ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL EYE INSTITUTE

FISCAL YEAR 1973

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

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TABLE OF CONTENTS

ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1972 - June 31, 1973

STATEMENT OF THE INSTITUTE DIRECTOR

During the past year the National Eye Institute has expanded its collaborative research program from a special initiative in glaucoma to studies related to retinal disease. The appointment of a Clinical Director for the Clinical Branch of the Intramural Program will make possible the rapid growth and strengthening of the research elements of the intramural program during the coming year. Finally, in conjunction with the National Advisory Eye Council, program planning efforts were instituted to make use of available resources in fulfilling the mission of the National Eye Institute, i.e. prevention, diagnosis, and treatment of visual disorders.

Carl Kupfer

Carl Kupfer, M.D.

ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1972 - June 31, 1973

REPORT OF THE DIRECTOR OF INTRAMURAL RESEARCH

Carl Kupfer, M.D.

The preceeding year has been noteworthy in the recruitment of Dr. Elmer J. Ballintine as Clinical Director of the Clinical Branch, National Eye Institute. As mentioned in my report of last year, the emphasis for the coming year will now center on the development of the Clinical Branch with recruitment of additional staff to embark upon a clinical research program in the areas of glaucoma, congenital cataracts, retinal vascular and degenerative diseases and ocular tumors.

ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1972 - June 30, 1973

REPORT OF THE CLINICAL DIRECTOR
Elmer J. Ballintine, M.D.

The program of the Clinical Branch is being developed so that the study of ocular disease in patients will be related to laboratory investigation. The work of the Clinical Branch is being pursued according to two main concepts. First, it is recognized that unique or fortuitous opportunities for research on eye disease arise when specially qualified investigators, promising new techniques, or potentially productive clinical situations appear. Our facilities must be arranged so that these opportunities can be explored promptly and effectively.

A second concept of research in the Clinical Branch recognizes that four important eye diseases account for a large fraction of ocular disability. These are glaucoma, diabetic retinopathy, retinal degenerations, and cataracts. A long range goal of the Clinical Branch is to recruit well-qualified investigators who will make investigation in one of these fields a major part of their careers.

In developing the opportunity for these lines of research, the Clinical Branch pursues several long-term activities within the National Institutes of Health Clinical Center. The consultation service performs ophthalmologic examinations for about 1200 patients per year who are being cared for by the other Institutes. In collaboration with the experimental pathology section of the National Eye Institute Laboratory of Vision Research, approximately 100 eyes are examined histopathologically each year.

There were over 2,500 outpatient visits during the year and 150 admissions to the inpatient division.

As an example of the participation of the Clinical Branch in a unique research opportunity, the study of diabetes among the Pima Indians by the Epidemiology Branch of the NIAMDD may be cited. Fundus photographs of several thousand subjects obtained biannually are examined for the presence of diabetic retinopathy and classified by the Clinical Branch. The Clinical Branch is also consulted in the design of the ophthalmologic aspects of the study. The Clinical Branch cooperates in a similar way with investigators in the cellular and tumor immunology section of the National Cancer Institute in a study of immunological aspects of ocular malignant melanomas.

The kinds of investigation being performed under research protocols are apparent in the individual project reports.

INTRAMURAL RESEARCH

Clinical Branch

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Choroidal-Retinal Degenerative Diseases

Previous Serial Number: Same

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

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

Cooperating Units: NEI(I)-73 CB 006(c)
Walter Reed Army Institute of Research (Division of Surgery), Washington, D.C., A.R. Rosenthal, M.D. and D. Huxoll, D.V.M., Study of Chloroquine Induced Damage to the Retina of the Rhesus Monkey

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 choroidal-retinal degenerative diseases. The night blindness syndromes, familial macular degeneration and effects of drugs toxic to the retina are being emphasized.

Methods Employed: Clinical studies utilize specialized tests of visual function (dark adaptation, cone thresholds, visual fields) electroretinography and photography of the fundus of the eye including fluorescein dye studies. Appropriate testing of relatives is undertaken to document genetic patterns. Controlled studies to evaluate vitamin A therapy in selected diseases are in progress. Surgical and electrophysiological studies in animals are continuing.. Side effects of several drugs are being evaluated. Extensive chart reviews are in progress.

