function of each layer of the ciliary epithelium, which has not been clearly shown, is to be demonstrated.

Methods Employed: Rhesus monkeys are used in this study. Fine structure of the ciliary epithelium is studied after alteration of the intraocular pressure in various conditions and durations.

The most extensively performed experiment during this fiscal year is the perfusion of the internal carotid artery with hypertonic solution. Two molar solutions of either urea or lactamide are perfused into the carotid artery. Also, intravascular Evans blue-albumin is used as a protein tracer for determining the integrity of the blood-ocular barrier. Intraocular pressure is measured following perfusion, and eyes are enucleated at various times from 1 minute to 3 months thereafter.

Major Findings: The intraocular pressure of the perfused eye falls from about 12.5mm Hg before perfusion to about 2mm Hg within one hour, remains at this low level for several days, and then slowly increases until it becomes normal 3 to 6 weeks later. One minute following hypertonic perfusion, diaphragms are absent from fenestrae of the capillaries of the ciliary body.

One day later, the fenestrae are obliterated entirely.

The ciliary epithelium is swollen one minute after perfusion, and intercellular spaces are expanded between the pigmented and non-pigmented layers. The necrotic change of the pigmented epithelium progresses until, after one day, macrophages have invaded the pigmented layer to take up the cellular debris. At this time, however, non-pigmented cells appear normal. As intraocular pressure approaches normal level at 3 to 6 weeks after perfusion, macrophages recede from the layer and connective tissue fills the sites they have occupied. It can be seen that the pigmented cell population is reduced by 50 to 80% below normal, particularly at the pars plana. The non-pigmented cells appear entirely normal.

Significance to Biomedical Research and the Program of the Institute: Findings in this investigation are totally new to the present knowledge of the glaucoma physiology. The accumulating information from this study is significantly approaching a newer understanding of the function of the ciliary epithelium.

Proposed Course of Project: By the simple perfusion of hypertonic solution, the pigmented layer of the ciliary epithelium seems to maintain the normal intraocular pressure. These monkey eyes will be used for various experiments to pinpoint the function of the non-pigmented epithelial cell.

Honors and Awards: None

Publications:

Okisaka, S., Kuwabara, T. and Rapoport, S.I.: Selective destruction of the pigmented ciliary epithelium in the ciliary body of the eye. Science, (in press) 1974.

Serial No. NEI-73 LVR 133
1. Laboratory of Vision Research
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Functions of Cell Microtubules and Microfilaments in Ocular Tissues

Previous Serial Number: Same

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

Other Investigator: Joram Piatigorsky, Ph.D. (NICHD)

Cooperating Units: Laboratory of Molecular Genetics, NICHD

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.3

Project Description:

Objectives: Our aim is to determine the contributions made by cytoplasmic microtubules and microfilaments to the normal differentiation, changes in shape, secretion cycles, movements, and contractions of ocular cells.

Methods Employed: Vinblastine sulfate, colchicine, and related drugs are used to selectively destroy microtubules in incubated eye cells. Cytochalasin B is used to selectively eliminate microfilament function. In collaboration with Dr. Joram Piatigorsky (NICHD), lens epithelial cells have been incubated in organ culture in the presence of insulin, cyclic nucleotides, theophylline, papavarine, and various growth media in order to obtain different degrees of cell development. The number and organization of microtubules are analyzed by high resolution electron microscopy.

Major Findings: Preliminary results suggest that, while microtubules are essential for cell elongation, their presence alone is not sufficient to effect elongation. Apparently, other factors are required to trigger the normal process which permits a lens epithelial cell to elongate and form a mature lens fiber.

Significance to Biomedical Research and the Program of the Institute: Drugs which destroy cell microtubules reversibly are among those which are

used commonly in cancer chemotherapy. It is essential for us to understand the precise functions of microtubules in ocular cells if we are to determine accurately the dosage levels which are safe for the maintenance of normal vision. Microtubules are conspicuous components of developing lens cells, inner segments, and filament regions of photoreceptor cells, and of nerve axons. It is conceivable that drug overdose could lead to cataract formation if such destroys the microtubules in an elongating lens cell during a critical period. Likewise, vision may be impaired by disruption of microtubules in photoreceptors and axons. This study should help determine if any of these ocular cells, which depend on microtubules and microfilaments for their normal function, may be sensitive to chemotherapy and other environmental or hereditary insults.

Proposed Course of Project: After the work on cultured lens cells is completed the efforst will be directed to investigating the role of microtubules in visual cells.

Honros and Awards: None

Publications:

Robison, W. G., Jr. and Charlton, J. S.: Microtubules, microfilaments, and pigment movement in the chromatophores of Palaemonetes vulgaris (Crustacea). J. Exp. Zool. 186: 279-304, 1973.

Harris, W. F. and Robison, W. G.,Jr.: Dislocations in microtubular bundles within spermatozoa of the coccid insect Neosteingelia Texana and evidence for slip. Nature (London) 246: 513-515, 1973.

Serial No. NEI-73 LVR 149
1. Laboratory of Vision Research
2. Section on Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

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

Previous Serial Number: None

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

Other Investigators: Toichiro Kuwabara, M.D.
David G. Cogan, M.D.
David A. Newsome, M.D.
R. Theodore Fletcher
Kenneth R. Kenyon, M.D.
Gerald J. Chader, Ph.D.

Cooperating Units: None

Man Years:

Total:	3.1
Professional:	2.8
Other:	0.3

Project Description:

Objectives: Our purpose is to investigate and define more precisely the intimate physical and chemical interrelationships that exist between the pigmented cells and the visual cells of the eye. Pathological studies of retinal detachments and of several other causes of blindness indicate that pigmented cells are essential to maintain the structural integrity of the retina, and to support the vital functions of the photoreceptor cells.

We propose to study how the pigmented cells perform their roles and what specific aspects are absent in various pathological cases.

Methods Employed: We have utilized an hereditary pathological condition as an experimental tool to investigate how the membrane discs of the rod outer segments are digested by the pigment epithelium. The Chediak-Higashi syndrome of humans represents a block in the digestive mechanisms of cells. The beige mutant of the C57 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 cytochemistry at both levels.

Major Findings: We have compared the digestive mechanisms and melanogenesis of the pigment epithelium of beige and normal (C57) mice with the intent of determining more precisely the origin of the defect in beige mice and with the hope of elucidating the normal processes of intracellular digestion and melanin formation. Cytochemistry at the electron microscope level has enabled us to localize within the pigment epithelial cells and choroidal cells the enzymes that are involved (acid phosphatases and catalases). Differences in the packaging of these enzymes by normal and mutant pigment epithelial cells permit a greater definition of the steps involved in the formation of lysosomes and melanosomes. There is more relation between these processes than previously known.

Little is known regarding the replacement of damaged pigment epithelial cells and the control of their normal development. In collaboration with Dr. Chader and others of this Laboratory we have found that cyclic AMP promotes a reasonable amount of differentiation in pigment epithelial cells which are dissected from chick embryos and grown in a tissue culture medium which retards differentiation in the absence of cyclic AMP.

Significance to Biomedical Research and the Program of the Institute: Only by clear definitions of the normal functions and healthy states of ocular cells can we provide precise diagnosis of pathological conditions and seek treatments free of undesirable side effects. Studies on pigmented cells of the eye contribute directly to our understanding and managing of many retinal and choroidal diseases.

Proposed Course of Project: Other mutants will be used as tools 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.

Honors and Awards: None

Publications:

Newsome, D. A., Fletcher, R. T., Robison, W. G., Jr., Kenyon, K. R. and Chader, G. J.: 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.

Serial No. NEI-71 LVR 026
1. Laboratory of Vision Research
2. Section on Neurophysiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Physiology of the Primate Visual System

Previous Serial Number: Same

Principal Investigators: Peter Gouras, M.D.
Francisco de Monasterio, M.D., Ph.D.
(Foreign Visiting Scientist)
Edward Famiglietti, M.D., Ph.D.
(Research Associate)

Other Investigators: Helga Kolb, Ph.D. and
Eleanor Collins of the Clinical Branch, NEI

Cooperating Units: Dr. Peter Padmos, Institute of Perception
Sosterberg, The Netherlands

Man Years:

Total:	2.5
Professional:	2.5
Other:	0

Project Description:

Objectives: To understand the neural organization underlying visual perception. We have been concentrating on the function and connectivity of single cells subserving the foveal area of vision in the retina, lateral geniculate nucleus and striate cortex of the rhesus monkey.

Methods Employed: Electrophysiological recording from single neurons of different levels of the visual system of anesthetized monkeys; correlation of responses of single cells in the layers and morphological cell types as seen by light and electron microscopy; the use of refined optical stimuli to quantitatively define the receptive field properties of cells.

Major Findings: During the past year experiments were completed which had been begun with Dr. Pieter Padmos of the Institute of Perception, Sosterberg, Netherlands on the analysis of graded visually evoked extracellular and intracellular responses from the foveal region of striate cortex of anesthetized, paralyzed rhesus monkeys. The findings show that the earliest electrical responses detectable in foveal striate cortex following light stimulation

is a graded extracellular potential which is positive at the cortical surface and negative in the grey matter and has a peak latency of about 60 msec. The response is similar at both the on- and the off- phase of a light stimulus. The relationship of this graded potential to the depth of the recording electrode, to the latency of extracellular impulses and to post-synaptic potentials indicates that it is generated by the depolarization of cortical cells. Action spectra obtained in the presence of strong selective chromatic adaptation indicate the participation of all three cone mechanisms in this response. Each cone mechanism contributes a similar potential to the response but antagonism between cone mechanisms is apparent. The proportion in which a cone mechanism contributes to the response varies from one area to another implying topographical differences in the representation of cone mechanisms in striate cortex.

These findings could be correlated with those obtained from extracellular recordings of single cells made in the same area of striate cortex which have revealed the following information. The majority of cells in layer 4B have opponent-color properties indicating that color opponency plays an important role in the early stages of visual processing in foveal striate cortex. In contrast to cells in the lateral geniculate nucleus many of these cortical cells receive center-surround antagonism from the same cone mechanism. Some cells show this spatial antagonism at threshold; others require supra-threshold stimuli for its demonstration. The majority of cells in layer 4B do not show orientation or directional selectivity. The proportion of cells with orientation and directional selectivity increases and the proportion of opponent-color cells decreases with increasing distance above and below layer 4B so that the majority of cells in the outer layers exhibit considerable spatial selectivity without apparent color opponency. These changing proportions suggest that the latter cells may be receiving their inputs from different types of opponent color cells making them sensitive to different types of color contrast but not to color per se. More opponent-color cells receive inputs from the red and green sensitive cone mechanisms than from the blue sensitive one. This difference is more marked in layer 4B than 3B suggesting that the latter cortical layer may be more involved in color vision than the former.

Dr. Francisco de Monasterio has been able to classify six different classes of ganglion cells in various regions of monkey retina including the foveola. Two of these classes have never been reported before neither in the retina nor lateral geniculate nucleus of this animal and are therefore of great interest as to their destination and role in the primate visual system.

Dr. Edward Famiglietti has collaborated with Dr. Helga Kolb in the combined use of Golgi, electronmicroscopy (EM) and serial section EM technique to work out the connections of rod and cone bipolar cells in cat and primate retina and to discover a unique pathway for rod signals to ganglion cells through a special amacrine cell.

Significance to Biomedical Research and the Program of the Institute: Such studies of retinal function at the cellular level should prove valuable for understanding vision and pathophysiology of retinal diseases.

Proposed Course of Project: To continue to explore the foveal-geniculo-striate projection system in the rhesus monkey.

Honors and Awards:

Invited lecture, Gouras, P.: Color and spatial specificity in foveal striate cortex. The Colloquium on Neurobiology in Medicine and Psychology. The University of Zurich, Switzerland, July 4, 1973.

Invited lecture, Gouras, P.: Color coding in striate cortex. Psychology Department, University of Maryland, College Park, Md. October 25, 1973.

Publications:

Gouras, P. and Padmos, P.: Identification of cone mechanisms in graded responses of foveal striate cortex. J. Physiol. (Lond.) (in press) 1974.

Gouras, P.: Opponent-colour cells indifferent layers of foveal striate cortex. J. Physiol. (Lond.) (in press) 1974.

Serial No. NEI-73 LVR-110
1. Laboratory of Vision Research
2. Section on Neurophysiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

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

Previous Serial Number: Same

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

Other Investigators: None

Cooperating Units: Eleanor Collins, Clinical Branch, NEI

Man Years:

Total:	1.1
Professional	1.0
Other:	0.1

Project Description:

Objectives: To understand how information about the external world is reflected in the neuronal firing patterns and morphological organization of rhesus monkey visual cortex.

Methods Employed: Extracellular recordings are obtained from single neurons in the visual cortex of anesthetized rhesus monkeys using glass micropipette electrodes. Each animal is refracted and supplied with contact lenses sufficient to focus his eyes on a tangent screen upon which visual stimuli are projected. Stimuli can be presented either manually or with a servomotor device specifically designed for this purpose. Independently variable stimulus parameters include shape, size, orientation, velocity and direction of movement, pulse duration, intensity, and color. Electrode recording sites can be marked by the injection of colored dye from the micropipette tip.

Major Findings: At least three independent information-processing systems can be distinguished in the primary visual cortex (area 17) of the rhesus monkey. The majority of color sensitive cells respond without regard to stimulus shape or direction of movement. Cells in a second group respond only to very slowly moving, precisely oriented light bars without regard to color or direction. Cells in a third group respond preferentially to stimulus movements in one direction, without regard to color or line orientation. Most cells in area 17

are thus specialized for the detection of a single stimulus feature at the expense of other features.

The color cells tend to be located in two distinct regions (layers 3B and 4B) near the termination sites of lateral geniculate axons, whereas directional cells are usually found in layer 4A, which contains axon terminals from adjacent cortical regions. Orientation-specific cells tend to occur in the upper- and lower-most layers (2, 3A, 5, and 6).

Significance to Biomedical Research and the Program of the Institute: It is essential that we understand the normal mechanisms of information-processing in the visual cortex 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 of Project: An attempt will be made to examine some of the outputs of area 17 using both anatomical and physiological techniques.

Honors and Awards:

Invited lecture: Functional specialization of visual cortex laminae in the monkey, at MIT Colloquium, Cambridge, Massachusetts, March 1, 1974.

Publications:

Leighton, S.B. and Dow, B.M.: Servo-controlled moving stimulus generator for single unit studies in vision. Vision Res. 13: 1195-1198, 1973.

Dow, B.M.: Functional classes of cells and their laminar distribution in monkey visual cortex. J. Neurophysiol. (in press).

Serial No. NEI-71 LVR 005
1. Laboratory of Vision Research
2. Section on Neurophysiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Electrophysiological Studies of Mammalian Retina

Previous Serial Number: Same

Principal Investigators: Ralph Nelson, Ph.D. (NIH Postdoctoral Fellow)
Peter Gouras, M.D.

Other Investigators: Helga Kolb, Ph.D.

Cooperating Units: Astrid Kafka, M.D., University of Vienna, Austria
Gerald Chader, Ph.D., Section of Biochemistry, LVR
Marshall Nirenberg, Ph.D., Laboratory of Biochemical Genetics, NHLI

Man Years:

Total:	1.5
Professional:	1.5
Other:	0

Project Description:

Objectives: To understand the functional organization of mammalian retina and its relationship to disease states.

Methods Employed: Recording from single cells in tissue culture or in isolated perfused or superfused mammalian retina; recording of multicellular responses from different retinal layers; examination of the retina by light and electronmicroscopy.

Major Findings: Drs. Nelson and von Lutzow have devised a preparation of the cat retina which has allowed them to make intracellular recordings from most of the neuronal types therein and to stain the neurons with procion yellow dye. This marks the first time that such techniques have been successfully applied to any mammalian retina. The responses of "A" and "B" type horizontal cells, horizontal, cell axon terminals, both rod and cone bipolar cells, two distinct types of amacrine cells, and several classes of ganglion cell have been identified.

Horizontal cells have been shown to have unusual properties which may prove to be broadly significant to our understanding of the central nervous

system. One such astonishing finding was that the axon terminal of the "B" type horizontal cell has responses which are unrelated to those found in its cell body. Whereas only 40% of the amplitude of the cell body response is generated by rods, 80% of the amplitude of the axon terminal response is generated by rods. Furthermore the rod signal of the axon terminal has been shown to be over 1 log unit more sensitive than that of the cell body. Thus the "B" type cell body and its axon terminal system appear to be functionally independent units. The "A" type horizontal cell presents a second challenge to our understanding of neuronal circuits. Although the elegant anatomical work of Dr. Kolb has shown that these horizontal cells are postsynaptic only to cones, nonetheless as in the "B" type cell body, about 40% of the responses of these units were generated by rods. This result may imply a kind of synaptic interaction which has not yet been identified electronmicroscopically. Other neuronal types are currently under intensive study and it seems possible that an exhaustive description of neuronal properties in the cat retinal may emerge in the next few years.

Dr. Gouras has been collaborating with Drs. G. Chader and M. Nirenberg on studying the electrical responses of cells in tissue culture to the application of various drugs. Membrane potentials and membrane properties of normal embryonic chick pigment epithelium in tissue culture have been obtained. The responses (Na^+, K^+, and Ca^{++} responses) of neuroblastoma cells have been studied during the application of drugs suspected to bind to specific receptors on the membranes of these cells as determined by biochemical analysis. It is planned to extend this work to retinoblastoma and hybrid retinal cells cloned in tissue culture as well as pigment epithelium of genetically abnormal retinae.

Significance to Biomedical Research and the Program of the Institute: Understanding the cellular physiology of the mammalian retina can only lead to a better understanding of abnormal states observed clinically.

Proposed Course of Project: To continue along the same lines.

Honors and Awards:

Invited lecture: (P. Gouras). Neurocircuitry of the retina. The Bioengineering Department, School of Engineering, University of Virginia, Charlottesville, Va., March 29, 1974.

Publications:

Niemeyer, G. and Gouras, P.: Rod and cone signals in S-potentials of the isolated perfused cat eye. Vision Res. 13: 1603-1612, 1973.

Niemeyer, G.: Intracellular recording from the isolated perfused cat eye. Vision Res. 13: 1613-1618, 1973.

Niemeyer, G. and Gouras, P.: The perfused mammalian eye as a preparation for electrophysiological studies. Doc. Ophthalmolocica, 10th ISCERG Symposium, Junk Publishers (Hague) 1973, pp. 261-268.

Hoff, M. and Gouras, P.: Tolerance of mammalian retina to circulatory arrest. Doc. Ophthalmologica, 10th ISCERG Symposium, Junk Publishers (Hague) 1973, pp. 57-63.

Gouras, P.: Synaptic transmission in the retina. In Dikstein, S. (ed.): Cell Pharmacology of the Eye. Springfield, Ill., Charles C. Thomas, 1974 (Chapter in press).

Gouras, P. and Chader, G.: Retinitis pigmentosa and retinol binding protein (Editorial). Invest Ophthalmol. (in press) 1974.

Kolb, H. and Gouras, P.: Electronmicroscopic observations of human retinitis pigmentosa. Invest. Ophthalmol. (in press) 1974.

OFFICE OF BIOMETRY AND EPIDEMIOLOGY

ANNUAL REPORT
OFFICE OF BIOMETRY AND EPIDEMIOLOGY
July 1, 1973 - June 30, 1974

REPORT OF THE CHIEF, OBE
Harold A. Kahn

During the year the staff of the Office was engaged in a wide variety of activities relating to methods of diagnosis, measurement of the extent of disease, identification of factors related to risk and objective comparison of alternative treatments. These involved a combination of direct research activities by the staff and assistance or consultation to others in the field of vision research. In addition, efforts were directed toward the education of research workers in clinical ophthalmology to appreciate the need for biostatistical and epidemiologic methods. Although the present staff is too small to make satisfactory progress with all of the projects already started or seriously proposed, educational activities are still considered to have one of the highest priorities. Major improvement in the quality of clinical eye research in the United States may well depend in large part on the success of this specialized educational effort.

Honors and Awards:

Harold A. Kahn received the DHEW Superior Service Honor Award.
Fred Ederer was elected a Fellow of the American Statistical Association.

Publications (related to project completed last year):

Ganley, J.P., Comstock, G.W.: Association of toxoplasmosis and cats. Letter to the Editor. Am. J. Epidemiol. 97:424, 1973

Serial No. NEI 72 OBE 100
1. Office of Biometry & Epidemiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Model Reporting Area for Blindness Statistics(MRA)

Previous Serial Number: Same

Principal Investigator: Harold A. Kahn

Other Investigators: Rita Hiller
Helen Moorhead

Cooperating Units: None

Man Years:

Total:	1.7
Professional:	0.3
Other:	1.4

Project Description:

Objectives: The purpose of the MRA study was to determine prevalence and incidence of bilateral legal blindness in the United States and its causes.

Methods Employed: This study was begun in 1962 by the National Institute of Neurological Diseases and Blindness in cooperation with the National Society for the Prevention of Blindness, the American Foundation for the Blind, and the U.S. Public Health Service's Division of Chronic Diseases.

Blindness registries from 16 states, which agreed to meet MRA standards, reported newly recorded cases of legal blindness, persons removed from the register, and those remaining on it at year end. NEI edited and tabulated these data in an annual report, furnished consultation to the registries on data collection and handling, and coded causes of blindness or reviewed cause coding done by the states in order to insure uniformity. During the past year major activity has centered on (a) the analysis of recent data and preparation of papers for the scientific literature, and (b) preparation of a simplified computer tape with common codes for all variables for all MRA States,to be used as a reference book in future years.

Major Findings: Blindness from diabetic retinopathy, although undoubtedly a present serious problem in the U.S.,is not currently increasing.
- Nonwhite females are subject to much higher risks of blindness from diabetic retinopathy than either white females or males of any race.

This higher risk is not completely explainable on the basis of known factors such as higher risk for diabetes and hypertension among nonwhite females.
- Blacks have a very much higher risk of blindness from glaucoma than Whites. We estimate a doubling of risk due to higher prevalence of underlying disease and a further doubling due to a combination of failure to receive early treatment and poorer response to treatment when given.

Significance to Biomedical Research and the Program of the Institute: Identification of high risk groups is obviously helpful in pinpointing further research opportunities as well as in public health applications.

Proposed Course: Except for special needs as may arise, we will discontinue collection and tabulation of MRA data for the next several years so as to release manpower for studies to follow up on research leads that have been derived from MRA. Two papers have been prepared: one is in press, the other is being circulated for critical comment.

Honors and Awards: None

Publications:

Kahn, H.A., Hiller, R.: Blindness caused by diabetic retinopathy. Am. J. Ophthalmol. (in press)

Serial No. NEI 72 OBE 101
1. Office of Biometry & Epidemiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: National Health and Nutrition Survey

Previous Serial Number: Same

Principal Investigator: Harold A. Kahn

Other Investigators: Helen Moorhead

Cooperating Units: Division of Health Examination Statistics
National Center for Health Statistics, HRA, DHEW

Man Years:

Total:	2.2
Professional:	0.2
Other:	2.0

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 geographical areas in the continental U.S., between the ages of 1 and 74, will be 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 persons were examined of a random sample of 17,072 in 33 geographic areas, a response rate of 71.6%. After receiving instruction in the protocol, ocular examinations were performed by house staff and research fellows from various academic institutions. 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. 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 study 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 a measure of the status of ocular health care, and it will provide 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. The next step planned is to tabulate and analyze interobserver variation as determined from the limited number of replicate examinations performed by NEI ophthalmologists on patients examined by the regular examiner. These results will point toward variables that should receive more--and those that should receive less--attention in subsequent tabulations. Analysis of data will be done by NEI and DHES in subsequent years.

Honors and Awards: None

Publications: None

Serial No. NEI 72 OBE 102
1. Office of Biometry & Epidemiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Framingham Eye Study (Contract NIH-NEI-72-2112)

Previous Serial Number: Same

Principal Investigator: Harold A. Kahn (for NEI aspects of the study)

Other Investigators: James P. Ganley, M.D., Dr.P.H.

Cooperating Units: Epidemiological Research Section, NHLI
Department of Preventive Medicine and Ophthalmology, Boston University School of Medicine
Department of Preventive Medicine, Harvard University School of Medicine

Man Years:

Total:	1.4
Professional:	1.0
Other:	0.4

Project Description:

Objectives: The aim of this investigation is to identify individuals among the Framingham Heart Study cohort who at the present time have 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) is being 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 twenty years on members of this group by the National Heart and Lung Institute.

Major Findings: In spite of great efforts to standardize examinations and control observer error, it is not clear that we can be uniformly successful in this regard. Individual variables for which observer error cannot be controlled will be restricted or avoided in later analytical tabulations; however, we will document the need for development of objective measuring

techniques for these variables and warn others to be critical as to their use in other studies.

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 begun in February 1973. Patient examination and data accumulation are expected to take approximately two years. Data processing is occurring simultaneously with data collection to provide early quality control monitoring. This is augmented by OBE staff conducting replicate patient examinations. Data analysis and publication is expected to require an additional two years. As of April 6, 1974, 2,140 persons, or 54% of the total cohort now alive, had been examined. We are just beginning an intensive "roundup" of all subjects still living in the Framingham area who have not yet been examined, including refusals, too sick to come in, hard to contact, etc. The program will include personal home visits wherever possible. Training in the reading of fundus photographs has begun and the procedure of independent reading with agreements accepted and differences adjudicated is to begin as soon as readers have been trained and tested.

Honors and Awards: None

Publications: None

Serial No. NEI 74 OBE 155
1. Office of Biometry & Epidemiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Comparison of Localized Treatments for Bilateral Disease

Previous Serial Number: None

Principal Investigator: Harold A. Kahn

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To write a pedagogical paper that will provide research workers in clinical ophthalmology with some insight into the relative merits of random allocation and matched pair allocation as alternative study designs.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: Clinical research in ophthalmology will benefit substantially from increased understanding and use of biostatistical and epidemiological techniques.