Major Findings: Approximately 350 patients were studied this year. The overwhelming majority presently are not curable. Nevertheless, most patients

were helped by a combination of genetic counseling, discussion of prognosis, and advice regarding rehabilitation. The study of chloroquine in monkeys has revealed early electronmicroscopic evidence of damage which cannot be studied in the human. It is partially reversible and occurs long before ERG changes are detectable. Chart reviews have revealed significant variation in severity of disease even within given families.

Significance to Biomedical Research and the Program of the Institute: This project is aimed at improving classification, prevention, and/or treatment of choroidal-retinal degenerative diseases via new diagnostic techniques, controlled therapeutic trials and animal experimentation.

Proposed Course of Project: Instrumentation has been devised to enable more exact studies of visual function in infants and children in order to extend the range of detection and longitudinal studies. More definitive therapeutic trials can be undertaken now that a technician has been establishing baseline, control and followup data. Animal and cell culture experiments are being initiated to enable collaborative efforts with ocular biochemists.

Honors and Awards: None

Publications:

Assessment of Ophthalmic, Endocrinologic and Genetic Findings in the Bardet-Biedl Syndrome, Donald R. Bergsma, M.D. and Kenneth S. Brown, M.D. Proceedings of Annual Birth Defects Conference, Johns Hopkins Hospital, Baltimore, Md., Human Genetics Branch, NIDR, (In press.)

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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.
Mitchel L. Wolf, M.D.

Cooperating Units: NEI(I)-73 CB 006(c)

Man Years:

Total:	1.25
Professional:	0.75
Others:	0.5

Project Description:

Objectives: The objective of this study is to properly classify, to clinically define, and to elucidate the cause, prevention and/or treatment of genetic and familial diseases affecting the eye. Please refer to the detailed description of a closely related project, NEI(I)-73 CB 038(c). This project involves a broader range of ophthalmic manifestation of genetic and familial disease.

Methods Employed: Clinical workups are tailored to each disease entity studied with emphasis on family studies, treatment when accepted methods are available, controlled therapeutic trials and genetic counseling. Biochemical tests are performed when applicable.

Major Findings: Approximately one hundred patients with familial and genetic disease involving the eye (excluding the choroidal-retinal diseases of project NEI(I)-73 CB 038(c)) were seen on referral or recall. Most patients are not curable but do benefit from a combination of palliative therapy, genetic counseling, and advice regarding prognosis and rehabilitation. One new syndrome has been discovered.

Significance to Biomedical Research and the Program of the Institute: This broadly defined project is focused on classification, etiology and treatment of diseases which interfere with vision. The common denominator

f familial and genetic occurance enables the marshalling of statistical nalysis, biochemical tests, genetic counseling, etc.

Proposed Course of Project: Tissue culture facilities are being developed o facilitate expanded collaborative efforts with biochemists.

lonors and Awards: None

'ublications:

The following articles contributed by Donald R. Bergsma, M.D. to the Birth Defects Atlas and Compendium, ed. Daniel Bergsma, M.D., The National Foundation-March of Dimes, Williams & Wilkins, Co., Baltimore, 1973 were partially written through the protocol: Study of Familial and Genetic Diseases of the Eye, NEI(I)-72 CB 039(c)

#	Page	Title
48	167	Anisometropia
106	225	Caruncle Abberations
169	285	Congenital Ectropian of Lids
179	295	Congenital Ptosis
203	317	Cyclopia
277	385	Epicanthus
291	399	External Ophthalmoplegia and Myopia
297	405	Familial Static Ophthalmoplegia
410	508	Hypertelorism
450	547	Jaw-Winking Syndrome
463	558	Lacrimal Canaliculus Atresia
464	558	Lacrimal Sac Fistula
509	594	Madarosis
579	657	Nasolacrimal Duct Impatency
619	694	Ophthalmoplegia Totalis with Ptosis and Miosis
683	751	Progressive Ophthalmoplegia
768	831	Supernumerary Puncta and Canaliculi

Serial No. NEI(I)-73 CB 139(c)

1. Clinical Branch
2.
3. Bethesda, Maryland

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

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

Previous Serial Number: None

Principal Investigators: Robert S. Brown, M.D.
Carl Kupfer, M.D.

Other Investigators: William R. Sullivan, M.D.
Walter J. Stark, M.D.
Fred Ederer

Cooperating Units: None

Man Years:

Total:	0.6
Professional:	0.6
Other:	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: Preliminary data indicates that both types of lenses provide relief from the painful symptoms of bullous keratopathy. Early analysis of objective parameters, such as stereo photographs, suggests that it will be possible to draw conclusions from the study which could not have been documented with subjective criteria alone.

Significance to Biomedical Research and the Program of the Institute: Carefully controlled, objective research on the therapeutic uses of the two principal hydrophilic lenses will provide data needed for the critical evaluation of this form of treatment in corneal disease.

Proposed Course of the Project: The project will continue.

Honors and Awards: None

Publications: None

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Argon Laser Photocoagulation of Retinal and Choroidal Diseases

Previous Serial Number: None

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

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.7
Professional:	0.7
Other:	0.0

Project Description:

Objectives: The project described here is another attempt at a controlled evaluation of argon laser photocoagulation as a treatment for diabetic retinopathy in patients who are not acceptable for the Cooperative Study and for the additional fundus diseases; sickle cell retinopathy, retinopathy following branch or central vein occlusion, senile macular degeneration, presumed ocular histoplasmosis syndrome, macular degeneration of angioid streaks, and central serous retinopathy. The untreated controls will also define the natural course of the disease which is incompletely described for several of these entities.

Methods Employed: The progress of these diseases and the effects of treatment will be assessed periodically by complete eye examinations including direct, indirect and slit lamp ophthalmoscopy, visual field examination, fundus photography and flourescein angiography.

The eyes of patients in the various disease catagories are assigned to treatment or control groups randomly. Laser photocoagulation spots are placed according to criteria defined in the protocols.

Major Findings: The number of patients in each catagory of disease is as yet too small to permit definite conclusions but the following tentative results can be sited. (1) Four patients with relatively symmetrical proliferative diabetic retinopathy who were not eligible for the Cooperative

Study were treated in one eye. In all cases, with followup ranging from six weeks to eight months, the treated eye has done better in all parameters: visual acuity, field, and fundus appearance. (2) Patients with background retinopathy, either symmetrical or asymmetrical, have not done so well. In no case has visual acuity improved, or even remained relatively stable by comparison with the untreated eye. (3) Treated patients with a variety of diseases of the pigment epithelium and choriocapillaris have in most cases responded with a rapid profileration of subretinal neovascularization or the appearance of new vessels following treatment when none were demonstrable before. This rapid proliferation did not seem to occur in the absence of laser treatment.

Significance to Biomedical Research and the Program of the Institute: These diseases have until the present time been untreatable; the argon laser offers a convenient and highly promising method of therapy which must be carefully and objectively evaluated. In addition, thorough studies of the natural history of these diseases must be obtained. Both of these objectives can be met by this project.

Proposed Course of the Project: These studies will be continued and expanded by recruiting patients through referral from ophthalmologists in the Washington area. An attempt will be made to arrange for area teaching hospitals which do not have laser facilities to enter their patients in the study and this way offer additional clinical experience to their residents. Preliminary arrangements have been made with the Department of Ophthalmology at Walter Reed Army Medical Center. Eventually, an attempt to create models of retinal disease in animals will be initiated.

Honors and Awards:

Sloan Foundation Lecturer in Retinal Physiology, The Bascom Palmer Eye Institute, University of Miami School of Medicine, March 1-3, 1973.
Lecturer in Retinal Physiology, The Wilmer Ophthalmological Institute, The Johns Hopkins University School of Medicine, February-April, 1973.