Proposed Course: To the extent that our limited resources permit, the Office of Biometry and Epidemiology will continue educational efforts directed toward increased utilization and appreciation of statistical and epidemiological methods in ophthalmological research. The paper has been accepted by Investigative Ophthalmology for publication as a Letter to the Editor.

Honors and Awards: None

Publications:

Kahn, H.A.: Comparison of localized treatments for bilateral disease. Invest. Ophthalmol. (in press)

Serial No. NEI 73 OBE 116
1. Office of Biometry & Epidemiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Systemic and Ocular Onchocerciasis

Previous Serial Number: Same

Principal Investigator: James P. Ganley, M.D., Dr. P.H.

Other Investigators: John E. Biles, World Health Organization

Cooperating Units: Parasitic Division, World Health Organization

Man Years:

Total:	0.02
Professional:	0.01
Other:	0.01

Project Description:

Objectives: Onchocerciasis is the second leading cause of blindness in Africa, and one of the major causes of ocular morbidity in the world. In an endemic area of Central Africa this disease may cause socioeconomic blindness in 6-8 percent of the total population, a rate for this disease alone that is 30 times greater than the prevalence of all causes of legal blindness in the United States.

To date this disease has been very difficult to control. The World Health Organization is about to begin a concerted effort, in cooperation with seven countries of West Africa, to control the disease in the Volta River basin.

This study team was detailed to the Upper Region of Ghana to collect pre-treatment baseline data on the prevalence of systemic onchocerciasis in the area, and the amount of blindness resulting from ocular involvement with this disease.

Major Findings: None (see Proposed Course)

Significance to Biomedical Research and the Program of the Institute: This study reveals the high frequency of systemic onchocerciasis and the severe ocular morbidity resulting from this disease. In an endemic area where the margin of survival is narrow, the added impact of severe visual impairment further hinders the socioeconomic development of large areas of Africa.

Proposed Course: Further work on the project was discontinued early in fiscal year 1973-1974 because of competing interests of the Office of Biometry and Epidemiology.

Honors and Awards: None

Publications: None

Serial No. NEI 73 OBE 117
1. Office of Biometry & Epidemiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Lymphocyte Transformation Response in Presumed Ocular Histoplasmosis

Previous Serial Number: Same

Principal Investigator: James P. Ganley, M.D., Dr.P.H.

Other Investigators: George Nemo, Ph.D.
George W. Comstock, M.D., Dr.P.H.
Jacob A. Brody, M.D.

Cooperating Units: Epidemiology Branch, National Institute of Neurological Diseases and Stroke
School of Hygiene and Public Health, Johns Hopkins University

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To determine if individuals with disciform type of presumed ocular histoplasmosis have a more reactive cellular immune system than individuals with peripheral scar type of disease or controls.

Methods Employed: Lymphocytes from individuals with the disciform disease were stimulated by a battery of specific and nonspecific antigens. The data from these cases were compared to matched controls without ocular histoplasmosis (age, race, sex) and to individuals with peripheral scars only.

Major Findings: Data are being analyzed.

Significance to Biomedical Research and the Program of the Institute: Patients with the symptomatic disciform type of ocular histoplasmosis are more reactive to histoplasmin skin tests and delayed skin tests than other uveitis patients without ocular histoplasmosis. This study hopes to determine whether this heightened response to skin test antigens is an innate function of the cellular immune system or reflects a more frequent exposure to Histoplasma capsulatum.

Proposed Course: The data are now being analyzed.

Honors and Awards: None

Publications: None

Section on Clinical Trials and Natural History Studies

Office of Biometry and Epidemiology

A major effort was exerted to improve the management and quality of the Collaborative Diabetic Retinopathy Study. The addition of Dr. Frederick L. Ferris,who site-visited each of the 15 clinical centers to replicate visual acuity measurements and to assist clinic staff in following protocol procedures and solving logistical problems, strengthened this effort. Certain sections of the Manual of Operations were revised to emphasize and facilitate masked visual acuity examinations and to prevent patients from dropping out of the study.

A major effort was also made to lay the groundwork for improving the quality of clinical research in ophthalmology through the AUPO Workshop on Clinical Trials held in November 1973. The editor of Investigative Ophthalmology has invited submission of the proceedings for publication.

The Statistical Center of the Cooperative Glaucoma Study has submitted a proposed contract to undertake, with the help of a qualified biostatistician, an analysis of the study's data collected over 13 years. If the contract is approved, the Section will maintain liaison with the biostatistician in the analysis of the data.

Publications:

Ederer F.: Letter to Editor. Br. J. Ophthalmol. (in press)

Serial No. NEI 72 OBE 105

1. Office of Biometry & Epidemiology
2. Section on Clinical Trials & Natural History Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Statistical Consultation, Collaborative Diabetic Retinopathy Study

Previous Serial Number: Same

Principal Investigators: Fred Ederer and Frederick L. Ferris (this pertains to statistical epidemiological consultation from the National Eye Institute only)

Other Investigators: Ophthalmologists from sixteen Clinical Centers and the Reading Center, and personnel from the Coordinating Center, University of Maryland

Cooperating Units: Sixteen medical centers 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. Statistical consultation is on matters of organization, design, conduct, data collection and data analysis. The 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 to protocol and completeness of patient studies and follow-up; advise on data editing, monitoring and analysis.

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. Statistical consultation is effected through participation in the development of the protocol and operations manual, participation in site visits to evaluate clinic performance, adherence to protocol, and reproducibility of vision tests, and membership on the Executive Committee, Data Monitoring Committee, and Policy Advisory Group.

The study's operations are directed by the Executive Committee, composed of Diabetic Retinopathy Study investigators. The Policy Advisory Group, composed of senior scientists in ophthalmology, diabetes, epidemiology, and biostatistics, is monitoring progress and advising both the National Eye Institute and the Executive Committee. Members of the Policy Advisory Group are not investigators in this study.

In 1971, ten Clinical Centers were selected to participate, two of which later dropped out. Eight more Centers were added in 1972. Patient recruitment began at four Centers in June 1972, at three Centers in September 1972, and at nine Centers in early 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. One additional Clinical Center dropped out in 1973, and in April 1974 the recruitment target was revised to 1,600, and the recruitment deadline extended until December 31, 1974. Through the end of March 1974, 1,000 patients had entered the study.

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. 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.

Proposed Course: Patient recruitment will continue until December 1974. Each patient will be followed for up to five years. The Coordinating Center is continually processing and analyzing the results, which are checked periodically by the Data Monitoring Committee. Expected completion date, including data processing and report writing, is 1981.

Honors and Awards: None

Publications:

Aiello, L.M., Berrocal, J., Davis, M.D., Ederer, F., Goldberg, M.F., Harris, J.E., Klimt, C.R., Knatterud, G.L., Margherio, R.R., McLean, E.N., McMeel, J.W., Myers, F.L., Norton, E.W.D., Patz, A., Prout, T., Riekhof, F.T., Straatsma, B.R., Tasman, W., vanHeuven, W.A.J., Watzke, R.C.: Diabetic Retinopathy Study. (Letter to the Editor) Am. J. Ophthalmol. 76:403-405, 1973

Aiello, L.M., Berrocal, J., Davis, M.D., Ederer, F., Goldberg, M.F., Harris, J.E., Klimt, C.R., Knatterud, G.L., Margherio, R.R., McLean, E.N., McMeel, J.W., Myers, F.L., Norton, E.W.D., Patz, A., Prout, T., Riekhof, F.T., Straatsma, B.R., Tasman, W., vanHeuven, W.A.J., Watzke, R.C.: The Diabetic Retinopathy Study. (Editorial) Arch. Ophthalmol. 90:347-348, 1973.

Serial No. NEI 73 OBE 128

1. Office of Biometry & Epidemiology
2. Section on Clinical Trials & Natural History Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Assessment of Evidence on the Value of Photocoagulation in Treating Diabetic Retinopathy

Previous Serial Number: Same

Principal Investigator: Fred Ederer

Other Investigators: Rita Hiller

Cooperating Units: None

Man Years:

Total:	0.4
Professional	0.4
Other:	0.0

Project Description:

Objectives: Photocoagulation treatment of diabetic retinopathy was introduced some fifteen years ago. This therapy is widely used and many dozens of papers on its results have been published, yet the true value of this treatment remains uncertain. Most of the reports are of uncontrolled studies. Progression of diabetic retinopathy does not follow a smooth, gradual course, but occurs unpredictably in sudden episodes. Dramatic and spontaneous remissions are not uncommon, making it impossible to evaluate the effect of treatment unless the results in a large number of treated eyes are compared with those in a comparable number of untreated eyes. Current opinions on the value of photocoagulation are largely based on unscientific evidence.

The main objective is to assess currently available evidence on the value of photocoagulation in the treatment of diabetic retinopathy. A secondary objective is to explore some statistical methods of analyzing visual acuity data in studies of this type.

Methods Employed: Criteria were established for evaluating the quality of studies of photocoagulation in diabetic retinopathy. The main requirements for good studies are: inclusion of a concurrent control group, random selection of eyes for treatment, masked evaluation of results of treatment, biometric

methods of data analysis, including complete accounting of patients treated, including deaths and losses to follow-up. The literature was reviewed and studies were characterized according to the listed criteria.

A punch card was prepared for each patient of three studies which published individual patient data, including visual acuity before and after treatment. Visual acuity was treated as a quantitative variable scaled in several different ways. Means and standard deviations were computed for visual acuity changes in treated and untreated eyes and differences were tested for statistical significance. Results were statistically adjusted for any pretreatment differences in visual acuity between treated and untreated eyes.

Major Findings: Only four published studies of photocoagulation in proliferative diabetic retinopathy were known to have used concurrent control groups. Three Zeiss photocoagulator studies followed patients up to four or more years. The fourth, a ruby laser study, followed patients less than two years. However, none of the reports specified that eyes were selected for treatment randomly, that visual acuity was measured by masked observers, or whether any patients had died or been lost to follow-up. In view of these defects, the true meaning of the results remains in doubt.

Four different methods of quantifying visual acuity yielded essentially equivalent results, suggesting that these methods may be of value in analyzing the results of studies of this type.

Significance to Biomedical Research and the Program of the Institute: The ongoing Diabetic Retinopathy Study, a large-scale, carefully controlled clinical trial designed to provide a definitive evaluation of the role of photocoagulation in diabetic retinopathy, is in progress. The study is planned to be completed in 1979. Until then, it is important to achieve the best possible scientific assessment of the value of photocoagulation in diabetic retinopathy from a careful reevaluation of currently available information--and that was the major objective of this project.

Proposed Course: A paper was presented at the ARVO meeting in Sarasota, Florida on April 25, 1974, and a manuscript has been submitted to Survey of Ophthalmology for publication. Except for a major revision of the manuscript requested by the editor, the project is completed.

Honors and Awards: None

Publications: None

Serial No. NEI 74 OBE 156

1. Office of Biometry & Epidemiology
2. Section on Clinical Trials & Natural History Studies
3. Bethesda, Maryalnd

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: AUPO Workshop on Clinical Trials

Previous Serial Number: None

Principal Investigator: Fred Ederer

Other Investigators: Carl Kupfer, Thomas Chalmers, Elmer Ballintine, Harold Kahn, Jacob Bearman and Matthew Davis

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To familiarize clinical investigators in ophthalmology with methods of good clinical trials.

Methods Employed: Six presentations (ethics, control group and randomization, objective measures and double-masking, reproducibility of measurements, writing a protocol, and practical applications) and a panel discussion were held on November 6, 1973 at the annual meeting of the Association of University Professors of Ophthalmology in Washington, D. C.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: New methods of conducting clinical trials, including new concepts in the ethics of such trials, have been developed during the past 25 years. While these methods have taken foothold among some medical specialties (mainly internal medicine), ophthalmological research, by and large, has remained uninvolved. If more ophthalmologists begin to use these methods, the quality of clinical research in ophthalmology will improve.

Proposed Course: The presentations and discussions were tape recorded and submitted to the authors for editing. All materials have been edited, and the manuscript has been submitted to Investigative Ophthalmology for publication. Project completed.

Honors and Awards: None

Publications: None

Serial No. NEI 74 OBE 157

1. Office of Biometry & Epidemiology
2. Section on Clinical Trials & Natural History Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Patient Bias, Investigator Bias, and the Double-Masked Procedure

Previous Serial Number: None

Principal Investigator: Fred Ederer

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To increase the awareness of clinical investigators of everpresent sources of bias and how to diminish their impact.

Methods Employed: Literature review.

Significance to Biomedical Research and the Program of the Institute: Awareness of important sources of bias in clinical research is inadequate among clinical investigators. Thus, relatively few published reports of clinical trials mention any concern about the possibility of these ever-present biases. Increasing such awareness can potentially result in marked improvements in the quality of clinical research.

Proposed Course: The paper was presented to the Technical Group of the Diabetic Retinopathy Study at its December 1973 meeting in Baltimore. Project completed.

Honors and Awards: None

Publications:

Ederer, F.: Patient bias, investigator bias, and the double-masked procedure in clinical trials. Am. J. Med. (in press)

Serial No. NEI 74 OBE 158

1. Office of Biometry & Epidemiology
2. Section on Clinical Trials & Natural History Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Confidence Limits on the Ratio of Two Poisson Variables

Previous Serial Number: None

Principal Investigator: Fred Ederer

Other Investigators: Nathan Mantel, George Washington University

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: The objective of this work is to set forth procedures for setting confidence limits on the ratio of two Poisson parameters or on a quantity proportional to the ratio of two Poisson parameters.

Methods Employed: Statistical theory.

Significance to Biomedical Research and the Program of the Institute: The need to set confidence limits on the ratio of two Poisson parameters arises in many scientific investigations. For example, the number of cases of blindness or the number of deaths in two reasonably large populations or blindness rates, or death rates, each age-adjusted by the indirect method, may be considered to follow the Poisson distribution.

Proposed Course: A manuscript has been accepted by the American Journal of Epidemiology and is now in press. Project completed.

Honors and Awards: None

Publications:

Ederer, F., Mantel, N.: Confidence limits on the ratio of two Poisson variables. Am. J. Epidemiol. (in press)

Section on Mathematical Statistics and Computer Applications

Office of Biometry and Epidemiology

The Section on Mathematical Statistics and Computer Applications was created near the beginning of fiscal 1974. This Section is responsible for consultation with respect to statistical aspects of laboratory investigations; for developing theoretical and mathematical concepts as necessary for various investigations of interest to OBE; for support of OBE investigations in terms of statistical computations and data organization, analysis and display; and for computer services required by OBE staff.

The Section provided consultation and collaboration with several investigators in the Clinical Branch of NEI: Dr. Douglas Gaasterland on studies of parameters of intraocular pressure; Dr. Gaasterland on a study of the utility of 125_I uptake in diagnosis of ocular melanoma; Dr. Donald Bergsma on evaluation of young normal volunteers' response and variation in measurement of dark adaptation and retinal function, visual fields, and electroretinograms; Drs. Robert Stark and William Sullivan on soft lens treatment of bullous keratopathy; and Dr. Sullivan on treatment of keratoconjunctivitis sicca.

Roy Milton also provided assistance to the Office for Extramural and Collaborative Programs of NEI in the planning and development of an information system for scientific program analysis of research grants.

As part of an effort to enhance the ability of the Section to respond with computer services for OBE, Roy Milton has participated in organizing a more general effort to promote awareness and cooperation among the statistical computing community at NIH. This effort is directed toward improving the local availability of statistical software and promoting related intra-NIH communication.

Project NEI 73 OBE 123, "Bayesian Confidence Limits for the Ratio of Poisson Parameters," was suspended pending further related research developments and availability of resources. It is anticipated that this project will be reinstated next year.

Serial No. NEI 73 OBE 120

1. Office of Biometry & Epidemiology
2. Section on Mathematical Statistics & Computer Applications
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Statistical Summary of Dark Adaptation and Retinal Function Clinical Data

Previous Serial Number: Same

Principal Investigator: Roy C. Milton, Ph.D.

Other Investigators: Ralph D. Gunkel, O.D., Clinical Branch, NEI

Cooperating Units: None

Man Years:

Total:	0.05
Professional:	0.05
Other:	0.00

Project Description:

Objectives: The objective of this study is to assess the epidemiologic value of dark adaptation and retinal function data, collected in the NEI Eye Clinic, which has previously been used primarily for clinical purposes.

Methods Employed: During 1971, about 200 persons were tested in the Eye Clinic for dark adaptation and retinal function by means of the Goldmann/Weekers Adaptometer, all tests being similarly administered. Data from stylus recording charts were transferred to computerized records for editing and analysis.

Major Findings: Reliable manual encoding of data from stylus recording charts into computer-acceptable format was found to be possible, and these encoding methods have been useful in subsequent evaluation of similar data. The variability present in this data does not permit useful summary by the available classification of subjects. While the present data have limited utility for and beyond clinical use, this critical examination has stimulated interest in the method for future consideration.

Significance to Biomedical Research and the Program of the Institute: This study is an effort to make maximum utilization of existing Eye Clinic data currently being routinely collected, and to suggest possible changes in Eye Clinic procedures and data collection which might enhance the value of the data beyond strictly clinical use.

Proposed Course: Project completed.

Honors and Awards: None

Publications: None

Serial No. NEI 73 OBE 119

1. Office of Biometry & Epidemiology
2. Section on Mathematical Statistics & Computer Applications
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Accuracy and Repeatability of Reading Fundus Photographs

Previous Serial Number: Same

Principal Investigators: James P. Ganley, M.D., Dr. P.H.
Roy C. Milton, Ph.D.

Other Investigators: Rodney Lynk, M.D.
Harold A. Kahn

Cooperating Units: Department of Ophthalmology, University of Wisconsin

Man Years:

Total:	0.2
Professional:	0.2
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 cooperative 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. J. 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: Preliminary analysis of intraobserver variability (repeatability) suggests that the physician readers exhibit somewhat less variability than the lay readers, but not for all lesions and seldom to a meaningful extent. Analysis is continuing.

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. If lay readers should prove to be comparable to physician readers in terms of accuracy and variability, they may be utilized to free the physician from this expensive and time-consuming procedure without loss of quality.

Proposed Course: Assessment and development of indices of agreement are nearly completed, as are analysis of interobserver variability and comparison with the standard readings. The project will be completed early next year.

Honors and Awards: None

Publications: None

Serial No. NEI 73 OBE 124

1. Office of Biometry & Epidemiology
2. Section on Mathematical Statistics & Computer Applications
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Prevalence, Incidence and Economic Cost of Eye Disease in the U. S.

Previous Serial Number: Same

Principal Investigators: Rita Hiller
Helen Moorhead

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.0
Other:	0.2

Project Description:

Objectives: To define the size and cost of the eye disease problem in the U. S. for purposes of program planning and reporting.

Methods Employed: The Professional Activity Study (PAS) was reviewed in depth to determine its potential for supplying data on eye conditions. Site visits were made to the medical record department in selected local PAS hospitals to investigate their data coding and transcription procedures. Additional data were collected from local PAS and non-PAS hospitals for comparison purposes, and PAS data on average length of stay and projected number of annual discharges were compared with other available sources.

Major Findings: PAS is a standardized format, centrally computerized, medical record information system with detailed data on approximately 35 percent of inpatient discharges from short-term hospitals in the U. S. It is acceptable for making national projections on an age and sex basis for selected inpatient eye conditions. It can provide no information on ocular morbidity treated on an outpatient basis, including cases treated in hospital emergency rooms without hospitalization.

Significance to Biomedical Research and the Program of the Institute: PAS is a readily available source of current information on inpatient ocular morbidity at what is expected to be a relatively reasonable expenditure of funds and manpower.

Proposed Course: There will be a continuing need for data on ocular conditions and their treatment. In the coming FY it is planned to: develop and implement methods for utilizing the massive PAS data base in estimating incidence and prevalence of selected inpatient ophthalmic conditions and procedures; to monitor continuously the emergence of new morbidity data bases with a potential for both in- and out-patient information such as those associated with implementation of the Professional Standards Review Organization (PSRO) law.

Honors and Awards: None

Publications: None

Serial No. NEI 73 OBE 121

1. Office of Biometry & Epidemiology
2. Section on Mathematical Statistics & Computer Applications
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Bayesian Statistical Theory and Methods: A Critical Study

Previous Serial Number: Same

Principal Investigators: Harold A. Kahn
Roy C. Milton, Ph.D.

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 critically review important literature on Bayesian statistical theory and methods, and to assess the relevance of Bayesian statistics to the work of the Office of Biometry and Epidemiology.

Methods Employed: The investigators meet in seminar for in-depth study of both theoretical and applied papers on Bayesian methods.

Major Findings: The practical relevance and implications of alternative statistical theories of inference are difficult to determine and evaluate, but efforts to study these aspects are essential to the ongoing development and application of statistical theory.

Significance to Biomedical Research and the Program of the Institute: Bayesian statistical theory is relatively recent in its application to biomedical research, and as a potentially promising new approach it deserves adequate evaluation and understanding in the ongoing effort to utilize the most appropriate methods of design and analysis in statistical investigations. Bayesian methods differ from classical statistical procedures in that probabilities derived from prior experience or subjective prior considerations are

combined with observed data to develop final probability statements about the data.

Proposed Course: This organized study will end this year.

Honors and Awards: None

Publications: None

Section on Ophthalmic Field and Developmental Research

Office of Biometry and Epidemiology

During the past year, technical staff of this section consisted of Dr. J. T. Schwartz, Section Head, and Mrs. Doris Collie, Ophthalmic Technician.

A. Studies in Final Phases or Completed During the Past Year

The data collection phase of a three-year study on the effect of treatment on the progression of myopia is now in its last year. In this study, the progress of myopia among individual twins receiving special treatment will be compared with the progress among their co-twins who wear standard spectacles.

Additional findings from a twin study on heritability of the influence of topically applied corticosteroid drugs on intraocular pressure were published. This investigation was designed to examine a popular hypothesis that the ocular hypertensive response to topical corticosteroids is inherited as a simple autosomal trait, a hypothesis which bears directly upon current concepts of the cause of chronic simple glaucoma. The findings of this study were at variance with a widely accepted genetic hypothesis. During the past year this study and also those which support the genetic hypothesis were discussed and critically reviewed at a workshop held among interested investigators in an effort to explain the disparity in findings.

Manuscript revisions were completed for a chapter on twin studies invited for inclusion in a forthcoming textbook on Genetic Aspects of Ophthalmology. The chapter includes a review of the modern world literature on methodology of twin studies and was prepared as an interpretive description of theory, applications and limitations of the twin heritability study model. The textbook is in press.

The size of the central cup of the optic nerve head is an important clinical parameter of glaucoma. An estimate of the influence of hereditary factors on the size of the cup/disc ratio in normal eyes is being studied using the twin heritability model. The association between cup/disc ratio and potentially influential demographic variables is also being studied. This analysis of data obtained on members of the Twin Registry for Eye Examinations is nearing completion. The findings suggest a significant contribution of genetic factors and a significant association of cup size with age and normal intraocular pressure.

Because of increasing clinical usage of cup/disc ratio measurements a retrospective analysis was undertaken to estimate the amount of measurement difference which might be associated with differences in the accustomed clinical methods employed by interested investigators. Those frequency distributions of cup/disc ratio which were published up to the present time were reviewed and examined on the basis of epidemiologic considerations. Average effects due to methodologic differences among observers appeared to be considerable. This study suggests that before specific values of cup/disc ratio can be regarded as clinically meaningful in themselves, methodologic differences need to be attended.

A study of inheritance of dermatoglyphic patterns, undertaken in collaboration

with the Children's Diagnostic and Study Branch of NICHD and the Department of Pediatrics, Medical University of South Carolina is nearing completion. Findings of this study will be reported at the forthcoming First International Congress on Twin Studies in Rome.

B. Studies in Progress During the Past Year

The ocular hypertensive steroid response has been reported to be associated with characteristics such as diabetes mellitus, thyroid disease, myopia, and size of the optic nerve head. To gain further understanding of the clinical significance of the phenomenon of steroid responsiveness, a correlative analysis of data obtained in the Twin Register for Eye Examinations is underway. Variables being examined include plasma cortisol, refraction, cup/disc ratio, hematocrit, glucose tolerance, uric acid, protein bound iodine, blood lipid fractions and blood pressure.

A study of the relative roles of hereditary and environmental factors in determining normal levels of ocular pressure is underway utilizing the data of our Twin Register for Eye Examinations. Such information should contribute to our better understanding of chronic simple glaucoma which is characterized by abnormal pressure levels.

Patterns of mixed hand-eye dominance have been incriminated in the etiology of dyslexia. An extensive review of the literature on clinical methods used to assess ocular dominance was undertaken during the past year and a detailed report is in preparation. Study of the inheritance of dominance patterns will be included in our next cycle of twin examinations.

C. Specific Investigative Proposals Prepared and Submitted During the Past Year

A possible study on glaucoma in collaboration with the University of Alexandria in Egypt has been proposed under the International PL-480 Program. The first objective of this study is an appraisal of the advantages and disadvantages of tonometric screening for glaucoma as a potential public health practice in this economically developing country. This objective would be of primary advantage to the local public health planners. The second objective is to define the ensuing natural history of individuals who exhibit ocular hypertension but whose eyes are otherwise normal. The natural future occurrence of glaucoma in eyes having elevated ocular pressure but no loss in visual field is not well understood today even though medical treatment is often prescribed for such individuals. The world ophthalmic community is in critical need of this information and it appears that the contemporary setting in Alexandria offers an unusually attractive opportunity to examine the issue.

A collaborative study within NEI has been proposed to assess carefully the recent findings reported with respect to inhibition of lymphocyte transformation by dexamethasone and theophylline. These findings appear to be offered as part of a revised hypothesis relating to inheritance of the ocular hypertensive response to topical dexamethasone and indirectly to inheritance of chronic simple glaucoma. Collaborators would include staff of the Office of Biometry and Epidemiology and the Clinical and Laboratory Branches of NEI.