Publications:

None

Serial No. NEI(I)-73 CB 141(c)
1. Clinical Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemistry of Vertebrate Retinal Receptor Outer Segments

Previous Serial Number: None

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

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.7
Professional:	0.7
Other:	0.0

Project Description:

Objectives: To study the biochemical properties and physiological function of rhodopsin and other vertebrate visual pigments; to identify other enzymatic functions in receptor outer segments and to study their role in the visual process.

Methods Employed: Outer segments were obtained from dark adapted fresh rat or frozen bovine retinas by ultracentrifuge flotation techniques. Suspensions of these segments were added to appropriate media containing adenosine diphosphate labeled with P_{32} and after incubation the amount of phosphate incorporated into the receptor protein was assayed by liquid scintillation counting. In other experiments, the proteins from this reaction were dissolved in sodium dodecyl sulfate and separated by electrophoresis on polyacrylamide gel. The various proteins separated by these methods were then assayed for the incorporation of P_{32}. The molecular weights of the proteins were also determined in duplicate electrophoresis gels.

Major Findings: Rhodopsin is phosphorylated by the gammaphosphate of ATP in a reaction which requires magnesium ion and is stimulated twofold by light. The reaction is probably too slow to be immediately involved in visual excitation, since maximum phosphorylation occurs after 10 minutes in the light at 37^{0}C. Only one site on the rhodopsin molecule appears to be involved. The reaction is distinct from other phosphorylations which occur in the rod outer segments and the specific substrate is probably pararhodopsin.

Significance to Biomedical Research and the Program of the Institute: The phosphorylation of rhodopsin appears to be too slow to subserve visual excitation, yet its stimulation by light suggests an important role in the visual process, perhaps in regeneration of rhodopsin.

Detailed knowledge of the biochemical details of retinal function is the basis for studying its alteration in retinal disease and in devising therapeutic hypotheses.

Proposed Course of the Project: Further studies of rhodopsin phosphorylation kinetics will be undertaken to determine which intermediate in the bleaching process is preferentially phosphorylated. Attempts will be made to see if visual pigments are phosphorylated in vivo. Amino acid analyses of phosphorylated visual pigments will be made to determine which amino acid residues and peptides are phosphorylated; these will be compared with phosphorylated sites in other phosphoproteins. Studies of visual pigment phosphorylation in species whose retinas contain a larger proportion of cones will be carried out. Molecular weight studies of visual pigments from several species will be performed, comparing results from SDS-acrylamide gel electrophoresis and Sephadex gel filtration.

Honors and Awards:

Rhodopsin phosphorylation study nominated for "Fight for Sight" award presented at Spring, 1973, National Meeting, Association for Research in Vision and Ophthalmology.

Publications:

Frank, R.N., Cavanagh, H.D., and Kenyon, K.R.: Light Stimulated Phosphorylation of Bovine Visual Pigment by Adenosine Triphosphate, J. Biol. Chem., 248: 596-609, 1973.

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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 Hendricks

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	1.1
Other:	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: In collaboration with Clinical Associates, routine psychophysical and other ophthalmic tests are conducted on appropriate patients. Findings are reported, discussed and entered in the patients medical records. There has been considerable discussion regarding color vision with Fogarty Scholar Dr. William Rushton of Cambridge, England and some progress in constructing an anomaloscope of his design. Numerous inquiries are answered regarding the modified adaptometer used for rod and cone threholds and the ganzfeld photostimulator for electroretinography.

Major Findings: Psychophysical tests were done on about 360 patients during the year in diagnostic or follow-up studies of retinopathies of either degenerative or toxic orgin.

Improved event-markers have been installed on the Eye-Trac machine and a number of recordings were made of eye movements, particularly in cases of nystagmus.

Adaptometer modifications and the ganzfeld photostimulator have been adopted at several institutions in this country and in Europe.

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 clinical program, but to the protocol for specific projects. One example is the development of a portable electroretinography unit for use in the study of childrens' retinal diseases under general anesthesia.

Proposed Course of Project: Clinic tests and modification of instruments should continue as dictated by patient material and current study projects.

Honors and Awards: None

Publications: None

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Anatomy and Pathology of the Mammalian Retina

Previous Serial Number: None

Principal Investigators: Helga Kolb, Ph.D.