D. Collaboration, Consultation and Services Rendered to Other Groups

Dr. J. Theodore Schwartz, Head of the Section on Ophthalmic Field and Developmental Research serves as consultant to the Department of Ophthalmology, USPHS Hospital, Baltimore, Maryland, as Ophthalmic Consultant to the Surgeon General's Medical Review Board, USPHS, and as a member of the Committee on Standardization of Tonometers, American Academy of Ophthalmology and Otolaryngology. He also serves as Clinical Assistant Professor of Ophthalmology, George Washington School of Medicine, Washington, D.C.; as ophthalmic consultant to the National Health Examination Survey, National Center for Health Statistics, HRA, DHEW, and as clinical consultant to the Public Health Department of Kent County, Maryland. This Section currently undertakes active collaboration with sections of the National Heart and Lung Institute, National Institute of Child Health and Human Development, the Department of Ophthalmology, University of Illinois, and the Department of Pediatrics, Medical University of South Carolina.

Serial No. NEI 70 OBE 001

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Twin Register for Eye Examinations (TREE)

Previous Serial Number: Same

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: Doris J. Collie

Cooperating Units: None

Man Years:

Total:	.55
Professional:	.10
Other:	.45

Project Description:

Objectives: To maintain 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: This Section has 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. During the past year address files were updated, contact with registrants was maintained. Reexaminations were provided for some members.

Major Findings: This register has provided a source of subjects for numerous studies described in earlier reports. Studies now in progress by this Section and relevant publications are described under individual project reports.

Work now in progress which originated in collaboration with other institutions compose 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 suggests a strong genetic contribution to the development of some palmar crease patterns and lends further support to the usefulness of the newly proposed classification. This work (HD-CP 14) is being 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.

During the past year, preliminary work was also undertaken to assess the feasibility of projects under consideration for the future. This included an extensive review of the literature on clinical methods used to assess ocular dominance. A detailed report is in preparation. Patterns of mixed hand-eye dominance have been incriminated in the etiology of dyslexia and a study of the inheritance of dominance patterns is being considered for inclusion in our next cycle of twin examinations.

Significance to Biomedical Research and the Program of the Institute: Comparison of agreement among monozygotic and dizygotic twins with regard to physical characteristics is valuable as an indication 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: It is proposed that this twin register continue to be maintained and expanded as a resource for direct and collaborative clinical investigation. It is planned that the following papers will be presented at the First International Congress on Twin Studies, Rome, Italy, October-November 1974. (Congress proceedings to be published.)

Plato, C.C., Schwartz, J.T., and Wertelecki, W.: Dermatoglyphic Investigations in Twins and Siblings.

Plato, C.C., Wertelecki, W., and Schwartz, J.T.: Normal and Aberrant Palmar Creases in Twins and Siblings.

Honors and Awards: None

Publications: None

Serial No. NEI 70 OBE 004

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effect of Treatment on the Progression of Myopia

Previous Serial Number: Same

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.55
Professional:	.20
Other:	.35

Project Description:

Objectives: To assess the effect of a specific treatment in retarding the progression of myopia.

Methods Employed: This is a three-year study among a population of 25 pairs of young, monozygotic twins who are similarly myopic. One co-twin receives standard spectacle correction as the control; the other is 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.

Photographic and ultrasound systems were assembled for the purpose of measuring the size of intraocular compartments and the curvature of refractive surfaces of the eye. During this past year all twins were reexamined at 6-month intervals. Participants have now been followed for 2 1/2 years.

Major Findings: Study in progress.

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 accommodation 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 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: The study population will receive its final examination early in the next fiscal year. A methodologic review of this unusual co-twin control design is being submitted for presentation at the forthcoming District International Congress of Twin Studies in Rome.

Honors and Awards: None

Publications: None

Serial No. NEI 73 OBE 127

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Methodology of Twin Heritability Studies

Previous Serial Number: Same

Principal Investigators: J. Theodore Schwartz, M.D.
Manning Feinleib, M.D.

Other Investigators: Morton F. Goldberg, M.D.

Cooperating Units: Epidemiology Branch, NHLI
Department of Ophthalmology, University of Illinois

Man Years:

Total:	.02
Professional:	.02
Other:	.00

Project Description:

Objectives: To prepare a thorough description of biases and other methodologic problems which might adversely influence the accuracy and validity of twin heritability studies.

Methods Employed: A review of modern world literature on methodology of twin heritability studies was undertaken by Dr. Schwartz and an interpretive description of theory, application and limitations of this investigative model was prepared in accordance with fundamental principles of population study design. A companion review of methods of data analysis was undertaken by Dr. Feinleib. Final manuscript revisions were completed during the past year.

Major Findings: Contemporary authors have expressed both favorable and critical views on various aspects of twin study methodology. Theoretical issues are sometimes raised, however, without considering their likely bearing, in a practical sense, on the outcome of twin investigations. Sources of bias in twin studies were observed to be divisible into two categories: those which tend to influence qualitative and quantitative assessment of heritability and those which tend to limit the application of twin findings to the world of non-twins. In this context, an assessment of each kind of bias arising from biologic, sociocultural and logistics issues was prepared in reference to the

topics: cause of twinning, unusual twin types, mutual fetal circulation, mirror imaging, perinatal factors, postnatal environment, logistics and supply problems, and determination of zygosity.

Significance to Biomedical Research and the Program of the Institute: This work brings together in a single manuscript a critical review and categorization of sources of potential biases in twin heritability studies along with sufficient description of relevant biology to place the biases in a practical perspective, and in a companion manuscript by Dr. Feinleib, the elucidation of contemporary methods of data analysis.

Proposed Course: Completed

Honors and Awards: None

Publications:

Schwartz, J.T.: The Twin Heritability Study. Part I. Perspective, Problems and Approach. In Goldberg, M.F. (ed.): Genetic and Metabolic Eye Disease. Boston, Little, Brown, 1974.

Serial No. NEI 74 OBE 159

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Influence of Methodologic Differences on Measurements of Cup/Disc Ratio

Previous Serial Number: None

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.15
Professional:	.10
Other:	.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 project documents the existence

of striking interobserver differences among those interested investigators who have published frequency distribution 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 description of this project is being prepared for publication.

Honors and Awards: None

Publications: None

Serial No. NEI 74 OBE 160

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Frequency Distribution of Horizontal Cup/Disc Ratio and Relationship Between Cup Size and Other Clinical Variables

Previous Serial Number: None

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:	.15
Professional:	.10
Other:	.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 on 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: The data analysis will be completed and a manuscript will be prepared for publication.

Honors and Awards: None

Publications: None

Serial No. NEI 74 OBE 161

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Association Between the Ocular Hypertensive Response to Topical Dexamethasone and Other Clinical and Laboratory Measurements

Previous Serial Number: None

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: Robert J. Garrison, M.D.
Manning Feinleib, M.D.
Frank H. Reuling, M.D.
Doris J. Collie

Cooperating Units: Epidemiology Branch, NHLI

Man Years:

Total:	.05
Professional:	.05
Other:	.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: Study presently in progress.

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 re-examine the relationship between steroid responsiveness and parameters of these disorders and other clinical and laboratory variables.

Proposed Course: Data will be analyzed during the next fiscal year.

Honors and Awards: None

Publications: None

Serial No. NEI 74 OBE 162

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Twin Study on the Inheritance of Normal Levels of Intraocular Pressure

Previous Serial Number: None

Principal Investigator: J. Theodore Schwartz, M.D.

Other Investigators: Frank H. Reuling, M.D.
Manning Feinleib, M.D.
Robert J. Garrison, M.D.
Doris J. Collie

Cooperating Units: Epidemiology Branch, NHLI

Man Years:

Total:	.05
Professional:	.05
Other:	.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: Study in progress.

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: Analysis of these data will be completed during the next fiscal year.

Honors and Awards: None

Publications: None

Serial No. NEI 74 OBE 163

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Twin Heritability Study of Horizontal Cup/Disc Ratio in Normal Eyes

Previous Serial Number: None

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:	.15
Professional:	.10
Other:	.05

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: Analysis 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: The analysis of these data will be completed and a manuscript prepared for publication.

Honors and Awards: None

Publications: None

Serial No. NEI 70 OBE 002

1. Office of Biometry & Epidemiology
2. Section on Ophthalmic Field & Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Heritability of the Effect of Corticosteroids on Intraocular Pressure

Previous Serial Number: Same

Principal Investigators: Frank H. Reuling, M.D.
J. Theodore Schwartz, M.D.

Other Investigators: Manning Feinleib, M.D.
Robert Garrison, M.S.
Doris J. Collie

Cooperating Units: NAS-NRC Twin Panel
Epidemiologic Research Section, NHLI

Man Years:

Total:	.10
Professional:	.10
Other:	.00

Project Description:

Objectives: To assess the role of genetic factors in determining the intraocular pressure response caused by topical application of corticosteroid eye drops.

Methods Employed: A sample of 80 pairs of monozygotic and like-sex dizygotic twins of age 15 and above were examined according to a standard protocol. Dexamethasone 0.1% eye drops were instilled three times per day for four weeks and the examination was repeated. Data were gathered on family history of various diseases, various measures of intraocular tension before, during, and after four weeks of steroids, and anatomical observations such as gonioscopy, corneal thickness, cup/disc ratio were recorded. In addition, blood chemistries including postprandial glucose and lipoprotein fractions were obtained. Physical examinations were performed by members of the Field Epidemiological Research Section of the NHLI.

The protocol for this study was approved by the NAS-NRC Follow-up Agency which granted access to those twins in their panel who reside in the

Washington-Baltimore metropolitan area. Five pairs of these twins are included in the study.

Major Findings: Low estimates of heritability were observed. Results of the study suggest that nongenetic factors play a major role in determining variation in the ocular response to a 4-week course of topical 0.1% dexamethasone. During the past year, detailed findings of this study were published. The findings of this study and also those which support the genetic hypothesis were discussed and critically reviewed at a workshop held among interested investigators.

Significance to Biomedical Research and the Program of the Institute: Correct assessment of the role of inheritance of the "steroid response" is of major importance insofar as this phenomenon has been described as being associated with the occurrence of chronic simple glaucoma, phenylthiourea taste testing, diabetes mellitus, thyroid function, and myopia. An important and widely held hypothesis regards chronic simple glaucoma as being monogenically inherited, based on an observed familial occurrence of steroid responsiveness. On the basis of the findings of this twin study, however, it seems evident that a theory of simple monogenic inheritance of the steroid response can be questioned. The results of the present study mark the need for further investigation of the determinants of this clinically important phenomenon.

Proposed Course: Completed.

Honors and Awards: None

Publications:

Schwartz, J.T., Reuling, F.H., Feinleib, M., Garrison, R.J., and Collie, D.J.: Twin study on ocular pressure following topical dexamethasone. Part I: Frequency distribution of pressure response. Am. J. Ophthalmol. 76:126-136, 1973.

Schwartz, J.T., Reuling, F.H., Feinleib, M., Garrison, R.J., and Collie, D.J.: Twin study on ocular pressure following topical dexamethasone. Part II: Inheritance of variation in pressure response. Arch. Ophthalmol. 90:281-286, 1973.

CONTRACT NARRATIVE

JOSLIN CLINIC (NIH 73 C 179)

Title: Etiology of Diabetic Retinopathy

Current Fund Allocation: $3,112

Objective: To discover factors related to retinopathy given the presence of diabetes of long duration.

Progress to Date: A small contract for a tabulation of Joslin Clinic population data with respect to duration of diabetes and presence of retinopathy has been completed. The results suggest that a large clinic such as Joslin would have enough cases with diabetes of long duration and without retinopathy to warrant proceeding further in study design.

Significance to NEI Programs and Biomedical Research: Diabetic retinopathy is an important cause of visual disability. Identification of factors that relate to the risk of retinopathy among long-term diabetics may be helpful both for control of the disease and for better understanding of it.

Proposed Course of Project: Proceed with the detailed design of a case-control study.

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: Steroid response has been measured in two groups: (a) those who were given this test several years ago, and (b) twin pairs. A substantial amount of data has been collected and are now being analyzed.

Significance to NEI Programs and Biomedical Research: The intraocular pressure response to steroids 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 of Project: A complete tabulation and analysis of results obtained is expected to be finished next year.

OFFICE OF SCIENTIFIC REPORTS AND PROGRAM PLANNING COORDINATION

ANNUAL REPORT
OFFICE OF SCIENTIFIC REPORTS AND PROGRAM PLANNING COORDINATION
NATIONAL EYE INSTITUTE
July 1, 1973 - June 30, 1974

The NEI Office of Information was redesignated as the Office of Scientific Reports and Program Planning Coordination during the year, reflecting both the reorganization of public affairs activities within HEW and the Institute's own decision to centralize program planning activities within the Office of the Director.

PUBLIC AFFAIRS CUTS AND REORGANIZATION

On July 19, HEW Secretary Weinberger issued a memorandum calling for the reduction in the amount of "self-serving, promotionally oriented material in Government." Each agency was allowed to present its case on which of its information activities could be considered "public affairs" (defined by HEW to include such public relations functions as publicity, press relations, films, exhibits, and other agency-initiated promotional projects) and which were not. The Eye Institute supported NIH's argument, which HEW eventually accepted, that its information program is largely concerned with answering public inquiries, scientific and technical reports, and consumer health education. Recognizing this, the Eye Institute, as well as other Institutes at NIH, were asked to identify staff members whose functions were associated with public affairs activities. One public affairs staff member from this Office was identified who, according to HEW guidelines, was transferred to another high-priority area (Extramural) within the Institute. The remaining staff were identified by the Institute has having non-public affairs information responsibilities, a classification which was accepted by NIH and the Department.

The above reclassification and transfer resulted in a centralization of all Institute/Division public affairs activities in the Office of the Director, NIH. Because public affairs was never a major NEI activity, the information activities of the Office have continued generally as before.

A second part of the public affairs reorganization was the reduction in the number of HEW publications. As a result of the Departmental request, NEI identified three of its twelve publications to be eliminated, but retained temporary authority to publish its experimental newsletter, 20/20.

Aside from consuming a considerable amount of staff time in responding to numerable memoranda from NIH and HEW, the impact of the public affairs reorganization on the National Eye Institute has been minimal: no high priority publications were eliminated, and we have been allowed to continue our information activities as in the past with a few minor exceptions.

PUBLIC INFORMATION

Publications

Partly as a result of uncertainty concerning the fate of publications during the public affairs reorganization, no new fact sheets or health education booklets on eye disorders were published during the year. An HEW-initiated project to produce a booklet on eye care (as reported in last year's Annual Report) was temporarily shelved due to uncertainties within the Office of Consumer Affairs, HEW, over the future of its consumer publications series of which the booklet was to be part. An attempt will be made during the coming year to publish the completed manuscript for this booklet as an NEI publication.

In cooperation with NEI Extramural and Collaborative Programs, two new folders on extramural support programs (Special Visual Sciences Research Award Program and Specialized Clinical Research Center Grants) were prepared. Two issues of 20/20 were mailed to the vision research community. Whether or not the Institute is permitted to continue this publication depends on the result of a readership survey which will have to be undertaken in the next issue prior to obtaining formal OMB approval.

The following number of NEI publications were distributed during the year:

Cataract (booklet and fact sheet)	3,926	Retinal Detachment	2,881
Diabetic Retinopathy	3,126	Refractive Errors	2,835
Retinitis Pigmentosa	2,841	Glaucoma	3,301
Macular Degeneration	2,849	Corneal Disease	2,673
Statistics on Blindness in the Model Reporting Area, 1969-1970			367
Corneal Disease Task Force Reprint			21
U.S. News and World Report Interview with Dr. Kupfer			200
Blindness and Services to the Blind in the United States (OSTI Report)			267
Security is an Eye Patch			48,120

Requests for the latter publication, supplies of which were inherited from the former PHS Neurological and Sensory Diseases Control Program, were stimulated by an announcement of its availability mailed to the nation's ophthalmologists by the American Association of Ophthalmology. Over 450 requests from practicing ophthalmologists for multiple copies were received, resulting in the near depletion of supplies of this very popular booklet. Over the past three years, the Institute has distributed over 240,000 copies of Security is an Eye Patch.

Public Inquiries

Approximately 800 written inquiries received by the Office required an individually written response, whereas the number of telephone inquiries

increased by approximately 33 percent: over 2,400 telephone calls were handled during the year. The majority of inquiries concerned cataract, both research and treatment. In particular, there was great public interest in phacoemulsification and the role of nutrition in cataractogenesis and prevention. Other areas of interest concerned laser treatment of glaucoma and diabetic retinopathy and the possibility of individuals coming to the National Eye Institute for treatment. Replies to approximately 160 letters from the public expressing concern with the Institute's support of diabetes-related research were prepared.

The Office responded to 35 controlled, written Congressional inquiries, and 96 telephone calls from Congressional officies.

Press Relations

Five press releases were prepared (new members of the National Advisory Eye Council, a warning of the possible damage to the eye when observing the Comet Kohoutek's approach to the sun, announcements of the appointments of Dr. Nusser and Mr. McManus, and Dr. Kinoshita's receipt of the ARVO Proctor Medal.) Eight stories were prepared for the NIH News and Features service, mailed to science writers in the professional and general press. Ten stories were prepared for the NIH Record. Announcements were mailed to the vision research journals concerning new NEI grants and awards, the availability to investigators of breeding pairs of rats with inherited retinal degenerations (produced under an NEI contract), and other items of immediate interest to the vision research community.

The Office assisted press representatives from the Associated Press, U.S. News and World Report, Readers Digest, the Blue Sheet, Woman's Day, Medical World News, National Enquirer, U.S. Medicine, and Medical Tribune.

Miscellaneous

In cooperation with the Office of Biometry and Epidemiology, the Office helped coordinate arrangements for presentation of a workshop on clinical trials, in conjunction with the winter meeting of Association of University Professors of Ophthalmology. The Office also coordinated arrangements with the Laboratory of Vision Research and Clinical Branch for a tour of the Institute by AUPO members.

A public relations proposal concerning the Framingham Eye Study was prepated by a member of the Office staff, Ms. Friedman Spellane. Its primary objective is to help enhance patient recruitment efforts for the study and to provide means for informing the professional and lay publics of the importance of this investigation. Thus far, a radio spot announcement was prepared and distributed in the Framingham and Boston areas, encouraging those who had participated in the Framingham Heart Study to take part in the Eye Study as well. Letters encouraging participation in the study have also been drafted and will be mailed to prospective participants. Finally, a supplement to the NIH science writers service, News and Features, is being prepared for distribution to the press and to members of the Study. This activity will be pursued further in FY 1975.

Four Search for Health columns were prepared for the NIH Office of Information concerning terminology relating to the eye, eye diseases, eye disease diagnosis, and methods of treatment which were mailed to weekly newspapers across the United States.

Arrangements were made in cooperation with the NIH Office of Information for Dr. Kupfer's appearance on a Tampa, Florida, television talk show in conjunction with the Spring ARVO meeting in Sarosota. The Office coordinated arrangements for interviews with Dr. Kupfer by the Readers Digest, U.S. News and World Report, U.S. Medicine, and the American Optometric Association News.

The Annual Save Your Vision Week Presidential Proclamation was prepared. NEI's contributions to the NIH Almanac and NIH Annual Report were prepared. The Office coordinated Institute submissions to the NIH Scientific Directory and Bibliography as well as this Annual Report.

Assistance was provided to the Director in the preparation of presentations before the Association of University Professors of Ophthalmology and the Association for Research in Vision and Ophthalmology.

PROGRAM PLANNING COORDINATION

Following recommendations of the NEI management conference held last September, the then Office of Information was designated as the coordinating point within the Institute for program planning and development activities. The Information Officer, Julian Morris, was designated as the Planning Coordinator. Although the Information Office had been actively engaged in planning activities, this designation reflected the Institute's desire to formalize its planning activities and provide better organization and followup.

The Office has thus had major responsibility for preparation of the NEI Forward (Long Range) Plan as well as special planning documents required by NIH and HEW, worked with the Executive Officer and Budget Officer in the drafting of narrative materials related to Appropriations Hearings, developed materials for various presentations to NIH staff regarding program plans, in cooperation with other segments of the Institute documented specific needs for additional funds and positions, coordinated arrangements for the Cataract Workshop, and assisted in the arrangements for the National Advisory Eye Council Program Planning Subcommittee's review of the Retinal and Choroidal Diseases, Cataract, and Corneal Disease programs.

For the coming fiscal year, the Office has major responsibility for coordinating the Subcommittee's review of the Glaucoma and Sensory Motor Disorders program and in following through on the Subcommittee's activities to the drafting and publication of its final report.

Honors and Awards:

Award of Excellence, Society for Technical Communication, Washington, D.C., Chapter, to Bonnie Friedman Spellane and Julian Morris "in recognition of outstanding achievement in technical communication" for the brochure Cataract: Focus on Research.

EXTRAMURAL AND COLLABORATIVE PROGRAMS

STATEMENT OF THE ASSOCIATE DIRECTOR FOR
EXTRAMURAL AND COLLABORATIVE PROGRAMS

A. Fiscal Year 1974 Funding

1. Research Grants

During the past year, the Institute has endeavored to realize three major objectives in funding of research grants: a) to support the largest possible number of high quality research projects through prudent program and fiscal management of available resources, b) to minimize harmful fluctuations in funding patterns, and c) to insure an adequate level of support for new research efforts as well as maintain a balanced program of on-going research.

The release of $1,406,000 in impounded FY 1973 funds, as well as an increase of $5,262,000 over the original FY 1974 budget estimates has enabled the Institute to accomplish these objectives without implementing a policy of fiscal negotiations which could jeopardize the further development of research in the visual sciences. The total funding distribution of research grants, including 8 competing renewals and 18 new grants awarded with impounded funds, is shown below.

Research Grant Support*
(Dollars in Thousands)

	Number	Total Amount Awarded
Prior year commitments	276	$16,261
Competing renewals	57	5,650
New awards	86	5,610
Total	419	$27,521

*FY 1974 Estimate: 4/13/74

In addition to the support of regular research grants, the Institute has initiated a "small grants" program, titled the NEI Special Visual Sciences Research Award Program.

The program is designed to encourage newly-trained investigators to remain active in eye research during the formative stages of their career. The awards are made for a non-renewable period of up to three years. Direct costs will be awarded in amounts up to $7,500 per annum, and may be used for such items as equipment, supplies, or technical support services. The first applications for this program were reviewed at the March 1974 meeting of the National Advisory Eye Council.

2. Training Programs

Although the Administration's decision to phase out the current training grant program is being implemented, the FY 1974 appropriation, together with the release of FY 1973 impounded funds, has enabled the Institute to award a total of 47 training grants at a support level of $3,857,000, including $954,000 of impounded funds, for 6 competing applications.

Fellowship support continued in the amount of $331,000 for commitments for 34 awards made prior to the phaseout of training programs, plus $500,000 released from impounded funds for the support of 41 competing awards.

In addition, the new fellowship program has been implemented this past year, with the first review of applications in April 1974.

3. Research Contracts

During FY 1974, 19 contracts were funded at a total cost of $2,200,000. Seventeen of these were exclusively for the Institute's collaborative study to evaluate treatment modalities for diabetic retinopathy. The remaining contracts were awarded for "The Development of a Large-Scale Method for the Preparation of Rod Outer Segments" and "The Biostatistical Analysis of Data Obtained from the Glaucoma Collaborative Study."

B. Organization and Staffing

1. Program Structure

Over the past year, the Extramural programs have been restructured to reflect more accurately the Institute's program priorities. The new major program areas, with estimates of FY 1974 support levels for research grants are as follows:

Program	FY 1974 Research Grant Funds
1. Retinal and Choroidal Diseases	$9,703,000
2. Corneal Diseases	5,148,000
3. Cataract	2,718,000
4. Glaucoma	3,348,000
5. Sensory-Motor and Rehabilitation	6,604,000

To achieve this programmatic orientation at the operating level, program directors have been assigned on the basis of the revised structure, and will be responsible for coordinating both contract and grant supported research in their designated programs. Similarly, the responsibilities of management personnel in the Contracts and Grants Branch have been revised to reflect the same program management framework.

2. National Advisory Eye Council

In order to establish a sound basis for Institute program planning activities in each program area, a subcommittee of the National Advisory Eye Council has been established to review current research support and, with the advice of scientific and technical experts in specific program areas, formulate recommendations regarding program balance to be transmitted to the full Council. Review of all programs is expected to be completed by November 1974.

3. Vision Research Program Committee

As part of the recent review of Federal advisory committees, the Institute proposed to eliminate training grant and fellowship review from the functions of the Vision Research and Training Committee, and broaden its sphere of activity to include not only the review of special project grant applications, but also participation in the analysis of extramural programs and in advising the Institute in its support of specific research areas. It was also proposed that the committee be renamed the Vision Research Program Committee, and a revised charter reflecting these changes was approved by the Secretary, HEW.

4. Establishment of Data and Analysis Unit

In response to increased data requirements for analysis, reporting, and management of Institute programs, a data and analysis unit has been established in the Office of the Associate Director. Initial efforts have begun to develop a comprehensive information system which will serve as a resource for extramural operations as well as provide analytical data for program planning, budget formulation, and reports.

5. Staff Changes

The following personnel have been appointed to key positions in the Extramural and Collaborative Programs during the past year:

Dr. Wilford L. Nusser	Chief, Scientific Programs Branch and Acting Program Director, Glaucoma Program
Dr. Luigi Giacometti	Program Director for Cataract and Corneal Disease Program
Dr. Israel A. Goldberg	Assistant to Acting Program Director, Glaucoma Program
Dr. John B. Mathis	Program Director for Sensory-Motor and Rehabilitation Program

RETINAL AND CHOROIDAL DISEASES

The NEI Retinal and Choroidal Diseases Program is divided into six disorder areas:

1. Circulatory Abnormalities
2. Vitreous Degeneration and Retinal Detachment
3. Developmental and Degenerative Abnormalities
4. Maculopathies
5. Tumors
6. Uveitis

CIRCULATORY ABNORMALITIES

These disorders include alterations in blood supply due to abnormal vascular development, obliteration, tortuosity, occlusion of blood vessels and hemorrhage. They may be due to environmental influences or heredity and include such clinical diseases as retrolental fibroplasia, diabetic retinopathy and macular degeneration.