Other Investigators: Professor B. B. Boycott, F.R.S. (London)
Peter Gouras, M.D.
Alan Laties, M.D. (Pennsylvania)
Edward Famiglietti, M.D., Ph.D.
Ralph Rosenthal, M.D. (Stanford)
Eleanor Collins
David Huxoll, D.V.M. (Walter Reed)

Cooperating Units: M.R.C. Biophysics Unit, Kings College, London, University of Pennsylvania Medical School, Walter Reed Army Medical Hospital

Man Years:

Total:	1.
Professional:	0.5
Other:	0.5

Project Description:

Objectives: To understand the connections of the neurons of the mammalian retina and to understand where the principal site of pathology is in certain toxicities (chloroquine) or genetic defects (retinitis pigmentosa).

Methods Employed: Ophthalmoscopy, fluorescein angiography and electroretinography were used in the chloroquine study. Routine histology, thin sectioning of plastic embedded retina and electronmicroscopy were employed to examine retinas with pathology. Experimentation on different fixations and variations of the Golgi silver method were employed to better stain the retina of the rhesus monkey for Golgi EM. Some tissues were examined by fluorescence microscopy.

Major Findings: During FY 73 new information was obtained

in the following investigations.

1. Chloroquine Retinopathy: Chloroquine hydrochloride was injected daily intramuscularly into rhesus monkeys. After one week of chloroquine the retina contained membranous cytoplasmic bodies (MCBs) in the ganglion cell cytoplasm. By nine months all the neurons were densely packed with MCBs and some degenerating ganglion cells were seen. The photoreceptors remained normal. The pigment epithelium after nine months of chloroquine showed signs of early pathology consisting of depigmentation and membranous whorls. The ophthalmoscopic appearance of the eyes was normal after as long as 18 months of the drug. There were also no changes in the fluorescein angiograms or the ERG recordings. The chemical analysis of the ocular tissues showed chloroquine accumulating in increasing amounts in the choroid and iris. Some animals had an eye enucleated after three weeks, three months or six months, and the drug was then discontinued. There were MCBs in the neurons at the time of enucleation but they had disappeared by three to six months after the termination of drug administration. This suggests that the MCB accumulating affect of chloroquine is a reversible affect. The pathology of chloroquine retinopathy is possibly analagous to the pathology of Tay-Sachs disease. The drug may be affecting critical enzymes in the lysosmes allowing accumulation of cell waste materials into the membranous whorls.

2. Retinitis Pigmentosa: A human eye with dominantly inherited retinitis pigmentosa has been examined by electronmicroscopy. The only photoreceptors remaining were the cones of the fovea. The discs of the outer segments were considered to have an abnormal appearance. The pigment epithelium of the fovea was abnormally crowded with lipofuscin granules. The pigment epithelium of the periphery of the retina was of a different morphological type and did not contain lipofuscin. Fluorescence microscopy demonstrated that lipofuscin containing foveal pigment epithelial cells can break away and migrate into the retina. The peripheral pigment epithelium did not fluoresce because of the absence of lipofuscin but it also has the ability to migrate into the retina to form the bone corpuscle pigmentation. We suggest that in human retinitis pigmentosa there is an overproduction of possibly abnormal outer segments which the pigment epithelium is capable of phagocysing.

3. Squirrel Monkey Retina: A Study of Dopamine Containing Cells: 5-hydroxydopamine (to increase the dopamine content) and 6-hydroxydopamine (to kill dopamine containing cells) were injected into the vitreous. Fluorescence microscopy showed that a certain population of the amacrines in the squirrel monkey retina contain catecholamines. The object of the drug injection was to see if any of the cells of the retina could be characterized by

electronmicroscopical evidence as containing dopamine as a transmitter. The findings were inconclusive probably because these techniques are not yet satisfactory for the CNS neurons. Further examination of these retinas is planned.

4. Golgi-EM of Rhesus Monkey: Fixation and Golgi impregnation techniques have now been improved to the extent that connections of the cells in the inner plexiform layer can be studied. A great deal of material is being accumulated and the characterization of the midget bipolar axon terminals is in progress. In addition, a new type of horizontal cell in the rhesus monkey retina has been examined by light microscopy and serially sectioned and reconstructed from electronmicrographs. The new type of cell has a different morphology in having finer dendrites and a larger dendritic field relative to the previously described horizontal cell. The new cell has lateral elements in some pedicles only and contacts between 12 to 18 cones in its dendritic field. Electronmicroscopy of the two types of horizontal cells found in the cat retina has demonstrated that both types have dendritic terminals ending in cones only. The B type cell has in addition an axon that ends in an axon terminal system that contacts rods only.

5. Centrifugal Fibers of Cat Retina: A study is in progress to investigate centrifugal fibers in the cat retina. Optic tract section has been performed and the eyes and lateral geniculate body are being examined with the EM at various intervals postoperatively.

Significance to Biomedical Research and the Program of the Institute: Such studies of the structure of the retina will provide valuable understanding of the connections of the cells within the retina and relevance to the higher visual pathways. Detailed knowledge of retinal structure is essential for understanding pathological alterations and functions.

Proposed Course of Project: To further understand the "wiring" of the retina in the primate. To understand how the first order neurons connect to the ganglion cells and the importance of the possible feed back from the geniculate to the inner plexiform layer. In addition we propose to finish the chloroquine project and possibly acquire relevant information on the human condition of chloroquine retinopathy.

Honors and Awards: None

Publications:

Boycott, B.B. and Kolb, H.: The connections between bipolar cells and the photoreceptors in the retina of the domestic cat.

J. Comp. Neurol. 148: 91, 1973.

Boycott, B.B. and Kolb, H.: The horizontal cells of the rhesus monkey. J. Comp. Neurol. 148: 115, 1973.

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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
Pharmaceutical Development Service, CC, NIH
Biomedical Engineering and Instrumentation Branch, DRS, NIH

Man Years:

Total:	3.2
Professional:	2.7
Other:	0.5

Project Description:

Objectives: The objectives of the Glaucoma Laboratory were outlined in the Annual Report for FY 1971. During FY 1972 it was possible to get a second clinical research laboratory operative, allowing expansion of the numbers of patients and volunteers being studied. There is continuing study of the parameters on intraocular pressure of normal young subjects.

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 examined before and after medication is given topically to one eye. Replicate measurements with sophisticated subjects are made. This laboratory is still the only one where a complete study of the aqueous humor dynamics can be carried out.

Major Findings: Completed this past year was a study on the effect of isoproterenol and norepinephrine on the parameters of intraocular pressure of young normal subjects. These results have been compared to those obtained

using epinephrine. A comparison of the parameters of intraocular pressure in older normal subjects with young and normal subjects is underway. The results indicate that with age the intraocular pressure stays constant. However, there is a decrease in facility of outflow with a calculated decrease in aqueous inflow. Finally, a study of the effect of pilocarpine on the parameters of intraocular pressure has been completed in normal subjects.

With the completion of studies on normal subjects, both young and old, research is now directed towards patients with ocular hypertension and frank glaucoma with increased intraocular pressure and field loss. Preliminary studies indicate that patients in this category may respond differently to medications than those in the normal group. In addition, the investigation of changes of parameters of intraocular pressure secondary to elevation of intraocular pressure with topical steroids in high response individuals has been studied in approximately six subjects. The predictions concerning the flow curves have been analyzed. Preliminary results indicate that as intraocular pressure increases, pseudofacility stays the same and the flow curve is displaced to the right along the flow-pressure curve.

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

Proposed Course of the Project: This project will continue, and will be extended in the numbers of volunteers and glaucomatous patients being studied.

Honors and Awards: None

Publications:

Gaasterland, D., Kupfer, C., Ross, K., and Gabelnick, H.L.: Studies of Aqueous humor dynamics in man. III. Measurements in young normal subjects using norepinephrine and isoproterenol. Invest. Ophthal. 12-4;267-279, 1973.