The preretinal vitreous monitoring of oxygen has been proposed as a useful method of studying a variety of conditions of retinal toxicity and degeneration in which retinal blood flow is an important factor. Dr. Noble David and associates[1-2-3] at the University of Miami have continued to use fluorescein densitometry for studying the relationship of blood oxygenation to retinal blood flow. The rhesus monkey has served as the animal model in which constriction of major retinal arteries and veins during hyperoxia and dilation during hypoxia were demonstrated. Retinal blood flow increased considerably in hypoxia and showed a moderate decrease in hyperoxia. These findings indicate that the retinal circulation parallels that of brain in adjusting to changes in the arterial oxygen partial pressure (pO_2) with compensatory changes in blood flow. These investigators continue to apply techniques for quantitative measurement of relative retinal blood flow to such variables as blood pressure, blood volume, hemorrhagic shock, and optic nerve atrophy.

The pathogenesis factors which determine the evolution of different vascular patterns and techniques of altering hemodynamics to prevent retinal complications in patients with branch retinal vein obstruction are being studied by Dr. Frank W. Newell and his colleagues[4-5] at the University of Chicago. The retinal vasculature of patients in these studies have been followed for periods up to five years utilizing fundus photography, fluorescein angiography and measurement of arterial perfusion pressure. The long-term fluorescein angiographic assessment and vascular dynamics of these patients indicate that they can be classified into four major groups: Group I includes patients in whom arterial perfusion pressure, retinal circulation time and visual functions remain normal; Group II includes patients in whom arterial perfusion pressure is normal, but there is some delay in the retinal circulation time; Group III includes patients who demonstrate an impaired arterial perfusion and retinal circulation time demonstrating marked venous stasis; and Group IV includes patients in whom arterial perfusion is grossly impaired and retinal circulation

time shows gross venous stagnation.

Careful quantitation of an experimental model of retinal ischemia and vascular proliferation may assist in the identification of factors associated with retinal vascularization and proliferation. Dr. Arnall Patz and associates[6] at the Johns Hopkins Medical School are conducting a study to determine the optimal pO_2 levels associated with production of retinal capillary and small vessel non-perfusion. The response is relatively linear up to a pO_2 of approximately 250 mm Hg. Sustained pO_2 levels in this range produced changes with the severity of capillary non-perfusion, as determined by fluorescein angiography, correspond with the degree of retinal ischemia produced.

In the course of study of retinal blood flow in choroidal and retinal vasculatures, Dr. Arnall Patz[7-8] and associates in collaboration with the Applied Physics Laboratory at Johns Hopkins University, have demonstrated that indocyanine green dye will permit infrared absorption angiography of the choroidal filling. The limitations of fluorescein dye are eliminated by use of indocyanine green. The application of infrared absorption angiography to the simultaneous study of choroidal and retinal circulation is in progress and will permit an investigation of the contribution of the choroidal and retinal circulation to retinal oxygenation. Routine choroidal circulation can be observed through simultaneous photography of choroidal and retinal circulations. Swelling of the optic cup*has been produced in experimental models by the use of intracranial surgery. Dr. Mark O. Tso[9] and associates at the Armed Forces Institute of Pathology have been using experimental models created by increasing intracranial pressure nonsurgically or by lowering intraocular pressure. The mechanism of breakdown of the optic nerve-blood barrier in the optic disc is currently being studied. The leakage of fluid from the vasculature of the optic disc and possibly from the peripapillary choroidal plexus in papilledema is being investigated by use of histochemical techniques which employ horseradish peroxidase as a tracer substance. Intravascular injection of this enzyme as a tracer will show areas of localized disruption of blood-optic nerve barrier.

The animal model for papilledema has been produced by x-irradiation of the right occipital lobe of rhesus monkeys. Of thirteen monkeys irradiated, four developed full papilledema, three showed mild edema of the optic disc while the remaining six developed atrophy of the optic disc. Those animals with papilledema also showed an increase in intracranial pressure. Electron microscopic study of the optic disc of animals with papilledema demonstrated swelling as well as fluid accumulation in the intercellular space. Extension of this work by use of animal models will be of assistance in understanding the pathogenesis of papilledema. The relationship between the peripapillary choroidal and the central retinal circulations under increases in intracranial pressure or decreased intraocular tension may be defined.

Dr. David J. Apple[10] and associates at the University of Illinois have designed a study to analyze the efficiency of argon laser burns in blood vessel closure or obliteration and to analyze factors relating the movement of fluid between various retinal and choroidal components. Monkeys have been subjected to photocoagulation treatment with subsequent histopathological and electron microscopic analyses. The results show that specificity of vascular

*Papilledema

effects ascribed to the argon laser is unfounded. The investigators have never found a destroyed or occluded vessel in the absence of significantly destroyed nerve fiber layer parenchyma. In addition, choriocapillaris closure has been a constant finding.

VITREOUS DEGENERATION AND RETINAL DETACHMENT

These disorders include rhegmatogenous and other retinal detachments due to vitreous fiber formation and shrinkage, and vitreous liquification and opacification.

Chemical Development

An increased understanding of the biological role of hyaluronic acid in the vitreous may lead to the possible use of this biopolymer in control of inflammation and regenerative processes. Further characterization of the structure of hyaluronic acid in solution and as a solid could be important in the possible therapeutic use of hyaluronic acid. In addition, the vitreous is useful in the study of biosynthesis of biopolymers. It may be viewed as a simple connective tissue. Dr. Endre A. Balazs[11] and associates at the Boston Biomedical Research Institute have been studying enzymes involved in the synthesis of vitreal components. They have also been investigating the role of salts such as sodium chloride on the swelling properties of vitreous gel. It has been suggested that the biopolymers may serve as volume regulators for gels such as vitreous.

Membrane Development

Investigation of vitreous membrane formation development as a consequence of hemorrhage and intraocular inflammation is in progress under Dr. David A. Swann[12] and associates at the Retina Foundation in Boston. They find that vitreous membranes could develop because of plasma clotting or aggregation of platelets. This group of investigators has conducted studies which document the effects of injecting platelets with or without plasma into the vitreous cavity. It now appears that when blood components are released into the vitreous cavity, intact platelets play a role in the instantaneous formation of membranes. The vitreous membranes can be formed in the absence of plasma proteins provided the platelets are physiologically functional. In some respects, platelet membranes resemble vitreous floaters which are seen clinically. Platelet-induced membranes are not accompanied by a proliferation of fibroblasts and fibroblastic membranes which may lead to retinal detachment.

Enucleated human eyes are being examined by Dr. Robert Y. Foos[13] and associates at UCLA. He has observed a prevalence of proliferative vitreoretinopathies which resulted from complications of ocular disease or surgery. This high incidence indicates the need for a better understanding of the underlying events and the relationship of these events to more complex lesions which lead to blindness. The proliferative lesions at the juncture of the vitreous and retina range from simple epiretinal membranes to processes in which both fibrous and vascular components lead to retinal detachment. Epiretinal

membranes are delicate strands on the surface of the retina. Their cause is poorly understood and has been the subject of study by Dr. Foos. They are found in association with developmental, inflammatory, vascular and mechanically produced lesions. The experience in Dr. Foos' laboratory indicates that the membranes originate in the glial cells which become mitotic through a variety of stimuli.

Dr. Miguel F. Refojo[14] and associates at the Retina Foundation, Boston, point out that in cases of severe vitreous traction there is need for a material which will tamponade the retina against the choroid during formation of choroi-retinal adhesion and will not pass through a retinal break. Dr. Refojo has been developing synthetic polymers similar to the natural vitreous body of the eye. The gel must be injectable and not hinder vision by scattering of light.

DEVELOPMENTAL AND DEGENERATIVE DISORDERS

Pigment Epithelium

This is a single layer of cells in the retina which contain varying amounts of pigment. These cells are firmly bound to Bruch's membrane which is bound to the choriocapillaris. The intimate relationship between the photo-receptors and their blood supply suggests that the pigment epithelium has a critical role in the maintenance of the photoreceptors. The pigment epithelium has been implicated in clinical problems of maculopathies, retinal detachment, visual pigment synthesis, removal of rod outer segment debris and information processing as implied by its contribution to the electroretinogram. Due to an awareness of the implications and of a critical role of the pigment epithe-lium, new concepts of the pigment epithelium involvement in retinal degenera-tive disorders are developing.

It is unclear whether the pigment epithelium follows the general biological phenomenon of phagocytosis or whether this structure had additional specializations which are characteristic of these cells. Dr. Joe G. Hollyfield[15] and associates at Columbia University have shown that phagocytic activity begins early in the amphibian embryo and is responsible for the removal of egg pigment which is eliminated from cells of retinal neuroepithelium. These investigators have explored the question of whether the phagocytic activity of the pigment epithelium is specific or an indiscriminate scavenging. The study has shown that pigment epithelial cells will phagocytize polystyrene spheres which have been injected into the space between the retina and pigment epithelium in the frog embryo, tadpole and adult, and therefore, the phagocytic activity is not restricted to rod outer segment discs.

The interphotoreceptor matrix appears to be synthesized by the neural retina and the pigment epithelium with possible contributions from the photo-receptors and Müller cells. Dr. Lynette Feeney[16-17] and associates at the University of Oregon Medical School have used radioactive precursors of proteins, glycoproteins and glycosaminoglycans, which are components of the interphoto-receptor matrix, to produce autoradiographs in an attempt to verify the site of matrix macromolecules. Autoradiographs of 10-day old and adult mice were

prepared for light and electron microscopy. Pulse label techniques show that although the apical microvilli and their cell coats in the 10-day old mice are proliferating maximally, the adult microvilli show a more intense labelling which probably relates to increased phagocytic activity.

Most of the studies of pigment epithelium have involved embryonic eyes as a source of explants or in situ observations of phagocytosis, proliferation or cell movement. Conclusions with regard to potential activities of these cells have been based upon histopathologic observations. Dr. Mark O. M. Tso[18] and associates at the Armed Forces Institute of Pathology collaborated with Dr. Daniel Albert and associates at Yale University and developed a method of organ culture of human pigment epithelium and choroid in an effort to study the reactions of this tissue to various stimuli. These investigators have shown that preparations of human tissue obtained postmortem and during surgical procedures may be maintained in culture for at least two weeks. The tissue does not proliferate or degenerate and appears to remain active and suitable for morphological studies. The culture system employed produced pigment epithelium which resembled the in situ tissue in that there is a persistence of apical villi, terminal bars, infoldings of basal plasma membranes, intact basement membranes and melanin and lipofuscin granules. The techniques may be useful to study the cytological behavior of pigment epithelium in vitro during various stages of retinal diseases.

Dr. Kenneth Brown[19-20] and associates at the University of California have concentrated their efforts upon the functional relations of photoreceptors to the pigment epithelium. They have described the anatomic relationship between the pigment epithelial cell and photoreceptors in frog retina. The pigment epithelium forms apical processes which, in the cat, ensheath the cone outer segment. Although the functional significance of this sheath is not understood, it is evident that the pigment epithelial cell relates to the cone as well as the rod. These investigators point to the similarities of the choroid plexuses of brain and the retinal pigment epithelium and choroid. A series of intracellular microelectrode experiments indicate differences and similarities in ion permeability and flux at apical and basal membranes of the pigment epithelial cell. Ouabain placed on the epithelial side of the tissue was found to depolarize the apical membrane but was ineffective when placed on the choroidal side. These observations indicate that the apical membrane, but not the basal membrane, contains a sodium-potassium pump. The physical condition of the pigment epithelium used in transport studies is examined by scanning electron microscopy. These investigators have developed a technique for vertically cracking the epithelial layer at cell junctions so that lateral surfaces of individual cells can be visualized.

MEMBRANES

The production of an electrical response to the absorption of light by photoreceptors is related to a system of light-induced membrane reorganization. Investigation of intact membranes and relevant membrane models may further knowledge of the mechanism of energy-transduction and transfer in photoreceptor membranes. The structure of photoreceptor membranes and visual pigment properties are the subjects of current investigations.

Dr. Kent Blasie[21] and associates at the University of Pennsylvania have approached the description of membrane structure and light-induced membrane phenomena at the submolecular level. These investigators have determined by x-ray diffraction the location of rhodopsin within the profile of photoreceptor disc membranes and forces responsible for this location, as well as the local arrangement of rhodopsin over the surface of the disc membranes. It is anticipated that analysis of diffraction data from the disc membrane profile will permit a detailed evaluation of light-induced changes in the membrane structure at the submolecular level. To date, data appear to fit a model of unbleached photopigment molecules which protrude into the aqueous layer and into the lipid core. Bleaching causes the photopigment to move deeper into the lipid core and in a transmembrane direction.

Dr. Wayne L. Hubbell[22] and associates at the University of California have been interested in the location of rhodopsin in the disc membrane. They have shown that the surface of the rhodopsin molecule is in direct contact with the hydrocarbon chains of the phospholipid of the disc membranes which it penetrates. The distribution of rhodopsin molecules in the plane of the membranes has been studied by techniques which reveal membrane surfaces. It has been found that the rhodopsin behaves as a solute which is regulated in its solution by light. Careful studies by this group indicate that rhodopsin completely traverses the thickness of the membranes. The process by which the light energy is transduced to produce the electrical response may relate to a permeability change in the disc membrane. The model systems of rhodopsin in a phospholipid bilayer permits the investigation of light-activated ion movement in membranes. Results of model membranes appear to be consistent with current interpretations of x-ray studies of intact photoreceptor membranes.

Dr. Richard A. Cone[23] and associates at the Johns Hopkins University are studying the effect of rhodopsin molecule on the general disc membrane characteristics. The rapid lateral diffusion of rhodopsin within the disc membrane has been observed by microspectrophotometric techniques. Their results indicate that the effective viscosity of the membrane permits rhodopsin to float freely in the lipid phase. These investigators find that rod outer segments offer the best model for the study of rapid lateral diffusion of proteins in membranes. By observing diffusion constants or rhodopsin, it will be possible to determine the effective radius of the molecule within the lipid phase of the membrane. This work implies that in the plane of the membrane, rhodopsin molecule must be slightly elliptical or nearly circular in shape. Rhodopsin may be thought of as a neuronal protein, in a neural membrane, and knowledge of its structure and function will reveal factors which influence transport of drugs, nutritional components and the spread of electrical energy in photoreception.

The question of rod outer segment disc organization and the localization of rhodopsin have also been investigated by Dr. Edward A. Dratz[24] and associates at the University of California. Preparations of intact discs and rod outer segment fractions were exposed to membrane-permeable and membrane-impermeable reagents which label amino acids. They found that half of the rhodopsin amino groups were labelled by the membrane impermeable reagent. These data indicate that rhodopsin must protrude into the aqueous medium between discs. It seems clear that rhodopsin exposes a hydrophilic surface on the outside of the disc. It is still unclear whether a single rhodopsin molecule transverses the disc membrane or whether rhodopsin molecules are situated on both sides of the

membrane. Factors which influence membrane structure and function will be more easily understood as more information about membrane proteins and ion transport is obtained.

VISUAL PIGMENTS

The question posed is how changes in molecular structure of visual pigment molecules relate with nervous excitation of the photoreceptor. Since structural changes in the photopigments are the first events in the transduction process, comprehension of visual pigment photochemistry will help to understand visual excitation. Correlations between ionization and hydrogen-ion concentration changes of rhodopsin and the late receptor potential have been investigated by Dr. Sanford E. Ostroy[25] and associates at Purdue University, and thermal intermediates of visual pigment bleaching have been isolated. The experiments show proton uptake during illumination and proton release during later processes. These reversable effects implicate hydrogen ion changes in visual adaptation. Furthermore, ionization changes may be a controlling factor for the existence of photopigment intermediates.

Dr. Bruce E. Goldstein[26] and associates at the University of Pittsburgh emphasize that in addition to morphological differences between rods and cones, rod and cone pigment regeneration in isolated retinas are different. Perhaps the observed effects are due to chemical differences between rod and cone photosegments. They have used the early receptor potential to show that the frog's green-rod and cone photopigments regenerate in the isolated retina whereas rhodopsin does not. It appears that photoproducts of cone pigment bleaching may decay faster than do rod photoproducts. Microspectrophotometric methods will detect rod photoproducts, but in the absence of cone photoproducts, they may have decayed to a non-visible portion of the spectrum. Differences between rod and cone photoproduct chemistry may be important in understanding why some degenerative diseases, such as retinitis pigmentosa, appear to be initially selective for rods.

MACULOPATHIES

Dr. Edward W. Norton[27] and associates at the University of Miami continue to study macular diseases in selected groups of patients with special emphasis on diagnosis, classification, natural history and hereditary and familial aspects. Wherever possible, histological correlations are made with the clinical observations. These investigators have contributed to the understanding of the pathophysiology of macular diseases. Among the specific diseases studied are cystoid macular edema, idiopathic central serous choroidopathy, and senile disciform macular degeneration.

Dr. John D.M. Gass[28], Bascom Palmer Eye Institute has reviewed 200 patients with macular drusen and disciform degenerative changes. He has concluded that all of these patients have familial diseases which affect the choriocapillaris and pigment epithelium. The involvement of the overlying retina is secondary. It is important to continue the study to determine whether there is evidence that any form of medical treatment alters its natural course.

Some clinicians have advocated a therapeutic approach to the problem of central serous choroidopathy and have employed photocoagulators. Dr. Ephraim Friedman[29] and associates at Boston University Medical Center are among those who have emphasized the need for controlled studies of "nontreatment". These investigators have presented clinical data from 27 patients with documented central serous choroidopathy who were not photocoagulated and whose condition was followed by fluorescein angiography. These investigators believe that in most cases the disease is benign and self-limiting, and therefore, may require no treatment by photocoagulation. They do recognize that in some cases photocoagulation may shorten the duration of a given episode of central serous choroidopathy; however, there are potential hazards in the treatment.

Dr. Samuel V. Vainisi[30] and associates at the University of Illinois have been concerned with the establishment of a breeding colony of baboons with heredomacular degeneration. They have been interested in the inbred colony of sixty baboons at the Brookfield Zoo. These animals are being examined, and behavioral, photographic, electroretinographic and fluorescein studies will be conducted in order to describe progressive lesions which might be detected in the macular region. These investigators observed behavioral abnormalities in a baboon which led them to suspect that the animal had visual acuity deficiencies. Clinical tests indicated that the animal had juvenile macular degeneration. It was planned that the animal would be useful in establishment of a breeding colony, however, she died of unknown causes. Histopathologic analysis confirmed the clinical diagnosis. It is assumed that the lesion may be inherited and indicates that the baboon may provide a nonhuman primate model for investigation of macular degeneration.

Further studies which involve the creation of experimental models in rhesus monkey in order to study various pathogenic mechanisms of macular degeneration are being conducted by Dr. Mark O.M. Tso[31] and associates at the Armed Forces Institute of Pathology. These investigators believe that one of the possible pathogenic factors of macular degeneration is chronic exposure to an excessive amount of light. They produced an experimental model in several stages of development by exposure of the macular region to the light of an indirect ophthalmoscope. The light provides a mild photic insult. Fluorescein angiography showed that leakage of fluorescein is the first clinical sign of damage to the macula. The leakage started 24 to 48 hours after exposure and is probably caused by a mechanism other than photocoagulation. These experiments demonstrate that a good experimental model can be produced.

TUMORS

Biochemical Developments

Retinoblastoma resembles other tumors such as lymphoma for which there is evidence which suggests a viral cause. Dr. Daniel M. Albert[32] and associates at Yale University School of Medicine argue for a viral etiology in retinoblastoma, although direct evidence for the role of a virus is lacking. Electron microscopic examination and immunologic methods have yielded little evidence

of viral infection. Based on biochemical methods, these investigators have been studying the RNA-directed DNA-polymerase activity in human ocular tumors. This enzyme is important because of its presence in animal RNA oncogenic viruses which have been analyzed. This enzyme activity has not been demonstrated in normal, rapidly dividing tissues but has been found to be present in ten specimens of retinoblastoma.

Ultrasonic Developments

At Case Western Reserve University, engineering and ophthalmic skills have been combined under the direction of Dr. Adnan Sokollu[33] and Dr. Edward W. Purnell. These investigators have developed a hand-held B-scan ultrasonic pulse-echo apparatus for the two-dimensional visualization of ocular tissue. The scan mechanism and display unit are sufficiently portable so that they can be rolled to the patient's bedside or into the operating room. The use of this portable unit in the operating room is the first application of B-scanning techniques in surgery. The B-scan ultrasonographic method is especially helpful in explorations of tissues behind the eye globe. A complete examination is made possible because the flexible hand-held device retains the capability of larger scanners to differentiate finer tissue details.

Further work on improving capabilities of ultrasonic diagnostic systems to ensure a more reliable diagnosis of ocular and orbital tumors is being conducted through the collaborative efforts of Dr. D. Jackson Coleman[34] and Dr. Frederic L. Lizzi at Columbia University and Riverside Research Institute in New York City. A large number of patients have been examined ultrasonically, and clinical studies have demonstrated the value of B-scan ultrasonic methods for patient evaluation. These investigators have designed a dual beam scanning system. The equipment being developed by this group will enhance tissue reflectance levels and separation of tissue planes which is useful in demonstrating variations in ocular tumor patterns and vitreous hemorrhage. The ultrasonic measurement of tissue characteristics is being studied in order to improve the diagnostic usefulness of these methods.

UVEITIS

Studies in progress in experimental animals indicate that prostaglandins are important in the development of inflammation in the human eye. As a response to mechanical trauma or induced uveitis, prostaglandin-like substances are released into the aqueous humor. Substances with prostaglandin-like activity are not detected in uninflamed eyes. Dr. Kenneth E. Eakins[35] and associates at Columbia University have been exploring the role of prostaglandins in the physiology and pathology of the anterior uvea. These investigators have used the rabbit to demonstrate that fluorescein-iris angiography techniques is useful for recording alterations in the diameter and permeability of the uveal blood vessels. Topical application of prostaglandins produces vasodilation and an increase in permeability of blood vessels at the base of the iris which are more sensitive to the actions of the prostaglandins. The vascular alterations produced by prostaglandins in the anterior uvea may be diminished

by prostaglandin antagonists. The action of prostaglandin-blocking agents may be of value in the control of the clinical condition of acute anterior uveitis.

Dr. Laszlo Z. Bito[36] and associates at Columbia University have been investigating the mechanism and characteristics of the prostaglandin transport system. It appears that a single episode of uveitis is capable of damaging an absorptive prostaglandin transport and its accumualation in the anterior chamber. The evidence suggests that there is substantial removal of prostaglandins from the aqueous humor in the posterior chamber by the ciliary processes. Since prostaglandins are associated with and can reproduce the symptoms of uveitis, the accumulation of prostaglandins may be of clinical significance.

Since the iris may modify the composition of aqueous humor, the permeability of the iris capillary network is of interest. Dr. Richard S. Smith[37] and associates at the Albany Medical College have been investigating the normal ultrastructure of the iris capillaries and their permeability to tracers to establish the anatomic location of vascular and epithelial barrier systems. Electron-dense, low molecular weight horseradish peroxidase will pass rapidly from ciliary capillaries and fill the extracellular space of the pigmented ciliary epithelium. Tight junctions are evident in the non-pigmented ciliary epithelium and may be the blood-aqueous barrier to low molecular weight substances. Peroxidase does enter the posterior chambers rapidly and the transport through the non-pigmented epithelium is hypothesized to have taken place by pinocytotic activity. Transport of water and of smaller molecules may occur by mechanisms not explained by these studies. Therefore, it has been shown the free movement of solutes between portions of the extracellular space in the eye is controlled by the presence of anatomic and physiologic permeability barriers. Vascular leaks occur in a variety of clinical conditions, such as central serous retinopathy, macular degeneration, diabetic retinopathy as well as uveitis. Production of a satisfactory experimental model for vascular leakage will permit studies of these conditions and enhance the understanding and treatment of these clinical entities.

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CORNEA

The cornea is composed of three main layers. The outermost layer consists of typical stratified epithelium; the middle, central portion or stroma, consists of a connective tissue, collagen fibers embedded in mucopolysaccharides. The third or inner membrane, called the endothelium, consists of one layer of flat cells bathed by the aqueous humor. Accumulation of water occurs in the stroma, while the outer epithelium or the inner endothelium provides the metabolic activities necessary to maintain the cornea dehydrated. The site of the transport process, as well as the mechanism by which water is removed normally from the stroma, are of critical importance for the understanding of corneal physiology. It is now established that a fluid pump which is concerned with the maintenance of the hydration of the stroma is located in the cornea endothelium. The mechanism driving this pump or how this pump operates is not understood. Dr. Jorge Fischbarg, Columbia University[1] has, however, recently discovered that the endothelium layer generates a small electric potential. Electrical potential across biological membranes are usually associated with active transport ions. This could form the basis for the water pump which is present in the endothelium. In support of this idea, Dr. Fischbarg has noted that several drugs which inhibit the fluid pump of the cornea also depress electrical potential. The electrical potential developed by the endothelium of the cornea has also been measured by Dr. David M. Maurice, Stanford University[2]. The magnitude and size of the pump according to Dr. Maurice suggests that it could be related to the active transport of ions.

The cornea is the only tissue with high collagen content that is transparent. The collagenous material of the stroma is made up of small fibrils which occupy about 1/10 of the total thickness of the cornea. The transparency of the stroma of the cornea was first explained as an interference effect based on the perfect regularity of the collagen fibrils--the lattice theory. According to this theory, the cornea is transparent because there is a "destructive" interference of the light scattered by the individual fibers.

Recent work by Dr. George B. Benedek has discounted the need for perfect regularity in the collagen fibers in order for the interference mechanism to succeed and has indicated that the cornea is transparent because the size of the fibrils and the spaces between them are of such values that the incident visible light is not scattered. The findings of electron microscopy substantiate this view, but the effects of fixation and embedding on the regularity of the fibrils are uncertain. Clouding of the cornea was ascribed to a disorganization of the fibril lattice and Benedek considered it a result of the forming of open spaces in the structure, the so-called lakes.

This question was partially solved by measurement of the angular and spectral distribution of the scattered light by Dr. Frederick A. Bettelheim[3] at Adelphi University. Dr. Bettelheim found that the light scattered in normal bovine and human cornea is largely due to the birefringence*. The intrinsic birefringence is the result of non-random distribution of collagen fibrils at different loci. However, in the visual path most species have minimal birefringence areas. The distribution of equal birefringent areas of the cornea in different species account largely for the adaptive processes that the species evolves within its environment. According to Dr. Bettelheim, there is

*Double refraction

a strong interplay between the two tasks of the cornea: mechanical protection and transparency. Molecular superstructures are built to accomplish both of these tasks and therefore, the molecular organization of the periphery and center of the cornea may be different.

Herpes simplex infection of the cornea is one of the most important ocular virus disease. Its importance stems not only from the frequency of initial attacks, but also from the likelihood of its recurrence with progressive scarring and morbidity, and also from the fact that this is one of the few virus diseases that may in some cases be successfully treated with chemotherapeutic agents. In 1961, Dr. Herbert E. Kaufman at the University of Florida showed that 5-iodo-2'-deoxyuridine (IDU) was a therapeutically effective antiviral agent in the management of herpetic keratitis. Since then, several other promising antiviral nucleotides have been synthesized and evaluated for efficacy in control of herpes virus. Among these are: (1) cytosine arabinoside (Ara-C), (2) adenine arabinoside (Ara-A) and (3) trifluorothymidine.

In 1973, Dr. Kaufman[4] reported that trifluorothymidine appeared to be the most useful of currently available antivirals for topical treatment. Trifluorothymidine is virtually non-allergic, is approximately ten times as potent as IDU and is much more soluble. Most importantly, the rapidity and regularity of ulcer healing with trifluorothymidine is far superior to that of IDU.

Recurrent herpes simplex is one of the major problems in ophthalmology both in terms of morbidity, and in terms of visual disability. Present evidence indicates that once someone has an initial attack of herpes simplex the odds are approximately 25% of having another attack within two years. If there has been more than one attack of corneal herpes the odds are approximately 43% of having another attack within two years. The pathogenic mechanism of the recurrence so characteristic of herpes simplex infection is unknown. Many hypotheses have been proposed to explain what appears to be latent herpes simplex virus infection in many experimental animals.

Recently, Dr. Anthony Nesburn, Estelle Doheny Eye Foundation Laboratory[5], has found that latent herpes simplex virus can be isolated from rabbit trigeminal ganglia between episodes of recurrent ocular disease, whereas no evidence of herpes simplex virus infection was found in any other tissue tested. The concept that virus might not be latent within the cornea epithelium but might come to the cornea from exogenous sources has raised new hope that such recurrence might be preventable by drugs. Dr. Kaufman reported that the prevention of recurrent herpes simplex might be possible through the use of interferon. Interferon inducers such as Poly-IC were studied by Dr. Kaufman extensively and found to be very effective in rabbits and other rodents. In monkeys and in man however, these interferon inducers seem to have only a minimal effect which disappears rapidly. Human interferon on the other hand, according to Dr. Kaufman, effectively prevents herpes infection in monkeys even when given as drops as seldom as twice a day. The finding that human interferon administered to monkeys protects against herpes is encouraging, and certainly justifies further attempts to define the therapeutic dose required, and the practicality of administration to man for the prevention of the herpes simplex keratitis.

In terms of applied clinical research, clearly one of the most exciting recent developments in corneal research is the therapeutic soft contact lens. The idea of covering the cornea and protecting it is not new, but the ability to protect the cornea with an optically clear dressing which is well tolerated has not been possible until the advent of the soft lens. Dr. Kaufman[6] developed and first reported the use of the soft contact lens for the treatment of cornea disease.

Recently, soft contact lenses made of hydrophilic gel have proved safe and effective for the treatment of bullous keratopathy. Bullous keratopathy is a condition in which the inside layer of the cornea, the endothelium, is damaged, either as a result of cataract extraction or because of degeneration with age. It is estimated that of the five hundred thousand cataract extractions done a year, approximately five percent result in bullous keratopathy with swelling and clouding of the cornea, blisters on the surface which can be excruciatingly painful, and reduce vision. Soft contact lenses create a smooth optical surface, improving vision in a significant proportion of these patients. In addition, in the vast majority of such patients these lenses, by preventing the blisters from breaking in a painful way, restore comfort to patients who might otherwise be in agony. They have been found to be effective not only in the approximately twenty-five thousand patients a year who develop bullous keratopathy after cataract extraction, but in thousands more who develop it from degenerations due to aging and other causes.

In addition to their general availability for the treatment of bullous keratopathy, these moist, optically clear, protective lenses have proven useful in many other kinds of corneal disease. In some patients with arthritis, and in others with aging alone, normal tear production becomes deficient and the eye dries and becomes scarred. Soft lenses have provided a moist protective blanket for such eyes. By protecting the surface of the cornea in a wide variety of other conditions, they have permitted corneal ulcers to heal. By creating a smooth surface without rubbing off the surface tissue after injuries and other conditions which create irregularities in the corneal surface they have prevented tissue damage while providing good vision. Good vision is present since their smooth spherical surface becomes the effective front surface of the eye.

Another exciting development stemming from basic research is the finding that the collagen fibrils of the cornea can be altered by heat so that the front of the eye changes its shape without the development of corneal scarring. A procedure called thermokeratoplasty, developed by Dr. Antonio Gassett and associate, University of Florida[6], has been used on dozens of patients with keratoconus, a bulging forward of the front of the eye which grossly distorts vision and can even ultimately rupture. This special heat probe irons the cornea back to its normal shape and permits these patients to function again without the risks and enormous expense of corneal transplantation. This procedure has shown a high degree of effectiveness.

It has been demonstrated that the corneal shape can be changed with heat, and this offers the possibility that, with further research, nearsightedness

may be effectively treated, and other kinds of optical corrections may be done in a relatively simple manner.

Several thousand corneal transplants are done in the United States each year, and although the donor cornea from a foreign donor is usually well accepted, between ten and fifty percent of corneal transplants may fail because of immune rejection. There is evidence in animals that it is possible to treat antibodies against this graft tissue chemically so that they will bond to the graft but not damage the transplant. When this is done, animal experience indicates that the graft is significantly protected. Dr. John Chandler[7] at the University of Florida College of Medicine has developed a "blocking" antibody in which corneal buttons are bathed prior to transplantation. The "blocking" antibody binds to and coats corneal and lymphoid cells and protects them from the cytotoxic antibodies.

Growth factors which specifically stimulate epithelial or mesodermal cell growth have been described by Dr. Virginia Weimar and Dr. Kenneth Haraguchi[8] at the University of Oregon Medical School. A multiplicity of mesodermal growth factors have been discovered by Dr. Weimar and Dr. Haraguchi. Certain combinations of these mesodermal growth factors also appear to block cell growth, and may have great potential for application in ocular and in other tissues. Poor cell growth could be overcome by application of these factors. It is anticipated that scarring in the cornea might be inhibited by localized application of antibodies of the mesodermal growth factors. The mesodermal growth factors, in particular, have been discovered so recently, that their necessary isolation and purification for further studies are still in progress. In connection with these studies, a computerized image analysis system has been developed by Dr. Weimar for evaluating connective tissue cell growth. This image analysis system provides a valuable new tool for the evaluation of the effect of various drugs on wound healing in connective tissues of all types.

It has long been suspected that collagenolytic enzymes were responsible for connective tissue destruction in various disease states in various parts of the body. However, only recently have studies of the origin of the perforation of the alkali-burned cornea indicated that the collagenase causes tissue destruction. Studies on the cell origin of the collagenase show that this enzyme was produced not only by corneal epithelium but also by the underlying stroma.

The isolation and characterization of corneal collagenases by Dr. Stuart Brown and associates, Cornell University[9] are extremely important steps in research aimed at finding methods for treating and preventing corneal scarring following corneal burns and herpes infections. As more is known about these collagenases, perhaps specific inhibitors can be found which will not have harmful side effects. Drs. Alan Sugar and Stephen Waltman, Washington University School of Medicine[10], have found that some of the collagenase inhibitors currently used for therapy have toxic effects on the cornea and can themselves cause scarring. The inhibition of corneal collagenases by serum antiproteases recently described by Drs. Michael Berman and associates[11] of the Retina Foundation appears to be very promising.

Trachoma-inclusion-conjunctivitis (TRIC) agents (chlamydia) are the most common causes of infectious eye diseases in the world. While the disease has almost disappeared from the more industrialized countries, it is still a severe problem in many developing countries. In some areas trachoma is holoendemic and, although recognized as a major public health problem, limited financial resources and other pressing medical problems often restrict the scope of trachoma control programs where they are most needed.

There are only six laboratories in the world today, three in the United States, Dr. J. Grayston at the University of Washington, Dr. R. Nichols at Harvard and Dr. C.R. Dawson at the University of California in San Francisco, with a major commitment to trachoma research. Recently a large trachoma research unit at the Lister Institute in London ceased operations after fifteen years of significant research in the field.

While trachoma is not an important public health problem for industrialized nations, only countries like the United States have the resources to sustain research in trachoma on a sufficient scale. Research aimed at controlling blindness due to trachoma can be justified on humanitarian grounds alone, but there is also a long-term economic benefit since developing countries can become self-sufficient sooner and will need less economic aid if the burden of economically blind is decreased and the number of productive adults is increased.

A substantial number of trachoma cases in American Indians have been reported. Since 1965 Dr. Chandler Dawson and associates[12-13], at the University of California San Francisco have been conducting controlled chemotherapy trials of trachoma among American Indians. The goals of Dr. Dawson are to evaluate the role of long acting tetracyclines in the therapy of trachoma, the role of type-specific-chlamydial antibodies in tears and serum of infected persons and to determine the effect of lysozyme on extracellular chlamydia. The results of these studies provide guidance for the Indian Health Service (USPHS) and Navajo Tribal Council in implementation of thrachoma control programs.

Ophthalmologists are accustomed to identifying signs of fully expressed inherited diseases in the eye: e.g. the macular cherry-red spot of Tay-Sachs disease and cornea verticillata of Fabry's disease. Physical signs of the carrier state of inborn errors of metabolism are much less frequently observed, and have never been described in some disorders. Accurate, safe, expeditious, and inexpensive tests that will identify heterozygous carriers are indispensable for genetic counseling.

Recently, such tests have been developed for Tay-Sachs disease and Fabry's disease. Dr. Edward Cotlier, University of Illinois Eye and Ear Infirmary[14], has shown that the specific enzyme deficiency that characterizes Tay-Sachs disease can be detected in human tears. Affected homozygous children show a virtual absence of the normal enzyme hexosaminidase-A, whereas asymptomatic carrier heterozygous individuals have an enzyme level that is intermediate between that of disease and normal individuals. Similar results have recently

been detected for the enzyme deficiency of Fabry's disease by Dr. Cotlier and associates[15]. Virtually no activity of the responsible enzyme, alpha-galactosidase-A was detected in the tears of affected male patients, whereas carrier female patients have intermediate reduction in activity as compared with normal individuals.

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CATARACT

One aim of lens research directly or indirectly, is the elucidation of the mechanism, or mechanisms, which result in loss of normal lens transparency. A prerequisite for this, however, is an understanding of the basis for the quite remarkable transparency of the normal lens. The transparency of the lens depends upon the maintenance of a high degree of order in the protein molecules of which it consists.

Until recently, no clear evidence existed to indicate what mechanism was responsible for the orderly configuration of the lens proteins.

Dr. Frederick Bettelheim, Adelphi University[1], has developed special non-destructive optico-mechanical methods that are useful in providing information on two facets of lens and cornea research: (a) transparency, and (b) super-molecular aggregate structures. Actually, the two facets are complementary. The optical properties, for example, provide structural information and at the same time, one can infer what process may lead to structural changes that cause cataracts.

In bovine lens (and later in human lens) Dr. Bettelheim found that the scattered light (opacity) occurs at low angles only and is due to density fluctuations alone. In dynamic experiments during which the lens is under periodic vibrations of set frequencies, he found that the structural changes in the vibrational cycle are quite different in the cortical and nuclear regions. From these findings, one could infer that cortical cataracts are of different nature (formation of lakes and separation of fiber cells) than those in the nuclear region (senile cataracts characterized by increasing size of aggregation of macromolecules in the fiber cell).

The behavior of alpha-crystallin protein in the lens and the mechanism by which it is converted to albuminoid and subsequently to the opaque material in cataract have long been of interest to many investigators.

Dr. Bettelheim's[2] work on the existence and function of phosphopeptides in the aggregation of alpha-crystallin appears to provide a breakthrough in the cataract field. He prepared for the first time an alpha-crystallin which gave by X-ray diffraction evidence of crystallinity. This was lost by the protein during dialysis, although the two phosphopeptides retained an altered X-ray diffraction pattern. The amorphous protein could then be recombined with the phosphopeptides to yield the original crystallin complex. The importance of this work depends upon the revelation of a new and unexpected factor in the control of alpha-crystallin organization on the macromolecular level. Until this paper appeared, it was tacitly assumed that the alpha-crystallin molecule carried within it the machinery needed to control its configuration. Although recent work by others has indicated a possible role for sugar alcohols, sugars and calcium ions in alpha-crystallin aggregation, this work on phosphopeptides views the problem from a fresh viewpoint.

There are many possible mechanisms of lens opacification, including any change capable of increasing the scattering or absorption of light. The theory of light scattering indicates that aggregation of the crystalline proteins in the lens can produce opacity as in the case of cataract.

Dr. Judith Jedziniak and associates of the Massachusetts Institute of Technology[3], have provided evidence which implicates calcium in the aggregation of alpha-crystallin of human lenses. The process is irreversible and produces molecules of a size which can affect light transmission. The authors equated calcium ion and total calcium in their discussions of cataractous and aging lenses and were able to demonstrate an effect of calcium which may have some pertinence to human cataract formation. They also found important evidence of compositional changes in the higher molecular weight alpha-crystallin aggregates. During aggregation there is an increase in leucine (which would depress solubility by making the molecule more hydrophobic) and a decrease in tyrosine (possibly because tyrosine is tied up in covalent linages peculiar to the aggregation process).

Dr. Abraham Spector, Columbia University[4], has continued his studies of the effect of aging on protein synthesis and protein structure of the lens and has attempted to correlate his findings with the development of geriatric cataract. He has demonstrated that the alpha-crystallin macromolecule of the lens increases in size as a function of aging. The only chemical difference noted between the large macromolecule and normal sized alpha-crystallin is the presence of 2-3% sugar and a decrease in solubility. This finding suggests that the insolubility of the alpha-crystallin as a function of aging may be associated with the development of geriatric cataracts.

Disordered carbohydrate metabolism has been long considered a potential cause of cataract formation. In 1957 it was found that an enzyme, hexokinase, has a key role in lens glycolysis. Since the action of hexokinase on glucose is the major controlling step in lens energy metabolism, the characterization of this enzyme is of great importance. A failure of the enzyme activity, for instance, can produce a significant metabolic stress on the lens. Dr. Leo Chylack, Massachusetts Eye and Ear Informary[5], has studied the hexokinase and found that it consists of two forms, only one of which is prominent in the human lens. The two forms of the enzyme (or isozymes) have a different distribution in the lens and different sensitivity to the glucose concentration. The potential significance of Dr. Chylack's finding is that for the first time there appears to be a chance of correlating changes in a major enzyme during the pre-cataractous stage with the subsequent development of cataract.

The lens contains the highest concentration of protein and glutathione of any tissue of the body. This unusual characteristic imposes a need for the continuous supply of amino acid for the synthesis of these compounds. Because of its avascularity the lens must derive most of the constituents required for metabolic energy and synthetic reactions from the surrounding intraocular fluids. Although the mechanism for synthesis of amino acids appear to be present in the lens, studies from Dr. Venkat Reddy, Oakland University[6], have revealed that transport mechanisms rather than synthetic processes play a major role in supplying amino acids to the lens.

Another aspect of lens enzymes and metabolism has been studied by Drs. William Rathbun and Katheryn Wicker, Univesity of Minnesota Medical School[7], in the past year. Dr. Rathbun has successfully demonstrated the activity of the enzyme γ-glutamyl transpeptidase in the lens, a key enzyme in glutathione metabolism. The function of glutathione in the lens and the details of its metabolism have long been mysterious processes because of the extremely high concentration of glutathione in all normal lenses and its loss in most cataracts without leaving a trace of catabolic products. The particular mode of glutathione metabolism is of timely interest in view of the fact that it has been found recently to be involved in amino acid transport in the kidney. If it so functions in the lens, Dr. Rathbun's findings would be highly pertinent since amino acids are so important in lens metabolism.

Dr. Kenshi Satoh and associates from the Juntendo University, Tokyo, Japan[8], have restudied the fluorescence of the human lens and obtained definitive results. There are two fluorescence bands: the purple (340nm) band is excited at 290 nm (in the long wave ultraviolet); the blue (420 nm) band is excited at 340 nm. The purple fluorescence is the major one in all protein fractions and its intensity is relatively constant with age. The blue fluorescence increases with age especially in the insoluble protein fraction. Although the application of this research to cataractogenesis is not yet apparent, there is much earlier work concerned with lens fluorescence as a diagnostic or predictive tool and more recent research is concerned with changes in certain amino acids which are correlated with the formation of dark nuclear cataract and changes in state of these amino acids (most importantly tyrosine and tryptophan). An important finding is that both these types of fluorescence are also found in the rabbit lens which is far removed from the human lens in many parameters. Thus it will be possible to carry out some experimental studies in an animal model.

The idea that exposure to sunlight may play a role in the formation of a type of cataract called brunescent cataract is an old one and is based chiefly on clinical evidence concerning the geographical distribution of such cataracts. Experimental evidence for this concept has been lacking until the recent work of Drs. Seymour Zigman, Joanne Schultz and Teresa Yulo, University of Rochester[9], who were able to demonstrate biochemical changes in the lenses of mice exposed chronically to long wave ultraviolet light, the only kind of ultraviolet which reaches the lens in vivo. After 16 weeks of light exposure, the mouse lenses began to show alterations in the protein fraction profile including particularly an increase in insoluble protein. Changes in permeability to amino acids and in their rate of incorporation into protein were likewise shown for dogfish lenses exposed in vitro to long wave ultraviolet light for 24 hours.

A search for early changes in the lens of cataractogenic agents has been pursued by Dr. John Kuck, Emory University[10]. Work is in progress to explore in experimental animals the combined effect of photosensitizing agents such as trimethylpsoralen and ultraviolet light and other potential cataractogenic agents like certain drugs and toxicants in food. One of the principal aims of Dr. Kuck's investigation is to determine if such cataractogenic processes are accompanied by permeability changes which can be monitored by tracer uptake before visible lesions become evident.

Dr. Robert Cowgill, Wake Forest University[11], studied a brown, melanoid pigment bound to the insoluble protein fraction of human cataractous lens. Dr. Cowgill demonstrated that the brown pigments are not the typical melanin pigment formed in other parts of the body. The pigment is a cross-linking bityrosin associated with the most insoluble proteins in the cataractous lens. The origin of the bityrosin is obscure. Dr. Cowgill, however, excluded the possibility that it originates from connective tissues and suggested that it probably arises from lens proteins present either in a normally or abnormally condensed state to permit formation of linkage between tyrosyl groups.

The morphogenetic sequences of lens development have been studied by Dr. Johan Zwaan, Children's Hospital Medical Center[12]. By immunoelectrophoresis techniques, he found that crystallins are tissue-specific proteins and their production begins at a relatively late stage of lens morphogenesis. Alpha-crystallin is the first protein to be produced by the lens, followed by beta-crystallin. Dr. Zwaan's work shed some light on the factors that are involved in the differentiation of the lens and of the genetic aberration that may disturb normal development. These findings are of interest in the study of congenital cataract.

Wound healing is a fundamental property that is indispensable for the maintenance of the normal function of an organism. The process of tissue repair encompasses some of the most fundamental problems in biology, i.e., the control of cellular migration, cell division, and differentiation. In addition to the repair process itself, injury presents a situation in which the trigger mechanism(s) of mitosis can be investigated and since the mitotic rate usually returns to normal upon removal of the stimulus, it provides a system to examine the mechanism(s) by which cells enter and leave the cell cycle. Dr. John Reddan's, Oakland University[13-14], studies are focused on the biochemical and morphological events that proceed and accompany injury-induced cell division and migration in the lens. Dr. Reddan found that mitosis can be induced in vitro in the central lens epithelium by the addition of insulin.

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GLAUCOMA

Glaucoma is a disease of the eye characterized initially by an abnormal elevation of the intraocular pressure. This is often followed by progressive loss of vision in parts of the visual field, and eventually by total blindness. As a leading cause of blindness, it represents one out of every eight cases of new blindness and it is estimated that one out of every 200 people in the United States over forty years of age has glaucoma. It often accompanies systemic disease, such as diabetes, rheumatoid arthritis and certain inflammatory conditions.

Studies of glaucoma may be divided into three categories: (a) those which aim to elucidate the mechanism by which intraocular pressure becomes elevated, (b) those aimed at understanding the mechanisms by which elevated intraocular pressure exerts its damaging effects upon the eye, and (c) those aimed at the improvement of diagnostic methodology and treatment procedures. The goal of much of this research is to acquire knowledge which will allow for the eventual prevention of glaucoma.

Aqueous humor, the fluid which fills the anterior segment of the eye, maintains the intraocular pressure. The fluid is produced in the ciliary body where it enters the posterior chamber, passes to the anterior chamber, and is drained primarily via the trabecular meshwork, where it enters Schlemm's canal on its way to the venous circulation. In angle-closure glaucoma, the aqueous outflow route is obstructed by apposition of the iris with the openings in the trabecular meshwork (the angle of the anterior chamber is closed). In primary open-angle glaucoma, the most prevalent form of the disease, the defect which leads to the abnormal increase in intraocular pressure is not as readily apparent. The central issues in much of the research on glaucoma are determination of the mechanisms involved in the production of aqueous humor (inflow) and determination of the normative and pathologic factors involved in regulating outflow.

A second aspect of glaucoma is the damage that excessively high intraocular pressure causes in the form of optic-disc cupping, optic nerve fiber loss, and the resultant visual field loss. The nerve fibers atrophy in the disc, retina and optic nerve. In addition, there is loss of the supporting astroglia in the optic nerve. It is usually assumed that the primary site of damage to the nerve fibers is at the optic nerve head, and that the loss of nerve fibers along the rest of the pathway occurs secondarily.

In addition to this concern with etiology, and with finding possible means of prevention, research in glaucoma is also directed at improvement of diagnosis and treatment. Methods are currently being developed to allow for more accurate measurement of intraocular pressure, aqueous production rate, facility of outflow, and extent of damage to the optic vasculature, the optic disc and the optic nerve. In addition, advances are being made in the development of improved pharmacological and surgical treatment of glaucomatous disorders.

AQUEOUS HUMOR DYNAMICS

It is well established that it is the relationship between rate of aqueous humor flow and resistance to its exit from the eye which establishes intraocular

pressure at a certain level above the pressure of the intra- and extra-ocular vessels. NEI supported investigators throughout the United States, and in Sweden, have been examining the dynamics of this system.

Aqueous Production

Dr. Richard Brubaker and his associates at the Mayo Foundation[1] have examined the filtration coefficient of the intraocular vasculature in monkeys. They conclude from their findings that differences in intraocular pressure between individuals, the therapeutic effects of hyperosmotic agents, and the diagnostically recognized effects of water drinking on intraocular pressure are all mediated by physio-chemical events at the intraocular blood-vessel wall. The role of neural factors in this process has also been demonstrated by Dr. Bernard Becker and associates at Washington University in St. Louis[2] when they found that the instillation of hypo-osmotic agents into the third ventricle of the rabbit brain (near the hypothalamus) resulted in an elevation of intraocular pressure. This elevation of intraocular pressure was not observed in single eyes which underwent optic nerve transection but remained in the contralateral nontransected eye.

Dr. Keith Green and associates at Johns Hopkins University[3-4] have developed a mathematical model which describes and measures the various factors involved in the production of aqueous humor at the ciliary body:facility, pseudofacility, capillary pressure, active secretion and the mean pressure index for filtration. The model has been employed[5] in examining the long-held view that active secretory processes are primary in aqueous humor formation and that passive, pressure-dependent factors (ultrafiltration) play only a minor role. Their analysis of perfusion data from the isolated rabbit ciliary body, from rabbits in vivo and from man, indicated that the reverse obtains; that ultrafiltration accounts for approximately 65% of aqueous production. However, the situation is still not clear, as researchers at Uppsala University present data to the contrary. Dr. Anders Bill[6] demonstrated that increasing the blood flow in the anterior uvea and ciliary process in monkeys does not yield automatic increases in the rate of aqueous humor formation. He concluded that in the monkey, at least, ultrafiltration does not play an important role in aqueous humor formation.

In many instances, the treatment of glaucoma involves the use of pharmacological agents which reduce aqueous production. Primary among these are adrenergic substances (e.g. epinephrine) and carbonic anhydrase inhibitors (e.g. acetazolamide [Diamox]). Although the clinical value of these agents has been recognized for many years, the biochemical mechanisms by which they lower intraocular pressure are just being determined. In many tissues adrenergic agents activate adenyl cyclase to produce adenosine 3', 5' -monophosphate (cyclic-AMP), and it is this "messenger" which actually mediates the physiological events which catecholamines initiate. Evidence has been reported in the past, by Dr. Marvin Sears at Yale University, that cyclic-AMP may play a central role in mediating the action of catecholamines on aqueous humor dynamics. Specifically, adrenergic agents which decrease intraocular pressure when administered topically to the rabbit eye, also increase the concentration of cyclic-AMP in the aqueous humor. In addition, intracameral injection of

cyclic-AMP lowers the intraocular pressure. Further research along these lines by Dr. Maurice Langham and his associates at Johns Hopkins University appears to demonstrate that the relationships between epinephrine, cyclic-AMP, and intraocular pressure are predominantly dependent on the alpha-agonistic properties of epinephrine[7-8].