Kupfer, C.: Clinical significance of pseudofacility. Amer. J. Ophth. 75-2:193-205, 1973.

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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
Other:	1.0

Project Description:

Objectives: To determine the pharmacodynamics of agents able to alter the intraocular pressure with a view toward finding more effective compounds and 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: Acetylcholine and Eserine (Ach+Es) produces a marked increase in the rate of aqueous humor production. Lowering of arterial perfusate pressure produces a linear decrease of the aqueous humor formation rate which indicates that the

mechanism of the Ach+Es response is to increase ultrafiltration. The data accumulated to date demonstrates that the agonistic actions of Ach+Es is on E-2 sites of sympathetic ganglion-like receptors.

Drugs which have been shown clinically to reduce eye pressure are being tested in the isolated eye preparation to determine what actions they have on the Ach-Es-induced aqueous humor response. To date, l-epinephrine, isoproterenol, pilocarpine, acetazolamide and ouabain have been examined. All of these agents cause a marked decrease in the rate of aqueous humor production. Pharmacodynamic studies indicate, with the possible exception of l-norepinephrine, that all of these agents reduce aqueous humor inflow by a direct agonistic action on E-1 sites of sympathetic ganglion-like receptors. We had shown earlier that each of these agents produced a vasoconstriction in the anterior segment of the eye by a direct action on ganglion-like receptors. The sites stimulated on the ganglion-like receptors identified as being responsible for the vascular response are similar to the identified sites affecting aqueous humor formation.

Summation of the vascular and aqueous humor data can be interpreted to suggest an afferent and an efferent arteriole-like system of the ciliary processes to affect ultrafiltration, similar to that found in the kidney glomerulus. Stimulation to produce constriction of possible efferent arterioles by Ach+Es (E-2 sites) would increase the transmural pressure of the ciliary processes to increase ultrafiltration. Similarly, stimulation to constrict afferent arterioles (E-1 sites) would decrease the ciliary process hydrostatic pressure and thus reduce the rate of ultrafiltration.

Significance to Biomedical Research and the Program of the Institute: The findings of ganglion-like receptors in the cat eye, which when pharmacologically stimulated or blocked, can moderate intraocular pressure and aqueous humor inflow represents a new concept in our understanding of the pharmacology of aqueous humor dynamics. These ganglion-like receptors could represent a physiological synapse for an axonal reflex type system for autoregulation of intraocular pressure or possibly represent a neurogenic pathway from the brain for such regulation.

Proposed Course of Project: Our efforts will be devoted to further clarify the pharmacologic properties of the ganglion-like receptors and to determine their physiologic significance. In addition, other animal species will be utilized to determine the universitality of these receptors.

Honors and Awards: None

Publications:

Macri, F.J.: Local ganglion-like stimulating properties of some adrenergic amines which affect blood vessels of the anterior segment of the eye. Invest. Ophthal. 11: 838, 1972.

Cevario, Stanley: A leak-proof needle useful for anterior chamber mixing or for multiple injections in acute experiments. Invest. Ophthal. (in press).

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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

Previous Serial Number: None

Principal Investigators: Walter J. Stark, M.D.
Douglas E. Gaasterland, M.D.
Mitchel L. Wolf, M.D.
Carl Kupfer, M.D.

Other Investigators: Barbara Rollins
Rachael Thrasher

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.4
Others:	.8

Project Description:

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

Methods Employed: Patients with suspected intraocular melanoma are admitted to the outpatient orientation service of the National Eye Institute. A thorough ophthalmologic and general history and physical examination are carried out. If the patient agrees to enter the research project, thyroid function studies are obtained and the patient is given two drops of (Saturated Solution of the Potassium Iodide) twice daily. Approximately two days after beginning the (Saturated Solution of Potassium Iodide), iodinated chloroquine containing 500 microcuries (.5 MCI) are administered by mouth. Gamma counting using the probe designed by the Nuclear Medicine Department of the University of Michigan is performed on the day of administration and then approximately every two to three days following administration of the radioactive material (counting is performed each day on inpatients). The duration of gamma counting varies slightly with the individual patients ranging from approximately 30 to 90 days after administration of the radioactive material.