It has been demonstrated by Dr. Monte Holland and associates at Tulane University that aqueous production is decreased and outflow facility is increased following chemical sympathectomy with 6-hydroxydopamine (6-HD) applied topically to the eyes of animals. Most importantly, they found that the beneficial pressure-lowering effects of epinephrine can be enhanced following this chemical sympathectomy. It has more recently been demonstrated by Dr. Albert Zeller at Northwestern University[9] that monoamine oxidase inhibitors have similar potentiating effects on the action of epinephrine on the rate of aqueous production and on intraocular pressure, in rabbits. These findings suggested that patients with glaucoma might be aided by combining 6-HD with topical epinephrine therapy. Dr. Monte Holland and his associates conducted a series of clinical studies with this pharmacological procedure on patients suffering from glaucomas that did not respond well to standard pharmacological treatment. In one study[10] they examined epinephrine dose-response relationships and demonstrated that the chemical sympathectomy was effective in lowering intraocular pressure over a 5-decade range of epinephrine concentrations. The results of a second study[11] showed that the supersensitizing effects of 6-HD obtain with a number of alpha- and beta-adrenergic amines without systemic side effects. However, epinephrine was found to be the most desirable agent because it was effective for a longer duration. In reviewing their experiences over two years with 92 patients (128 eyes), Holland et al.[12] conclude that 6-HD chemical sympathectomy is an adjunct for therapy which provides the patient with uncontrollable glaucoma a medical alternative to surgery.

Researchers at the University of Alabama, Washington University and Uppsala University have been examining the role of steroids in glaucoma. It has recently been reconfirmed by Dr. Ralph Levene and his associates[13] that steroid glaucoma can be consistently produced in rabbits by topical treatment with a 1% dexamethasone solution over a period of a few weeks. Examination of steroid effects in humans provided even more exciting results[14] when it was found that 85 patients with primary open-angle glaucoma had significantly higher plasma cortisol levels following orally administered dexamethasone than did 77 normals. In addition, the data on plasma cortisol level indicated a possible trend with age for the glaucoma patients but not for the normals. It has also been demonstrated by Dr. Bernard Becker and his associates at Washington University that patients with primary open-angle glaucoma are more sensitive to steroids in non-ocular systems as well as ocular systems[15-16]. Clearly, a systemic endocrine marker provides the potential for differentiating individuals with glaucoma.

Differential responding to steroids might also provide a methodology for identifying individuals who may be predisposed to elevated intraocular pressure and glaucoma[17-18]. Dr. Becker and his associates have defined three groups of normal individuals in terms of their response to adreno-cortical steroids--high,

moderate, and low responders. This response can be blocked to different degrees in each of the three groups by pre-treatment with a cortisol antagonist. It was found that "blocking" occurs earlier in glaucoma patients and in high-cortisol responders than in subjects who are low or moderate responders. These data are interpreted as providing further evidence for the theory that genetic factors relate the response to steroids and glaucoma.

Research at Johns Hopkins University, Washington University, and the Medical College of Georgia has demonstrated that intraocular pressure can also be affected by the administration of a number of autacoids (hormones) including prostaglandins (PG). The prostaglandins are a group of fatty acids which are known to antagonize the lypolysis induced by adrenergic agents. Their biochemical effects are exerted on adenyl cyclase (the target of epinephrine and a number of hormones) and on an enzyme involved in the production of cyclic-AMP.

Dr. Keith Green at Johns Hopkins[19], employing the isolated rabbit ciliary-body preparation presented data to indicate that PG enhances the permeability of the ciliary membrane which in turn results in elevated intraocular pressure. The primary site of action appears to be on the filtrative channels of the ciliary body. Dr. Tzu Chiang at the Medical College of Georgia[20] also demonstrated an increase in intraocular pressure with PG's in anesthetized rabbits, and in addition showed that other autacoids, in contrast, caused a lowering of pressure. He also reports that plasma PG-E1 levels in patients with open-angle or narrow-angle glaucoma were found to be higher than in non-glaucoma patients.

The ocular hypertension responses to some PG's, can be antagonized by a number of agents. Dr. Chiang demonstrated this to be the case in rabbits that were pretreated with either epinephrine or progesterone [21-22-23]. Dr. Becker and his associates at Washington University [24-25-26-27] have demonstrated that imidazole (a histaminic substance which enhances the reduction of cyclic-AMP) and indomethacin (an analgesic) were also effective in reducing the ocular pressure induced by PG's.

Dr. Keith Green[28] has also examined the effects of Δ-tetrahydrocannabinol (THC, a marihuana derivative) on aqueous humor production. THC was found to decrease the secretion and increase the filtration rate in the isolated rabbit ciliary body preparation. When live rabbits were injected with THC, intraocular pressure was found to decrease and both total outflow facility and aqueous protein level were found to increase. Dr. Green reports that the data available to date suggest that the response to THC involves vasoconstriction in the ocular blood vessels yielding a decrease in blood pressure, and thus a decrease in formation of aqueous at the ciliary body. The vasoconstriction obtained with THC may also have enhancing effects on intraocular pressure reduction by increasing aqueous outflow.

Aqueous Outflow

Mechanisms involving the structures in the angle of the anterior chamber--the trabecular meshwork and Schlemm's canal--are involved in the control of aqueous humor outflow from the eye to the venous circulation. Research with

monkeys by Dr. Anders Bill and associates at Uppsala has recently shown that an initial effect of constant artificial intraocular pressure elevation is a progressive increase in outflow facility[29]. However, this enhancement of outflow is only short-lived; outflow facility decreases after about two hours of maintained pressure elevation. Scanning and transmission electron microscopy indicate that the initial facility of outflow is due to damage ("punching holes") in the endothelial walls of the meshwork and of Schlemm's canal. By 24 hours, the defects are occluded by endothelial cells, blocking outflow. Along similar lines, Dr. Morton Grant and associates[30] at the Massachusetts Eye and Ear Infirmary have demonstrated that the rate of aqueous humor outflow through the trabecular meshwork and Schlemm's canal can be increased in enucleated monkey eyes by mechanically pressing the lens posteriorly. This effect was graded, reversible, and repeatable as long as the tissues in the angle of the anterior chamber were not damaged.

Employing the technique of suction gonioscopy, Dr. Bernard Becker and his associates at Washington University have examined the phenomenon of blood reflux into Schlemm's canal[31]. They find that the frequency of occurrence of blood reflux in patients is correlated with intraocular pressure level, facility of outflow, and corticosteroid responsiveness. An additional preliminary finding requiring further investigation was that the frequency of blood reflux in high corticosteroid responders was similar to that in glaucoma patients.

Efforts to improve the adequacy of the outflow channels make use of drugs or surgical procedures. The latter are usually reserved until all attempts at pharmacological maintenance of intraocular pressure have failed. Parasympathetic (cholinergic) agents, such as pilocarpine and anticholinesterases, are the medications primarily employed. However, employing a technique developed for the determination of small amounts of epinephrine in the presence of a large excess of other catecholamines[32], Dr. Ernst Barany and his associates at Uppsala presented evidence to indicate that epinephrine (an adrenergic) also increases outflow facility.

Advances in therapy with pilocarpine have been made using appliances that are easily placed directly on the eye. Dr. Bernard Becker and his associates have employed soft contact lenses, presoaked in pilocarpine. In a study with monkeys[33] they measured the amount of tritium-labelled pilocarpine entering the aqueous humor. It was found that there were higher and more prolonged concentrations of pilocarpine in the aqueous with this system than with pilocarpine eye drops. In a second study[34] soft-contact lens delivery of pilocarpine was examined with primary open-angle glaucoma patients who were otherwise uncontrolled on maximum medicinal therapy. It was reported that approximately half of these patients benefited from this new therapeutic technique.

The soft-contact lens technique is not without caveats. Apparently, larger doses of medication are pulsed into the eye in a relatively shorter period of time than with conventional eye-drop therapy. This raises the possibility for the occurrence of toxic effects. Another device which appears to be enjoying more stable effects employs a thin plastic wafer placed under the lower eyelid which allows a constant release of the appropriate dosage. This device, called the "Ocusert," has been developed by the Alza Corporation.

Dr. Mansour Armaly of George Washington University[35] reports that the magnitude of the mean percent reduction in outflow pressure in a group of glaucoma patients using the Ocusert for pilocarpine administration, was comparable to that with eye drops, and that the hypotensive effects were significantly greater.

With increasing detailed knowledge of the morphology and anatomy of the drainage system, investigations are concerned with the development of new surgical techniques. An example of such a project can be found at the Massachusetts Eye and Ear Infirmary. There, Dr. Morton Grant and his associates have attempted to devise a method of quantitative perfusion of excised segments of the angle of the anterior chamber. These experiments provide evidence that flow through this structure is very sensitive to slight physical distortions[36]. By perfusion of enucleated eyes, this research group has discovered several factors in microsurgery of Schlemm's canal that have not previously been recognized[37]. Namely: (a) in the current surgical procedure of probe trabeculotomy ab externo there is a strong tendency for channels opened by the surgery to close again, apparently as the disrupted trabecular meshwork tissues go back into place; and (b) passing the probe inside Schlemm's canal damages the outer wall of the canal, causing changes which tend to hinder aqueous outflow through the collector channels. In addition, in comparing a number of trabeculotomy techniques in enucleated eyes, these investigators find that internal trabeculotomy yields a greater increase in aqueous outflow than does trabeculotomy ab externo and suggest, therefore, that it is more promising for clinical use.

Dr. Grant has also been examining the feasibility of cyclocryotherapy in the treatment of a number of types of advanced, inadequately controlled glaucoma[38]. At the end of follow-up periods of approximately one year, the procedure was found to have maintained a reduction in intraocular pressure in more than one half of the cases. Complications did occur in 16 percent of the cases, with significant decreases in vision in 5 percent of the patients.

Considerable research is being conducted in a number of centers to examine various types of laser surgery in the treatment of glaucoma. It is hoped that laser energy, and the concomitant benefits of laser surgery in general, can be employed in effectively decreasing intraocular pressure.

NEUROLOGICAL AND VASCULAR EFFECTS

One of the most puzzling aspects of glaucoma is how elevated pressure within the eye causes the destruction of the optic nerve. For any given level of pressure, some patients have more resistant optic nerves than others. There is considerable evidence that optic nerve destruction and the visual-field loss of glaucoma may be caused by changes in the blood circulation of the eye. Thus, it is important to evaluate the eye's circulatory system as well as the intraocular pressure in studying glaucoma.

Evidence for reduced vascularity in glaucoma comes from research at Uppsala University and at George Washington University. Dr. Anders Bill and his associates have shown that blood flow through the optic nerve head can be

improved by reducing intraocular pressure and possibly also by raising blood pressure[39]. In addition, they have also shown[40-41] that topical administration of pilocarpine and neostigmine in monkeys results in an increase in blood flow through the anterior uvea. Dr. Mansour Armaly[42] employed heat conductance as a measure of blood-flow rate in cats and monkeys. He reports that remarkable changes occur in both the choroid and optic-nerve circulation as intraocular pressure is manipulated.

Recent studies by Dr. Douglas Anderson at the Bascom Palmer Institute at the University of Miami have been concerned with the acute effects of intraocular pressure on the optic nerve. Intraocular pressure was elevated by means of a manometric system through a small needle inserted into the anterior chamber in monkeys, and various parameters of optic disc function were studied. In one experiment[43], it was determined that the resultant ischemia produced graded effects on the optic disc which were correlated with the induced intraocular-pressure level. However, these were also accompanied by graded effects on the outer retina. Even though the disc and the outer retina are served by the same blood supply, the latter effect is not a common observation in glaucomatous damage. Thus, these data suggest that non-ischemic pressure-induced effects may be involved in the neuropathology associated with glaucoma. In a second experiment[44], ischemic changes in the optic nerve were studied by recording optic tract responses to flashes of light directed into the eye. It was found that elevation of intraocular pressure did not diminish nerve fiber conduction until the intraocular pressure was elevated to diastolic blood pressure. These data suggest that when intraocular pressure is elevated, the nutrition to the disc can be adequate for normal metabolic functions, and that the disc is not starved until intraocular pressure is considerably elevated.

Dr. J. Terry Ernest at the University of Chicago[45] describes a double-cannula technique for measuring, in cats, the tension of the optic disc while manipulating the perfusion pressure. His studies indicate [46] that the blood circulation at the optic disc autoregulates; i.e. it can adapt to short-term decreases in perfusion pressure. This finding is supported by a study involving fluorescein angiography of the disc[47]. Dr. Ernest suggests the possibility that high intraocular pressure may contribute to the breakdown in the autoregulatory mechanism of the optic disc circulation.

A group of studies by Dr. Bernard Becker and his associates in St. Louis, [48-49] has been directed at examining drugs which may protect the retina and optic nerve from damage despite the presence of anoxia due to reduced blood circulation. These studies stem from the suggestive results of an earlier pilot study with humans in which diphenylhydantoin (DPH, an anticonvulsive) appeared to protect 1/3 of the glaucoma patients treated from field loss. Basically, employing an in vitro rabbit retina preparation, it was found that one effect of hypoxia was a reduction in the amplitude of the electroretinogram. With the administration of DPH, this reduction in amplitude was inhibited. Further studies are being conducted to examine the possible therapeutic role of DPH in greater detail.

Dr. Morton Grant at the Massachusetts Eye and Ear Infirmary[50] reports an interesting condition in which obstruction of retinal blood flow results in elevation of intraocular pressure. He has identified and documented, in seven

patients, a new clinical entity which involves a reversible unilateral shallowing of the anterior chamber, with angle closure, resulting from occlusion of the central retinal vein.

IMPROVING DIAGNOSIS

The basic tools employed in the diagnosis and measurement of glaucomatous pathology are gonioscopy, tonometry and tonography, perimetry, ophthalmoscopy, and angiography. Much current research is being directed at improving these methodologies.

Schiotz tonometry is employed to ascertain intraocular pressure by measuring the depth of indentation of the cornea when a standard weight is applied. In contrast, Goldman applanation tonometry measures intraocular pressure as the force required to flatten a standard area of cornea. Schiotz tonometry is usually performed with the patient lying down, and applanation tonometry with the patient sitting. Dr. Douglas Anderson and Dr. Morton Grant[51] have compared the two techniques in 906 patients and report the following: (a) Pressure measurements with both techniques are affected by the position of the patient. (b) Pressure changes with position were greater for patients under medication than for those not under treatment. Most importantly, (c) Schiotz measurements are not as accurate as applanation measurements and can be relied on for only rough estimates. In this regard, Dr. W.K. McEwen[52] has described an information-processing methodology that can be employed to improve significantly currently used data reduction techniques for Schiotz tonography. However, there are other problems with Schiotz tonometry and most current efforts (for example those by Dr. Richard Brubaker at the Mayo Foundation and Dr. Irvin Pollack at Johns Hopkins University) are being directed at improving applanation techniques[53-54].

Because one of the first signs of neural damage is excavation and cupping of the optic disc, evaluation of the disc is most important in the diagnosis and treatment of glaucoma. For example, Dr. Bernard Becker and his associates at Washington University[55] have recently measured the vertical-horizontal ratio of the optic cup in glaucomatous and normal eyes. Vertical elongation was observed in 33 percent of the glaucoma patients, but in only 4 percent of the normals. Investigations directed at improving the evaluation of the optic disc are being conducted with NEI support at a number of research centers. Among these are studies involving mathematical analyses, computer mapping, and laser contour angiography of the optic nerve head.

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SENSORY-MOTOR DISORDERS AND REHABILITATION

INTRODUCTION

When light falls on the retina of the eye, a nerve impulse is generated which passes back through a succession of structures in the brain eventually arriving at a region called the visual cortex. Curiously, the cortex is that part of the brain farthest from the eye. It is in the visual cortex that the perceived image is reconstructed from the pattern of nerve impulses arriving from the eye. In addition to constructing the visual image, the brain must also control the motion of the eyes so that the image remains centered on the most sensitive portion of the retina. Further, the muscle controlling the lens shape must be activated for the maintenance of a focused image on the retina, the diameter of the pupil must be controlled for regulation of the amount of light falling on the retina, and the motion of the two eyes must be coordinated so that a single image is perceived. Much of the essential information controlling the function of the various ocular muscles is best defined as feedback information. It represents the completion of a loop which begins with the stimulation of the retina by light.

In this program area, there are studies of disorders of this oculomotor feedback system, such as strabismus (squint). Also found here are studies on disorders of perception, such as amblyopia. In addition to those activities which have a direct clinical impact, many studies are supported which provide more fundamental knowledge of the architecture of the visual pathways in the brain and the innervation of the muscles controlling movement of the eyes. Finally, there are studies on perception, the integrated functioning of the entire visual system to produce an image with shape, color, and three-dimensional form, an image which may be moving and which must be distinguishable against a background of many other shapes.

These fundamental studies provide essential information about the structure and functioning of the normal human visual system and thereby lay the groundwork necessary for clinical investigations into the cause and treatment of abnormal or disease conditions. Furthermore, information about the function of the visual nervous system can often be applied to questions concerning the nervous system and the neuromuscular systems of the body in general.

EYE MOVEMENT

When a person is asked to fixate, he rotates his eye causing the image of the fixation target to fall on his fovea. If he is then asked to maintain fixation on the target, his eye makes a consistent but idiosyncratic pattern of slow and fast miniature movements. In collaborative experiments conducted at the University of Maryland, Northeastern University, and George Washington University Medical Center, Dr. Robert Steinman, Dr. Genevieve Haddad, Dr. Alexander Skavenski, and Dr. Dianne Wyman have investigated the degree to which these miniature movements are involuntary, spontaneous, or reflexive[1]. Their results indicate that the miniature, rapid jumps of the eye (saccades) made during fixation are probably not different from the characteristics and probable function of large saccades made during visual exploration. Secondly,

the investigators found that with practice both human and monkey subjects could learn to suppress the frequency of miniature saccadic movement during fixation. This finding suggests that these movements are simply "busy work" and are not required to prevent the target image from fading. Furthermore, they do not appear necessary to stimulate any feedback system required to keep the eye in place for long periods of time. This finding begs the question: since miniature saccades are such a common feature of visual fixation in humans and primates, and yet have no apparent value in the visual process, why do we all exhibit them?

It has been generally believed that there is a minimum or threshold movement of an observed target necessary to stimulate an eye tracking movement. Displacements smaller than this threshold have been thought to produce no responding saccadic eye movement. If true, this threshold confounds our understanding of oculomotor control during maintained fixation of a stationary target since it requires that fixation errors introduced by a sudden movement of the target are somehow different from fixation errors produced by the oculomotor system itself. Dr. Dianne Wyman and Dr. Robert Steinman have shown that human subjects are able consistently and accurately to correct for miniature target displacements[2]. Further, the oculomotor system apparently uses saccades primarily to correct for the very small visual errors produced by these target steps. These findings demonstrate clearly that the error signals generated by small step displacements of the fixation target are not lost to the sensory subsystem, but are received and then transmitted to a processing system which, in turn, commands the motor control subsystem to make a corrective response. The correction of very small target displacements did not require practice by the subjects and was not dependent on their prior experience. These studies indicate that the apparent contradiction between the sensitivity of the oculomotor system to small errors during maintained fixation of a stationary visual target and its insensitivity to much larger errors during small step tracking is not a real one. The saccadic control system seems to make no distinction between intrinsic fixation errors (produced by the oculomotor system itself) and extrinsic fixation errors (produced by sudden target displacement). The subject makes small corrective saccades regardless of how the fixation error is produced. If there is a threshold target displacement, it is probably smaller than 1.7 minutes of arc. This result simplifies the task of understanding oculomotor control, because we need not figure out why experimentally produced visual errors would be less effective stimuli than errors produced by the subject's own eye movements.

In another set of experiments, Drs. Skavinski, Haddad, and Steinman together with Dr. Richard Stansberry have investigated the variety, frequency and magnitude of eye movements when the fixation target does not fall on the fovea[3]. Their results show that both saccades and intersaccadic drifts become larger as the target is moved from the fovea into and across the retinal periphery. This change in the pattern of eye movements was accompanied by a marked reduction in fixation stability, to and including the point at which the target did not even fall on the peripheral retina. Secondly, the saccade rate observed was not influenced markedly or systematically by any of the

stimulus conditions, suggesting that saccades are not initiated by a retinal reflex, although the size and accuracy may have depended on the quality of the visual or proprioceptive signal that made the subject aware of the direction in which he was looking.

Currently the correction of nonaccommodative strabismus (cross-eye or squint) involves surgical manipulation to loosen or tighten extraocular muscles to balance the forces and align the eyes. Drs. Allen Scott, Arthur Rosenbaum and Carter Collins of the Smith-Kettlewell Institute of Visual Sciences of San Francisco have conducted a study designed to evaluate the effects of various neurotoxic agents injected into specific extraocular muscles of the monkey in an attempt to seek pharmacologic alternatives for the connection of strabismus[4]. Of the various drugs injected (di-isopropyl-fluorophosphate, a-bungarotoxin, botulinum neurotoxin, Type A, and alcohol) only the botulinum neurotoxin demonstrated prolonged paresis of the injected muscle without serious local side effects, and without serious systemic effect. The duration of the effect extended from 2 weeks to a permanent effect (8 months) depending on the dose injected, and permanent alignment changes after temporary muscle paralysis were a common occurrence. A technique now has been developed for the precise localization of the muscle to be injected without the need for direct exposure of the muscle or conjunctiva. Botulinum toxin is believed to act presynaptically as a powerful blocker of cholinergic transmission. This effect does not appear to involve interference with impulse conduction in the motor nerve or the inhibition of synthesis or storage of acetylcholine. Neither the nerve nor the muscle suffers impairment of electrical excitability or conductivity in the presence of complete neuromuscular blocks produced by botulinum neurotoxin. The changes observed in nerve or muscle are considered secondary atrophic consequences from the loss of cholinergic transmission. The clinical relevance of the use of botulinum neurotoxin or other drug injections into extraocular muscles awaits appropriate human trials.

Drs. Bruce Pachter, Jacob Davidowitz and Goodwin Breinin of the New York University School of Medicine have conducted a light and electron microscopic study of the morphological changes associated with the myoneural junction in dystrophic mouse extraocular muscle[5-6]. They have found abnormalities of the muscle fiber in the subjunctional region in association with both normal and disrupted terminal axons. The most frequently observed myopathic change was the presence of extensive areas of densely packed vacuoles beneath the junction. Fibers containing such conglomerations of vacuoles also manifested swollen sarcoplasmic reticulum. Degeneration of terminal axons was evidenced by swelling, a decrease in the number of synaptic vesicles, and fragmented mitochondria. These findings suggest an involvement of the myoneural junction in the etiology of muscular dystrophy in the extraocular muscles of the mouse, a conclusion that is in contrast to the traditional belief that muscular dystrophy is a genetically determined primary myopathy in which abnormalities of motor innervation are secondary to the degeneration of the muscle fibers themselves. Several reports have appeared in the literature which suggest that denervation is a common occurrence and that the abnormality may be occurring in the vicinity of the neuromuscular junction. In other studies, these same investigators have

shown that various morphological disruptions are associated with gross changes in fiber diameter over hundreds of microns in dystrophic extraocular muscles. Electron microscopic examination revealed a continuum of different kinds of structural changes related to such variations in fiber diameter. These data indicate that a large assortment of ultrastructural changes can occur simultaneously in the same cell. The investigators have speculated that the initial insult to the cell may be an injury to the plasma membrane, perhaps at the level of the myoneural junction. As the membrane begins to break down, secondary and nonspecific changes occur in subcellular organization. These observations in the mouse are expected to tell us much about the pathology of human extraocular muscle dystrophy.

Since all oculomotor neurons participate simply to produce a required muscle tension without regard to the type of movement, the supranuclear organization must be arranged to produce the observed motor neuron behavior. Several lines of research have shown that each of the types of eye movement (saccadic, pursuit, convergence) are governed by separate neurological substrates at higher central nervous system levels. Several laboratories have reported data indicating that inputs from these separate systems are integrated into a common neural output at or before the level of oculomotor neurons. The question is whether this integration takes place at the motor neuron itself or at some supranuclear interneuronal level. Dr. Edward Keller at the University of California, Berkeley, has studied the behavior of both the abducens and oculomotor nucleus motor neurons during accommodative convergence eye movements in the alert monkey[7]. The observed behavior was compared with that of the same neurons during versional (parallel or conjugate) eye movements. The behavior was found to be identical, indicating that oculomotor unit discharge is determined by fixation angle without regard to the type of movement used to reach that angle. An analysis of the unit firing rate also suggested that the separate inputs controlling vergence (antiparallel or disjunctive) and version eye movements are probably combined at some supranuclear level before the motor neuron. The present study of motor neuron response during vergence adds quantitative detail to our understanding of the final level of the oculomotor system. The results showing that inputs from vergence and version systems are integrated at some neural level before the motor neuron were not entirely anticipated since various studies have demonstrated the independence of disjunctive and conjugate eye movement. The vergence and version control signals seemed likely candidates for summation at the oculomotor neurons.

Dr. Alexander Skavenski and Dr. David Robinson in studies conducted at the Johns Hopkins University and School of Medicine have investigated the role of the abducens neurons in the vestibulo-ocular reflex[8]. The eye position in space is the mechanical difference between the head position in space and the eye position in the head. Head motion could disturb vision if compensatory eye movements were not generated by the brain. Head rotations produced as an animal moves in its environment lead to compensatory eye movements which prevent images from sweeping across the retina too quickly. To insure that eye position in the head is just equal and opposite to the head position in space,

the brain must be able to sense head position. The resulting response is known as the vestibulo-ocular reflex. These investigators studied the motion of the eye of an experimental monkey in the light and in the dark when the head of the animal was rotated in a measurable fashion. Simultaneously, they measured the discharge rate of the abducens motor neuron. The relationship between discharge rate and eye position did not change when eye position was determined by either visual or vestibular stimulation. Further, no difference was found in the relationship between discharge rate modulation and eye velocity when the latter was induced by visual or vestibular stimuation. These results suggest that motor neuron behavior is determined only by eye position and velocity and is not determined by the type (saccade, pursuit, vergence or vestibular) of eye movement that created the position or velocity. Changes of eye position in the head were found to be equal and opposite to changes of the head position in space over the range of about .01 or 1.5 Hz during sinusoidal rotations of the head without vision. The investigators have suggested that there exists in the brain stem a neural integrator between the vestibular and oculomotor nuclei which converts head velocity signals to eye position signals. The phase shift observed in these experiments between the head velocity and the discharge patterns of motorneurons can be anticipated only if such a neural integrator is postulated in the path of the vestibulo-ocular reflex.

Drs. Albert Fuchs and U. Buttner of the University of Washington have been interested in the modulation of visual information processing by eye movements. During eye movements the preceived visual world seems to be stationary, whereas a moving object presented to a stationary eye causes the perception of movement. In both cases, a movement of retinal image occurs. Previous physiological studies in other laboratories have suggested that the dorsal nucleus of the lateral geniculate is the earliest station along the visual pathway where visual and eye movement interaction occurs. The investigators at the University of Washington have now studied the discharge patterns of single cells in the lateral geniculate nucleus and the pregeniculate nucleus of the alert monkey[9]. These discharge patterns were analyzed during spontaneous saccadic eye movements and unit activity was recorded either with the monkey in the dark or subjected to short flashes of light. Eye movements were carefully recorded and compared to the discharge patterns of the recorded cells. Nearly all of the units isolated in the lateral geniculate nucleus exhibited no change in activity with a saccadic eye movement in the dark or during light flash stimulation, and the investigators have concluded that no visual and oculomotor interaction occurs at the lateral geniculate nucleus. Of the 55 neurons isolated dorsal to the lateral geniculate nucleus and believed to lie largely in pregeniculate, 39 exhibited a clear change of activity with all saccades in the dark. These units either were usually silent and exhibited a burst of activity with a saccade or discharged at spontaneous rates and exhibited a pronounced inhibition with saccades. However, the activation or suppression of activity began an average of 80 milliseconds after the saccade. Of the 39 units, 26 also responded in some manner to the flash stimulus. These results indicate that the pregeniculate nucleus receives both an oculomotor and a visual input. However, the saccade-related unit discharges appear to occur

too long after the movement either to participate in saccadic suppression or to aid in differentiating those movements of retinal image due to eye movement from those due to movement of the visual world, per se.

Drs. Samuel Ron and David Robinson of Johns Hopkins University have conducted a quantitative investigation of the direction and type of eye movements evoked by stimulation of each subdivision of the entire cerebellum in the alert, intact monkey[10]. Each subdivision was systematically explored and eye movement and stimulus current were accurately measured and recorded. Three regions of the cerebellum were found to participate in oculomotor control. Within each region the type of eye movement was the same but the direction varied with stimulus location so that all eye movement directions were represented in each region. Saccadic movement was found to be evoked from the vermis, lobes V-VII. The directions of the saccades varied with electrode placement. Saccade amplitude was independent of pulse frequency, pulse width or pulse length, but, above threshold, increased with increasing stimulus current. Another region involved was the hemisphere, crus I and II and lobulus simplex, from which saccades similar to those in the vermis were evoked in addition to smooth movements whose velocity increased with an increase in all stimulus parameters. Smooth movements and saccades usually occurred together and usually had the same direction. The third region was the vestibulocerebellum: the floculus, nodulus and uvula. Nystagmus (a rhythmic oscillation of the eye) was evoked from this region. All other structures of the cerebellum were considered unrelated to the oculomotor system because no eye movements could be evoked by a stimulus current of 1 milliamp. Overall, these results present a fairly coherent picture of the types of eye movements that are associated with the various cerebellar divisions. The investigators hope that these results will clear the way for more complex experiments that will provide a new understanding of the role of the cerebellum in the control of eye movements.

In studies conducted at the University of California, Berkeley, Drs. Gerald Westheimer and Sidney Blair investigated the role of the brain stem and cerebellum in the control of eye movement. They found[11] that stimulation of certain regions of the brain stem of alert monkeys caused an inhibition of saccadic eye movements. For the duration of the stimulation, no saccadic movements were carried out, regardless of the visual stimulus. However, no interference with smooth pursuit eye movements or convergence eye movements was evident, nor did accommodation or vestibular eye movements appear to be affected. These results, as have others, suggest that saccadic eye movements are different from other classes of eye movement. The function of the cerebellum in the control of eye movement was studied by Drs. Westheimer and Blair in cerebellectomized monkeys. These animals showed an inability to maintain an eccentric gaze. A saccade into the peripheral field was always followed by a drift back toward central gaze. Two other abnormalities of eye movement control were apparent: the absence of all smooth pursuit movements and, during the first week after cerebellectomy, an absence of convergence movements. This latter defect was partially overcome at longer postoperative times. However, these monkeys never fully recovered the ability to maintain a converged position of the eyes. Other types of eye movement did not appear to be affected: saccadic movement was perfectly normal, and no abnormality in any phase of the vestibulo-ocular response was observed. These investigators have suggested that there

is something special about straight ahead gaze in oculomotor function since after cerebellectomy this is maintained when gaze in no other direction can be. The motor neurons from oculomotor nuclei recorded in alert cerebellectomized monkeys fired at their usual, quite rapid rates when the monkey was looking straight ahead, and their discharge rates were found to be modulated in the expected way during saccades and drifts. Thus, it appears that gaze-holding failure is not due to an inability of the motor neuron itself to continue firing. Furthermore, the results of this study argue that the mechanism for maintaining a steady impulse rate in oculomotor neurons during straight ahead gaze does not reside in the cerebellum.

In studies with Dr. Suzanne McKee[12], Dr. Westheimer has shown that smooth pursuit eye movements involve an element in their neural control that is not describable as the equivalent of a steady state eye position or a simple function of its rate of change. These findings are in conflict with Donders' Law which states that the orientation around a fixation axis is always the same no matter what movement proceeded the arrival of the eye in a given fixation position. The data from the work of Westheimer and McKee show that Donders' Law does not hold for the pursuit system. Not only may the observed tortion associated with a moving eye differ from the tortion found during steady fixation, but the angle and the size of the tortion also varies with the direction of the motion. These investigators do not as yet have sufficient data to formulate general rules regarding the direction and extent of this additional tortion engendered by smooth eye movements. These studies, electrophysiological evidence, and subjective observations suggest that smooth eye movements, unlike saccades, are not compensated for in space perception. Apparently, significant differences exist in the neural substrates of smooth tracking and saccadic eye movements.

Dr. Barbara Gordon of the University of Oregon has studied the responses of neurons in the superior colliculus in unanesthetized cats[13]. The receptive field properties of units in the superficial layers were found to be similar to those previously described for anesthetized animals. The least sensitive portions of the receptive field, however, disappeared under anesthesia. Thus, anesthesia has the effect of decreasing the size of the activating region of the receptive field. Visual receptive fields were found in these studies to be much larger in the deep collicular layers than in the superficial layers. Some deep layer receptive fields included the entire contralateral visual field. Most deep layer units responded to a wide range of stimulus sizes and shapes, were directionally selective (responding only to movement with a horizontal component toward the periphery of the contralateral visual field), and responded maximally to rapid stimulus movement. Many deep layer units, especially those in the lateral portion of the colliculus responded to auditory or somatic stimuli. A number of units responded to both auditory and visual stimuli, and a few responded to both somatic and visual stimuli. These receptive field properties are consistent with the notion that the colliculus may be used in the control of head and eye movements made in response to moving stimuli. The cells described in Dr. Gordon's studies may provide information that enables an animal to track visually a stimulus regardless of whether that stimulus initially impinges on its visual, auditory, or somatic sensory system.

Studies conducted by Drs. Westheimer and Blair[14] suggest that the motor pathway for accommodation (the alteration of lens shape) does not have a synapse in the ciliary ganglion while that for pupil constriction does. This conclusion comes from investigations which indicates that the sphincter iridae and the ciliary muscles of the alert monkey differ in their frequency response to intracranial electrical stimulation of the third nerve. Furthermore, local application of nicotine to the ciliary ganglion abolishes pupil constriction, but not accommodation, upon electrical stimulation of the third cranial nerve proximal to the ciliary ganglion. These results are very suggestive that there exists an uninterrupted neuronal path to the ciliary muscle and conflict with generally accepted theories of the pattern of innervation of the intraocular muscles.

VISUAL NERVOUS SYSTEM

Physiological studies of neuronal activity in the visual cortical area of the monkey have yielded considerable information on the functional organization of these areas. The study of area 17 has shown that at least two systems of neurons occur, arranged in a series of columns extending from pia to white matter. One system has common receptive field properties within each column whereas the other system is aggregated according to eye dominance. A horizontal organization is also evident which corresponds to cortical layering, separating simple monocular responses in lamina IV of the cortex from complex binocular responses in several laminae. Dr. Jennifer Lund of the University of Washington School of Medicine has conducted anatomical studies designed to establish morphological correlates for these physiological findings[15].

She has attempted to determine if there is some simple basic plan of neuronal organization in the visual cortex which might correlate with and be testable by physiological studies. The monkey was chosen as the experimental animal because the primary visual cortex (area 17) of the primate is more sharply divided into a series of clearly limited laminae than is that of the cat or rat, and these divisions provide a useful set of morphological landmarks.

Dr. Lund has found three basic cell groups in area 17: pyramidal neurons, stellate neurons with spiny dendrites, and stellate neurons with spine-free or sparsely spined dendrites. These three neuron groups show different distributions in depth from pia to white matter and differ in their relationship to the zone of concentrated termination of geniculocortical axons. The neuron type most closely related to the laminae receiving a heavy geniculocortical projection is the spiny stellate cell. This cell type is restricted to lamina IV. Pyramidal neuron cell bodies are almost totally excluded from lamina IVC which contains the broadest band of geniculocortical axon projections. The apical dendrites of lower pyramidal neurons bear many fewer spines in lamina IVC than in lamina V and VI. The basal dendrites of upper pyramidal cells spread superficially and deep into lamina IVA. The area of thalamocortical fiber termination, rather than within it. Spine-free stellate neurons occur at all cortical levels and the sparsely spined varieties have not been found in lamina IV but occur in other laminae. These and subsequent anatomical

studies are expected to provide us with a road map of this portion of the brain. A clear understanding of the neuronal interconnections should help to explain many of the findings of visual neurophysiologists and point the way toward further productive physiological studies of the visual process in the cortex.

The longest fibers of the optic tract reach to the upper layers of the superior colliculus of the midbrain. Dr. Gerald Schneider of the Massachusetts Institute of Technology has studied the effect of lesions of the superior colliculus in newborn hamsters on the formation of abnormal retinal projections[16]. The normal terminal area for optic tract fibers was destroyed in newborn hamsters. The animals were allowed to grow to maturity, and the distribution of the optic tract fibers was studied at that time. Considerable evidence of termination was found in areas normally devoid of such optic tract terminations: the remaining tissue of the colliculus and the thalamic nucleus. An abnormally high density of terminations was found in part of the ventral nucleus of the lateral geniculate body. These thalamic regions received connections from the superior colliculus in the normal animal. If the superficial layers of the superior colliculus were destroyed unilaterally at birth, axons from the eye contralateral to the lesion not only reached the area of early damage, but formed an abnormal decussation, crossing the tectal midline to terminate in the medial zone in the undamaged colliculus. Axons from the two eyes appeared to compete for terminal space in this intact colliculus, for they terminated in a nonoverlapping manner, and if the axons from the eye contralateral to the remaining colliculus were eliminated at birth, the anomalously recrossing axons increased in quantity and spread across the entire superior colliculus on the "wrong" side of the brain. Hamsters with such an anomaly showed wrong-direction turning in response to visual stimuli in a large part of the visual field. These studies provide new information on the factors which control the routing of afferent retinal fibers during the development of mammalian visual system. Lesions studies such as these can give important insights into the factors which control development in the normal system.

Dr. Frank Walsh of the Johns Hopkins School of Medicine and Dr. Richard Lindenberg of the Baltimore City Morgue have completed the first volume of Neuropathology of Vision: An Atlas[17]. This two volume set is intended to supplement major clinical texts by providing visual assistance to students and all physicians interested and engaged in the diagnosis of lesions involving the visual pathways: the optic nerve head, orbital optic nerve, intracranial optic nerve, chiasm, optic tract, lateral geniculate body, optic radiation, and calcarine cortex. In each case, the pathology of the structure is discussed and illustrated extensively. The authors of this atlas hope that their publication will serve to integrate basic information regarding neuroophthalmological diagnosis now found in scattered publications throughout the literature.

The retinal ganglion cell serves to integrate the responses of several retinal photoreceptors and is thus the first locus of visual image processing. Current theory holds that the output of a given ganglion cell is controlled by two spatially overlapping mechanisms: a "center" mechanism and a "surround"

mechanism. For an on-center, off-surround ganglion cell, the center mechanism would cause excitation during the period when the light is on and inhibition after the stimulus is terminated. The surround mechanism would cause inhibition when the light is on and excitation after stimulus termination. The response intensity curves, which are used to describe the fields for both mechanisms, have their maximum strength in the center of the receptive field. The curve for the center mechanism has a higher mean and lower standard deviation than that for the surround mechanism.

Given these spatial and temporal characteristics of the center and surround mechanisms, it would be expected that the response pattern to a stationary stimulus would be dependent upon the stimulus location in the receptive field. According to the model, three basic response patterns should be observed for an on-center, off-surround ganglion cell: (1) in the center of the receptive field, a stationary target should produce an on-response; (2) in the distant regions of the receptive field periphery, an off-response should be observed; (3) in the region closer to the receptive field center, a double, on and off, response should be elicited.

Drs. Ray Winters, Terry Hickey, and J. Pollack, at the University of Miami, have examined the effect of variations of stimulus distance (from the receptive field center) and target intensity upon peripheral response patterns of single on-center retinal ganglion cells[18]. Their results revealed two types of on-center units. One group responded to annuli of light flashed outside the receptive field center region with bursts of activity at both the onset and termination of the stimulus. These cells were, thus, on-center, on-off surround cells. A second group of cells gave responses that are similar to those predicted by the spatially-overlapping, dual mechanism theory: they were on-center, off-surround cells.

In related studies, Dr. Winters, together with Terry Hickey and D. Skaer, has examined the effect of varying the size of the stimulating flash of light on the responses to these two types of retinal ganglion cells[19]. The stimulating flashes were annuli of constant inside diameter but varying outside diameter. The two groupsof cells could be distinguished on the basis of their responses to changes in annulus size. Regardless of the location of the stimulus in the receptive field periphery, the first group of cells showed spatial summation of both the on-excitation and off-excitation responses. The effect of stimulus size on the responses of the second group of cells was more complex, appearing to be dependent on the location of the stimulus in the receptive field periphery. If the inside portion of the annulus was near the receptive field center, in the on-off zone, then small increases in stimulus size produced an increase in the strength of the excitation whereas large changes in stimulus size led to a decrease in the strength of the on-excitation. Off-excitation, on the other hand, was a function of spatial summation across the entire receptive field periphery. Thus, the center and surround mechanisms appears to be spatially coextensive for the first group of cells but not for the second, at least in the receptive field periphery. These results add a new element of complexity to the picture of the retinal ganglion cell integrative function.

Dr. Duco Hamasaki and associates at the Bascom Palmer Eye Institute in Miami have embarked on a study of the neurophysiological impact of visual deprivation on each of the visual centers from the retina to the cortex. In a recent study[20] they have detailed the response pattern of two types of ganglion cells in the normal (nondeprived) cat retina. One type of ganglion cell exhibited the so-called sustained response: when a small stimulating target was move to the peripheral, inhibitory, portion of the receptive field of the ganglion cell, a reduction in the firing rate of the cell was noted. Then, as the target moved from the surround to the center of the receptive field, the firing rate increased to a maximum and remained at an elevated level for the duration of the time that the target was in the center of the receptive field. The second type of ganglion cell, the transient response cell, showed no evidence of entry inhibition. However, as the stimulus reached the center of the receptive field, the firing rate increased suddenly and then dropped back to the spontaneous level almost as rapidly. The investigators have determined further that with increasing stimulus intensity, there is a linear increase in the maximum firing rate but no significant change in the size of the receptive field center. This observation indicates that the response properties of the center and surround components of the receptive field must be changing proportionately. These studies serve as the controls against which these investigators will compare data from their studies on ganglion cells from visually deprived animals.

The superior colliculus of the cat receives visual inputs from both retinae and from ipsilateral visual cortical areas[17-18-19], and the Clare-Bishop area. The cells in the most superficial collicular areas respond best to moving visual stimuli, and a large proportion are selectively responsive to stimuli moving in particular directions within well-defined receptive fields. Furthermore, most cells are stimulated equally well by the two eyes despite the predominantly contralateral retinal input to each half of the colliculus. Two investigators, Drs. Larry Palmer and Alan Rosenquist, working in the laboratory of Dr. James Sprague at the University of Pennsylvania have identified those cells in the striate cortex of cats which are responsible for the cortical stimulation of the superior colliculus[21]. By stimulating these cells electrically in the reverse direction, that is, by applying electrical stimuation to various points in the superior colliculus and determining those cells in the striate cortex which responded, these investigators were able to plot the receptive fields and study the general physiological properties of these corticotectal neurons. They were found to lie in layer V and were what are commonly called "complex" cells. They had large receptive fields and responded maximally to a slit or edge stimulus moving slowly across their receptive field. Most were binocular, and direction and orientation selective. However, most of the cells identified did not show improved response summation with stimulus length when slits parallel to the receptive field axis were substituted for spots as stimuli, in contrast to the behavior of most other cells in the striate cortex. Also, most of these units responded very well to small moving spots as stimuli. The data from these experiments are consistent with that obtained from ablation studies in that the properties of the corticotectal cells identified are precisely those which are lost in the colliculus following removal of cortical area 17. Apparently direction selectivity and the effectiveness of the ipsilateral eye in driving collicular units are dependent on binocular, direction-selective inputs from the striate cortex.

A study of the development of visual information processing capabilities in the superior colliculus of the neonatal kitten during maturation has been conducted by Drs. Barry Stein, Elemer Labos, and Lawrence Kruger of the University of California, Los Angeles[22]. These investigators found that information processing in the superior colliculus advances with age, and a distinct maturational sequence can be demonstrated for some neuronal response characteristics. Single neurons of the superior colliculus were studied from late fetal stages up to 8 weeks of age. During the first few days of life, few active neurons were found, and those which were encountered had significantly lower rates of spontaneous activity than that found in the adult. Prior to seven days of age, visual stimuli proved ineffective although somatic and acoustic stimuli were capable of exciting some neurons in the deeper laminae. Between 7 and 9 days of age, visually responsive neurons were monocularly activated by the contralateral eye and best excited by stationary visual stimuli. Those neurons responsive to moving stimuli at this developmental stage were most effectively excited by slowly moving targets. Neurons responsive to movement only were absent. From 10 to 13 days until 7 to 8 weeks of age, the proportion of neurons binocularly activated, selectively responsive to the various parameters of movement and responsive to movement but unresponsive to stationary light, all increased progressively. Stationary light became a relatively less effective stimulus than movement in neurons responsive to both stimuli, and a tendency for neuronal fatigue with repetitive stimulation decreased. Although each property did not become apparent simultaneously, in each neuron, maturational changes in neuronal specialization paralleled the sequence of events in the development of visually guided behavior and may reflect the maturation of the corticotectal pathway in the kitten.

The difference between the responsiveness of superior colliculus neurons to stationary light in the immature and adult cat is striking and has prompted a detailed examination of "static" properties in young kittens in order to quantify the stimulus parameters affecting response latency during critical stages of development. Drs. Stein, Labos and Kruger[23] have now shown that the minimum as well as the range of latencies of on- and off-responses to large stationary stimuli gradually diminishes in the period from 1 to 8 weeks. By the end of this period, the latency values are comparable to those of mature animals. In thesestudies the off-latency displayed a wide range of change related to manipulation of stimulus variables and could be eliminated independent of the on-response by reducing stimulus intensity and duration. The investigators found that the minimum stimulus duration and intensity required to elicit a response gradually diminished during the developmental period.

In studies conducted at Harvard University, D. Van Essen and J. Kelly, working in the laboratories of Drs. David Hubel and Torsten Wiesel have provided evidence that the shapes of cells in the visual cortex of cats may correlate with their response properties to various types of visual stimuli[24]. The experimental procedure involved extracellular recording with microelectrodes from a number of cortical cells. Each time a cell was reached, its receptive field was mapped by a procedure involving the recording of its responses to spots and slits of light projected onto a screen within view of the experimental

animal. After the receptive field characteristics had been determined, the microelectrode was advanced slightly to penetrate the cell. The impaled cell was then stained with Procion yellow injected through the hollow electrode. After sacrificing the animal and sectioning its brain, the investigators were able to identify the stained cells and determine their shape. Although the technical complexity of the experiment limited the number of cells mapped and stained, the results of the experiment suggest that, in the Hubel and Wiesel terminology, "simple" cells are probably stellate in shape and "complex" cells are likely to pyramidal. "Hypercomplex" cells were identified in only two cases, and one was stellate and the other pyramidal. These studies, which provide a correlation between a functional property and the appearance of a cell, are likely to prove of fundamental importance for future studies in many parts of the nervous system.

Because of their relative simplicity and the accessibility of their neural elements, the visual systems of several crustacea have been extensively studied as models for various elements of visual information processing in mammals and man. In studies conducted in the laboratory of Dr. C.A.G. Wiersma at the California Institute of Technology, Drs. Hugo Arechiga and Keiji Yanagisawa have investigated the response to light of the visual interneurons of the crayfish[25]. These investigators have found that illumination of areas of the eye outside of the receptive field of a given interneuron results in an inhibition of the light-stimulated response of the interneuron. Regardless of the distance between the inhibitory light and the receptive field, no decrement of inhibition was found. Neither was there a tendency for inhibition to decrease with time. The threshold for eliciting inhibition from a given area upon another was much higher than the threshold for excitation of the impacted area. Dark adaptation of the eye caused a widening of receptive fields with a corresponding reduction of the inhibitory area. This effect of dark adaptation on lateral inhibition is remarkably similar to that described some years ago by Horace Barlow and coworkers for the cat. Since in the crayfish, every field but the receptive one acts as an "off" inhibitory surround, the change in responsiveness found by Arechiga and Yanagisawa can be attributed primarily to an expansion of the excitatory field rather than to a reduction in the inhibitory strength of the surround. This change in field organization might be neural in nature, as postulated by Barlow for the cat, or more likely, the results of a peripheral mechanism, such as pigment migration in the photoreceptive units.

Because the ground squirrel has an all cone retina, it is the experimental animal of choice when one wishes to study vision without the confusing influence of the achromatic rods. Dr. Charles Michael of Yale University School of Medicine has studied the response properties and receptive fields of opponent-color and opponent-contrast cells in the lateral geniculate nucleus of the ground squirrel[26]. The majority of the color-sensitive neurons found had the common concentric center-surround receptive fields consisting of one blue-green opponent-color system in the center and the opposite type in the periphery, i.e. a given cell might respond when the center of its receptive field was stimulated by a green light but be inhibited when the same photoreceptors were stimulated with blue light; stimulation of cells in the surround elicited an exactly opposite spectral response. Because of their double opponent fields, these geniculate cells were optimally influenced by the simultaneous presentation of two

different colors: one covering the field center, the other illuminating the surround. Dr. Michael also found opponent-contrast neurons with receptive fields of the same center-concentric surround arrangement. These cells had very strong antagonistic surrounds and consequently were insensitive to diffuse light. The opponent-color and opponent-contrast cells seem to be segregated into clusters in the geniculate. This investigation has speculated that the double opponent-color cells probably receive excitatory inputs from two sets of optic tract fibers with simple circular fields and with opposite types of blue-green, opponent-color properties. A single afferent fiber has a receptive field which coincides with the cell's field center. The fields of a second set of fibers are distributed in an annular fashion around the cell's center; they collectively form the surround of the cell's receptive field. An additional two groups of optic tract fibres with the opposite types of opponent-color organization and with the appropriate field positions in the center or the surround may make inhibitory synapses on the geniculate cell. The opponent-contrast cells probably receive inputs from two sets of optic tract fibers with center-surround receptive fields. A single excitatory afferent has a receptive field center which coincides with the cell's field center. The surround fibers encircle the cell's field center and collectively form the surround. They are inhibitory and probably make presynaptic contacts on the terminal of the central fiber. These studies have further shown that two general types of fibers efferent from the retina are sharply segregated. The directionally selective axons travel only to the superior colliculus while the contrast-sensitive and opponent-color sensitive units go only to the lateral geniculate nucleus. These studies have enlarged our understanding of the mechanism of chromatic stimulus integration in the visual nervous system. Dr. Michael has provided a model "wiring diagram" which may represent the general pattern in other species with color vision, including man.

Drs. Russell DeValois and Herman Morgan at the University of California, Berkeley, together with Dr. Max Snodderly at the Massachusetts Institute of Technology, have been studying the visual system of the macaque monkey and comparing their results with those obtained for human observers in similar psychophysical experiments. The rationale for the studies is the need for an experimental animal with visual system properties close to those of the human, on which electrophysiological studies of individual cells in the visual nervous system can be performed. In one study[27], the visual acuity of human and monkey subjects was tested with gratings of various luminance and contrast qualities. The human and macaque observers gave results which were identical in form and quite similar in absolute values. In another set of studies[28], Drs. DeValois and Morgan, together with Martha Polson, William Mead and Elaine Hull, tested the spectral sensitivity of human and macaque observers under scotopic and photopic conditions. The results show a striking similarity between the species. The relative sensitivities for the two species across the spectrum were virtually identical, and the absolute sensitivities were remarkably close. These results suggest that both the absorption function of the photoreceptive pigments in the two species and the way in which different receptor responses are combined neurally are quite similar. Finally, Drs. DeValois and Richard Marrocco[29] have recorded the responses of 53 cells in the lateral geniculate nucleus of the macaque monkey. For each cell, the responses to stimuli varying in wavelength and purity (spectral bandwidth) were recorded.

The type of response of any given cell was determined by the stimulus wavelength. Some cells were stimulated by blue and inhibited by yellow, while others gave the reverse response. Still others represented a red/green opponent system. The response magnitude of any given cell, whether excitation or inhibition, was determined by the purity of the stimulus. The responses of these cells, taken together, are entirely consistent with the spectral sensitivities obtained for this same monkey species in the psychophysical experiments. Thus, this work contributes to our understanding of the role of these lateral geniculate cells in monkey, and perhaps human, perception of color.

Dr. Gunter Von Noorden of the Baylor College of Medicine has demonstrated[30] that unilateral lid closure of visually immature rhesus monkeys causes irreversible amblyopia in all animals so treated between birth and nine weeks of age. If the treatment is delayed until twelve weeks of age, no amblyopia appears. During the age of susceptibility, Dr. Von Noorden found that periods of occlusion as brief as 2-4 weeks were effective in causing severe amblyopia. Amblyopia also appeared in those monkeys which were made strabismic during the first week of life. Correlation of these data with those obtained from human patients indicates that the human visual system remains sensitive to unilateral lid closure (induced for therapeutic purposes) for a longer period of time than the monkey's. These results with the monkey are particularly provocative because of the similarity of the visual system in this animal to that of man. In other studies[31], this investigator has examined the histologic changes in the visual nervous system resulting from unilateral lid suturing or artificial strabismus, again in the immature rhesus monkey. Sections from the retina, lateral geniculate nuclei, and areas 17 and 18 of the visual cortex from monkeys with behaviorally demonstrated amblyopia were compared with similar tissues from normal monkeys. The only histologic change observed in the deprived monkeys was a significant reduction in cell section area in all layers of the lateral geniculate nucleus that received input from the deprived or deviant eye. These results also suggest that deprivation amblyopia and strabismic amblyopia result from a common mechanism, and reemphasize the importance of early correction of visual deprivation in human infants. Dr. Von Noorden suggests that therapy involving unilateral lid closure in infants is contraindicated except in extraordinary cases.

At Stanford University, Dr. Kao L. Chow is engaged in the collection of normative data on the ontogenesis of receptive field characteristics of neurons in the rabbit visual system. In addition, he is searching for possible neuronal changes as a consequence of abnormal rearing conditions or brain injury. In an investigation conducted with Drs. Paul Grobstein, Peter Spear and Lawrence Mathers, Dr. Chow has found[32] that in young rabbits, before the age in which the eyes open, only three of the seven types of receptive field cells described for the adult visual cortex are detectable. The remaining four types, which share the property of radially asymmetric fields, appear later, coincident with a decline in the percentage of cells that are visually responsive but not classifiable as to receptive field type. These findings suggest that in the rabbit, the receptive field organization of many striate cortical neurons is unlike that of the adult until at least three weeks after birth. Dr. Chow has developed the hypothesis that a major part of the transition from diffuse

to specific cortical receptive field cells may involve the late addition of synapses from inhibitory interneurons intrinsic to the cortex. These studies may provide additional information on the anatomical and physiological correlates of early visual deprivation since a significant amount of cortical development, including the appearance of asymmetric receptive fields, takes place after the time when patterned visual experience begins. In another approach to the same problem, Drs. Henry Ralston and Kao Chow[33] studied the synaptic plasticity in the lateral geniculate nucleus in rabbits subjected to unilateral visual cortex ablation. This experimental preparation provides a model for examining reorganization of optic tract axons which have been deprived of their normal postsynaptic membranes. In animals examined up to 14 days after cortex ablation, there was extensive cell damage and loss of axodentritic synapses of cortical origin which constitute the most numerous synaptic type in the normal lateral geniculate nucleus. For those animals with a longer survival time, few neurons and dendrites remained in the lateral geniculate nucleus. The fine structure of the nucleus was characterized by a shift from axodentritic synaptic contacts to a tenfold increase of axoaxonal synapses, which became the dominant population. The evidence developed by these studies indicates that there are new synaptic contacts formed between surviving axons in the degenerating lateral geniculate nucleus, as a consequence of the loss of postdendritic membranes. The new synapse formation is guided by the normal rules of axoaxonal organization indicating a maintenance of recognition of appropriate membranes for synaptic contact in the reordered lateral geniculate nucleus. In other studies, Drs. Chow, Mathers and Spear[34] have found that rabbits enucleated shortly after birth and allowed to survive for up to six months show marked changes in the architectonics of retinal axon distribution. While no evidence was found of axonal sprouting in the lateral geniculate body, uncrossed retinofugal axons had spread throughout the lateral half of the superior colliculus in areas normally innervated only by the contralateral eye. When the neurons in the area of new axonal growth were tested for stimulation by light or electric shock to the optic nerve, very few functional cells could be found. All the responsive neurons were judged to be located within the limits of the normal ipsilateral projection. Thus, it appears that while anatomic evidence of spreading of the uncrossed retinal input is clear, the functional significance of this new growth has yet to be demonstrated.

If the visual experience of young cats is modified substantially, the population of neurons in the primary visual cortex is found, upon subsequent examination, to be abnormal. The abnormalities can be generalized as a change in the selective responsiveness of the neurons to favor the patterns of experience they have received. To produce these effects, the modified experience must occur in the "critical period" (about 3 weeks to 3 months in cats). These changes in the synaptic organization of the cortical cells appear to be permanent, but have so far been studied only some time after the experience that caused them. In studies conducted at the University of California, Berkeley, Drs. John Pettigrew, Horace B. Barlow and Carl Olson, have shown that single neurons in the kitten visual cortex can be induced to increase their responsiveness to repeated stimuli applied while the neurons are under observation[35].

These short-term changes are in the same direction as the permanent modifications produced in whole populations of neurons following experimental manipulations during the critical period of cortical development. However, the changes observed by these investigators were less pronounced and probably transient. These experiments demonstrate the rapidity with which changes in the organization of the visual pathway can occur in visually immature animals.

In studies conducted at the University of Washington, Drs. Raymond Lund, J.T. Cunningham and Jennifer Lund have shown that the removal of an eye in newborn rats results in an increase of uncrossed projections from the intact eye to the superior colliculus and lateral geniculate body[36]. The additional uncrossed projections arise from areas of the retina which would normally project only contralaterally and terminate in regions of the geniculate and colliculus which, in turn, would normally receive only crossed optic axons. The increased projection does not occur in animals enucleated at 10 days or older. These studies were stimulated by the well-documented observation that although the mammalian central nervous system has only a limited ability to regenerate damaged elements following specific lesions, functional recovery of substantial portions can sometimes follow a lesion. This observation suggests a reorganization or reinforcement of alternative pathways. In younger brains the degree of recovery after damage appears much greater than in adults. These investigators have interpreted their findings as an indication that at the time of the lesion, many axons in the neonatal brain have already crossed the optic chiasm, but others have yet to arrive. When the later-growing axons reach this region in the operated animals, the normal guidance factors determining that they should cross to the contralateral side are no longer present. These axons then distribute, some contralaterally and others ipsilaterally, or they distribute all to the ipsilateral visual centers. This apparent reorganization of growth patterns is probably in contrast to the plasticity demonstrated in the adult after eye removal which seems to be largely the result of a takeover of postsynaptic sites vacated by degenerated optic terminals. The results of these studies also argue that the critical period during which deprivation or distortion of visual input permanently affects the organization of visual centers might be a reflection of the time course of axonal output of late-developing neuron populations, rather than interference with the elaboration of synapses from axons already synaptically connected. Such an hypothesis is consistent with the limited time course of these periods of susceptibility.

Drs. Frank Baker, Peter Grigg and Gunter Von Noorden, in studies conducted at the Johns Hopkins School of Medicine, have examined the response characteristics of neurons in the striate and prestriate cortex of normal macaque monkeys and those that had been raised under conditions of form deprivation or strabismus[37]. Most neurons were best activated by visual stimuli that had elongated borders of light and dark which removed through the receptive fields of the cells perpendicular to the long axis of the stimulus. The neuronal responses depended on the size, shape and orientation of the stimuli. In normal animals, most of the cells could be stimulated from either eye, although in the striate cortex about 25% of the neurons were monocular, and in the prestriate cortex there were only 4% monocularly driven cells. Binocular stimulation of the

binocular cells in these regions of the brain revealed two classes of neurons. In the first class were those cells that gave a binocular response almost equivalent to the monocular response from the dominant eye and for which variation in a relative position of the stimuli on the two retinas, that is, the ocular disparity, produced little change in the response. This type of neuron was seen in both striate and prestriate cortex. In the second class of binocular cells, seen only in the prestriate cortex, the number of action potentials evoked by the stimuli depended upon ocular disparity, and for a majority of these cells the response was markedly facilitated by slight binocular disparities. Using these results from normal animals as a baseline, the investigators turned their attention to visually deprived animals which shortly after birth had undergone lid closure or eye surgery to produce a strabismic condition. The acuity in each eye of every animal was tested during adult life. In general, the neurophysiological results demonstrated changes at the single neuron level which paralleled in degree the behavioral changes: in animals with very poor acuity in the deprived or strabismic eye, the principal neurophysiological correlate was the presence of cortical cell population that was stimulated almost totally by the normal eye alone. These results suggest that visual deprivation of whatever type causes a reordering of the architecture of the higher visual centers.

PERCEPTION

A number of studies in the literature have demonstrated that the main function of eye movements occuring during fixation is the maintenance of seeing. Experimental procedures exist for stabilizing an image on a given portion of the retina despite eye movement. When this is done, the image contrast and edges within the stimulating target fade within a few seconds. Rapid, miniature movements of the eye are not effective in maintaining clear visibility of a stabilized image. However, large, slow movements over one minute of arc in magnitude and less than 10 Hz do help to maintain the visibility of the target. Dr. Ulker Tulunay-Keesey of the University of Wisconsin has previously shown that flickering a stabilized image without moving it, thus causing a temporal change of luminance over the same retinal receptors, also helps to prolong the duration of target visibility. This effect is dependent on the use of low flicker rates. In work recently reported[38], Dr. Keesey has studied the ability of human observers to see a flickering bar of light which was only slightly brighter than an illuminated background. The experimental procedure involved a variation of the ratio of target bar to background luminance as well as the rate of flicker frequency of the bar. These studies indicate that within the frequency range used, the most effective frequency for visibility was the lowest one, 0.4 Hz, regardless of contrast. Visibility declined as the frequency increased. At lower frequencies, contrast appeared relatively ineffective in maintaining visibility; however, at higher flicker rates, at which the effectiveness of flicker was reduced, contrast appeared to be the only variable determining the level of visibility of target. These results confirm once more that slow drifts of the image across the retina are the main factor in keeping the target continuously visible. In other studies with Dr. Angel Vassiliev[39], Dr. Keesey has shown that photopic visual sensitivity is regulated

by the movement of the eye, i.e. by the movement of the image on the retina. When the image was free to move across the retina, as in normal viewing conditions, an incremental flash upon a concentric background was detected at levels of luminance which depended on the extent of the retina illuminated by the background. This is the well known desenitization-sensitization effect, described by Westheimer. Stabilization of the image of the flash and the background on the retina greatly reduced the dependence of sensitivity upon illuminated area. Dr. Keesey has speculated that retinal sensitivity is mediated by both the "sustained" and "transient" systems in normal vision and primarily by the "sustained" system when the images are stabilized on the retina.

When different images are presented to the two retinas of human subjects, the visual system strives to produce a single perceived image. This fusional response is believed to be made of two distinct components: (1) a central fusional response whose magnitude is limited to the extent of the so-called Panum's fusional areas and (2) a motor response in the form of compensatory eye movements. Dr. Andrew Kertesz of Northwestern University has reported that retinal images which differ by a rotational component are fused by a purely central mechanism without any evidence of compensatory eye movements[40]. Thus, cyclofusional stimulation appears to offer a valuable approach to the study of the central components of the fusional response, i.e. the dynamic aspects of Panum's fusional areas. The central response involves detection or assessment of the disparity and the processing of the disparity in the interest of binocular vision. Dr. Kertesz has conducted several experiments designed to explore the processing of disparate retinal images by the central fusional mechanism. In one study[41], he has developed data which suggest that the prime strategy of the central mechanism is to bring the largest possible portion of the two retinal images into correspondence. Given this portion, the visual system appears to discard the rest of the stimulus pattern as noise which is not a valid part of the stimulus. When the fusional mechanism is operating, this noise is not perceived by the subject. Dr. Kertesz suggests that two central mechanisms could be postulated to explain the visual system's approach to assessing the retinal image disparity: a mechanism that is tuned to orientational disparities, or one that serves to detect positional disparities. He has now produced data[42] to support his contention that it is the disparity introduced by the stimulus at each pair of retinal points in the two eyes that the fusional mechanism responds to, primarily. The angle of displacement between the two images does not appear to be as important a stimulus to the visual system. These results correlate with those of other investigators who have demonstrated binocularly-driven cortical disparity detectors in the visual cortex of the monkey and cat.

Psychophysical studies on the mechanism of steropsis conducted by Drs. Whitman Richards and David Regan at the Massachusetts Institute of Technology have raised the possibility that two separate mechanisms function for the processing of binocular cues for depth[43]. The experimental subjects were asked to fixate on a given point in space and were presented with stimuli consisting of parallel bars. The viewing system was arranged so that each eye could view only one of a pair of bars, and the two eyes were required either to converge

or diverge in order to produce a single perceived image. The MIT group has found a number of subjects who are capable of processing one type of stimulus, e.g. convergent disparities, but not the other. When the ability to process both convergent and divergent disparities is present in the individual, the existence of two separate mechanisms can still be demonstrated by plotting field maps for stereopsis for each type of disparity. Extensive measurements on one observer have shown that the zone of the visual field over which convergent disparities are processed may differ quite distinctly from the zone over which divergent disparities can be integrated. Furthermore, Dr. Richards has found that for those individuals who possess one or the other of the stereo-processing mechanisms, a contrast reversal of the stimuli and backgrounds may lead to a reversal in apparent processing mechanisms[44]. Thus, those who are able to process convergent disparities presented as light bars on a dark background, when presented with dark bars on a light background, may lose the former inability to process divergent stimuli but now lack the ability to process convergent stimuli. Dr. Richards has suggested that such reversals in depth with contrast may be the result of interactions between the center and surround components of the disparity mechanism.

Dr. Patricia Ondercin, with Drs. Nathen Perry, Jr. and Donald Childers of the University of Florida have studied the phenomenon of ocular dominance[45]. They have found that dominance appears to be a continuous function which is normally distributed in the general population. By placing lenses of various dioptric powers in front of one or the other eye of the test subjects, the investigators were able to establish, enhance, or decrease ocular dominance by modifying the image clarity in one eye. These results indicate that dominance, as measured by dichoptic stimulation, is not a static characteristic even in adults. In these experiments dominance was shifted to the nondominant eye even in subjects showing strong ocular dominance under normal viewing conditions.

In a joint experiment conducted by Drs. Joseph Sturr and Davida Teller at Syracuse University and the University of Washington, respectively, the interaction of the two eyes was evaluated when a test stimulus was presented to one and an inhibitory stimulus to the other[46]. These studies were conceived as a further test of the physiological site of the Westheimer effect. Dr. Westheimer has shown that the threshold of perception for a small test spot located in the center of an illuminated disc varies with the diameter of that disc. The threshold for the test spot rises with increasing disc diameters, reaches a maximum for an intermediate disc diameter, and the falls again. This desensitization effect has been attributed to the existence of an antagonistic surround in the receptive fields of neural units within the retina. Concentric receptive fields are known to occur in various vertebrate species at the bipolar, ganglion, and lateral geniculate levels as well. The studies of Sturr and Teller were designed to expose the possible interactions of stimuli to the eyes of the higher centers of the visual nervous system. In their studies, the test flash was presented to one eye and the sensitizing disc or annulus was presented to the other. With steady presentation levels, dichoptic interactions were found to be small or nonexistent. However, under transient conditions, dichoptic and monoptic effects were remarkably alike. These results

are consistent with the conclusion that steady state disc annulus interactions and transient disc annulus interactions probably occur at more peripheral and more central locations in the visual nervous system, respectively.

Frank Bagrash, a student of Dr. James Thomas of the University of California, Los Angeles, has conducted a series of experiments which suggest that the human visual system contains tunable channels which can affect the transformation of the photic stimulus into a perceived image[47]. The experimental procedure involved measurement of threshold intensity for light discs of varying diameters. When the subject briefly observed an adapting disc of given diameter at suprathreshold level, his subsequent threshold of perception for a test stimulus was markedly increased when the test stimulus was of the same size as the adapting stimulus. However, when the test stimulus differed markedly in size from the adapting stimulus, no significant difference in threshold intensity was observed between control and adapted trials. In other experiments, annuli were substituted for the adapting disc. The data of these experiments suggest that adapting area is not the sole determinent of the observed effect. The spatial distribution of the adapting area and its interaction with intensity both appear to play a role. Dr. Bagrash argues that the effect of size tuning on threshold intensity can best be accounted for by some multiple, as opposed to single, channel tuning mechanism interpretation. These investigators, together with those in several other laboratories around the world, are exploring the implications of psychophysical studies which suggest that the visual nervous system "tunes in" those portions of a stimulus which are of interest and "tunes out" much of the additional visual information presented simultaneously.

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CONTRACT NARRATIVE

YALE UNIVERSITY (NIH-NEI-71-2512)

Title: Development of Drugs Useful in the Treatment of Glaucoma and their Evaluation in Animals and Man

Current Fund Allocation: $144,870 for the period June 28, 1973 through June 27, 1974

Objective: The objective of this contract is to conduct a systematic search for adrenergic drugs with potential use in the treatment of open-angle glaucoma. Compounds which interfere with the re-uptake and binding of norepinephrine and epinepherine and those which interfere with enzymatic degradation of norepinepherine may enhance and prolong the action of these drugs.

Progress to Date: The contractor is completing studies to: (1) evaluate penetration of topically administered adrenergic drugs; (2) study the site of action of adrenergic drugs; (3) determine the role of catechol-o-methyl transferase in the inactivation of catecolamines; (4) assess the efficacy of amitriptylene; and (5) evaluate analogues of cyclic AMP.

Significance to NEI Programs and Biomedical Research: The successful development of more effective means of drug therapy for glaucoma patients would represent a significant development in the treatment of this serious visual disorder.

Proposed Course of Project: These studies will be completed in FY 1975.

CONTRACT NARRATIVE

WASHINGTON UNIVERSITY (NIH-NEI-71-2514)

Title: Evaluation of the Effectiveness of Diphenylhydantoin (DPH) in Reversal of Recent Glaucomatous Field Defects

Current Fund Allocation: $271,094 for the period June 24, 1971 through June 23, 1975.

Objective: This project is a clinical trial to determine 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 clinical protocol includes the normal testing and management of ocular hypertension.

Progress to Date: Forty-six patients are currently included in the study. A preliminary analysis of data has been completed for the first 20 patients and the results transmitted to a biostatistician for evaluation. However, results of the study will be masked from the investigators until 50 patients have been studied, unless there is either a significant adverse reaction to treatment or clear-cut evidence that one treatment methodology is superior to the other.

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 visual disorder which is one of the major causes of blindness and visual disability.

Proposed Course of Project: It is anticipated that an additional year will be required for completion of this study.

CONTRACT NARRATIVE

GEORGE WASHINGTON UNIVERSITY (NIH-NEI-72-2114)

Title: Study and Improvement of Surgery on the Outflow Channels in Glaucoma Eyes

Current Fund Allocation: $479,796 for the period June 30, 1972 through June 29, 1975

Objective: The objective of this contract is to develop new chemical and mechanical methods to reduce the high resistance to outflow in glaucoma eyes.

Progress to Date: The contractor has continued his studies of the effects of perfusion on monkey eyes. Studies on the effect of hyaluronidase on the trabecular network and development of microsurgical techniques for glaucoma have also been undertaken. Calibrations of a fluorimetry device for measuring fluorescein concentration has been accomplished and preliminary readings made.

Significance to NEI Programs and Biomedical Research: This project is part of a major special emphasis program of the Institute - the improvement of the prevention, diagnosis, and treatment of glaucoma. Successful development of more effective chemical and mechanical techniques for treating glaucoma would be of significant importance to this effort.

Proposed Course of Project: It is anticipated that this research will be completed by June, 1975.

CONTRACT NARRATIVE

THE UNIVERSITY OF UTAH (NIH-NEI-73-2115)

Title: Development of a Non-Invasive Sensing System to Measure Intraocular Pressure Continuously

Current Fund Allocation: $95,927 for the period June 1, 1973 through May 31, 1974.

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, the need entails the development of a noninvasive 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 made substantial progress in the design and development of this system. The system envisaged 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. Design phases for the transducer housing, cabling, tooling, assembly, and gaging are complete. The system transmitter has been built and operated and is complete except for final testing with the transducers. The receiver circuitry has been built and a preliminary version is ready for testing with other components of the system.

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 cause artifactual alteration of the record). This condition impedes accurate and adequate diagnosis and hinders administration of timely pharmacologic therapy. The successful development of a noninvasive system for continuous monitoring of intraocular pressure will largely overcome these obstacles, and will help to elucidate the physiological and anatomic 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 of Project: The next phase of this project will be devoted to the testing and evaluation of the system in dogs. It is expected that a three-month extension of the project will be required for the completion of this test phase.

CONTRACT NARRATIVE

UNIVERSITY OF CALIFORNIA AT SAN FRANCISCO (NIH-NEI-73-2116)

Title: Small Scale Test Procedure on the Reliability and Visability of Observing Nerve Fiber Bundle Defects as an Early Diagnostic Procedure for Visual Field Defects

Current Fund Allocation: $24,802 for the period June 1, 1973 through May 31, 1974

Objective: The objective of this project is to determine whether the technique of observing nerve fiber bundle defects by direct ophthalmoscopy using red-free illumination (and subsequent documentation of the defects by fundus photography) is as reproducible and also as specific and sensitive a diagnostic technique as perimetry to determine nerve degeneration at an early stage in glaucoma.

Progress to Date: The contractor has adhered to the timetable established for this study. Approximately 60 patients have been recruited for the study and are now completing the examination protocol. Preliminary analysis of the data is underway and a final report for the project is anticipated within the next few months.

Significance to NEI Programs and Biomedical Research: The principal criteria used in establishing the diagnosis of glaucoma are findings of an elevated intraocular pressure, glaucomatous disc cupping, and a glaucomatous defect with visual field testing. Glaucomatous levels for these criteria are difficult to establish generally. This is particularly true of visual field testing, which, though very important in diagnosis, is costly and time consuming. Red-free light ophthalmoscopy may open the way for a less costly, more accurate and sensitive screening device. It could be used in the standard examination to substantiate other testing procedures and to replace field testing when this is impossible (age factors, language barrier, etc.)

Proposed Course of Project: It is anticipated that this pilot study will be completed early in FY 1975.

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

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 identified over 500 possible dyes, pigments, and stains for testing and evaluation as indicator substances for study of retinal and choroidal circulation. Over 400 of these substances have been ordered and received. These dyes have been tested for solubility in water, in acid and base solutions, ethanol, ethelene glycol and dimethyl sulfoxide. Those substances with suitable absorption spectra are tested for fluorescence. Approximately 50 dyes have been found to be soluble and have suitable fluorescent spectra. Thirty of these dyes have been tested in rodents for gross toxicity, and one half of these were found to be toxic.

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 diseased eyes have not been adequately quantitated. Although fluorescein angiography has contributed significantly 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 of Project: Remaining dyes will be tested for solubility, fluorescence, and gross toxicity during the immediate next phase of the study. In addition to compiling detailed data of these tests, a complete toxicity testing protocol for comprehensive screening will be developed.

CONTRACT NARRATIVE

GEORGE WASHINGTON UNIVERSITY (NIH-NEI-73-2131)

Title: Development of a Clinically Useful, Objective, Photographic Technique for Fluorescein Angiography

Current Fund Allocation: $85,591 for the period June 18, 1973 through June 17, 1974.

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 proceeded on schedule. Testing and final refinement of the system is now underway, and a detailed protocol for clinical evaluation of the modified camera has been developed.

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 of Project: It is expected that an additional six months will be required to complete this project.

CONTRACT NARRATIVE

THE WHITTAKER CORPORATION (NIH-NEI-73-2132)

Title: Development of a Clinically Useful, Objective, Photographic Technique for Fluorescein Angiography

Current Fund Allocation: $90,967 for the period June, 30, 1973 through June 29, 1974.

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 is completing 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. A protocol for the clinical evaluation of this focusing device has been completed and will be implemented in the next phase of this study.

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 of Project: It is expected that an additional six months will be required for completion of the automatic focusing system and its evaluation in a clinical setting.

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

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 continued to produce RCS animals for use in different breeding schemes and has distributed several breeding pairs to interested investigators. Several litters of "intermediate disease" rats have been produced and are ready for classification by eye histology. Overall progress has been outstanding.

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 have 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 of Project: Under this contract, genetically stable breeding stocks of six (6) congenic inbred strains of RCS rats with retinal degeneration will be developed. Approximately 50 breeding pairs of each substrain will be available for distribution at different times in the contract period.

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