Major Findings: To date we have had nine patients with pigmented lesions of the fundus which either represent very small slow growing melanomas or nevi. One of these patients has shown an elevation in count over the eye containing the pigmented lesion. We have had four patients with histologically proven melanomas of the uvea. Three of these patients have shown a statistically significant difference in counts over the two eyes with the eye containing the tumor having the greatest number of counts. In the one patient in which the difference in counts between the two eyes was not statistically significant, it appeared that the patient did not receive the full dose (500 microcuries) of I-125 chloroquine analog. In this patient, the counts were approximately 1/4 to 1/3 what we have obtained on the other patients.

Significance to Biomedical Research and the Program of the Institute: With this limited number of patients it appears that the I-125 chloroquine analog is a useful test to employ in patients with a questionable diagnosis of intraocular melanoma. It seems, however, that very small lesions which do not warrant enucleation but which might represent early melanoma, do not give a positive test.

The I-125 iodinated chloroquine analog has the advantage over other radioactive materials used in the diagnosis of intraocular melanoma, in that counting can be carried out reliably without surgical incision of the conjunctiva. It appears that its major disadvantage is that small posterior pole lesions probably will not concentrate adequate material to give a positive test.

Proposed Course of Project: Patients will continue to be enrolled in this study during the next year. It is possible that with the baseline material we have we can detect a change in counts if there is a questionable change in the fundus appearance of patients already tested.

Honors and Awards: None

Publications: None

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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

Previous Serial Number: None

Principal Investigators: Walter J. Stark, M.D.
David Newsome, M.D.

Other Investigators: None

Cooperating Units: Paul Terasaki, Ph.D. and Gerhard Opelz, M.D.
UCLA, Los Angeles, California

Man Years:

Total:	1.5
Professional:	.5
Other:	.5

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 were evaluated preoperatively for the presence of lymphocytotoxic antibodies. Attempts were made to obtain lymphocyte typing of all donors. Postoperatively the patients were followed with serial serum testing for the formation of lymphocytotoxic antibodies.

Major Findings: Fifteen patients had technically successful corneal transplants in this study. Serum testing for lymphocytotoxic antibodies in all fifteen patients was negative prior to surgery, and no patients developed lymphocytotoxic antibodies during the postoperative period. The followup has ranged from six months to 24 months. Eight patients developed evidence of immune allograft reaction which was treated successfully with systemic corticosteroids. These eight patients were negative for lymphocytotoxic antibodies prior to surgery and only one patient developed lymphocytotoxic antibodies at the time of immune graft reaction. We had 12 patients who developed clinical evidence of immune graft rejection with subsequent graft failure. Six of these patients had no lymphocytotoxic antibodies prior to surgery and five of the six patients developed lymphocytotoxic antibodies at the time of or following graft rejection. Three patients were presensitized prior to keratoplasty as evidence by having lymphocytotoxic antibodies. These three patients maintained their lymphocytotoxic antibody following keratoplasty and the graft failed from rejection despite immunosuppressive therapy. Three patients in the study were not tested prior to keratoplasty, but lymphocytotoxic antibodies were present in the serum at the time of or following immune graft rejection. These three patients had no prior history of exposure to foreign HL-A antigens.

Significance to Biomedical Research and the Program of the Institute: See Progress Report by Dr. David Newsome and Dr. Walter Stark on demonstration of HL-A antigens on cultured corneal cells in vitro.

Our study shows that cytotoxic antibodies directed against HL-A antigens can develop an association with corneal graft rejection. Since the HL-A antigens have earlier been demonstrated on cultured corneal cells this seems to provide evidence for the importance of HL-A antigens as histocompatibility antigens in corneal transplantation.

Of practical importance for corneal transplantation is our finding that presensitization to HL-A antigens in the form of lymphocytotoxic antibodies parallels the results in kidney transplants. In fact, all three patients in our study have had detectable lymphocytotoxic antibodies in their serum prior to surgery had graft failure despite immunosuppressive therapy.

Our study shows that keratoplasty in unfavorable cases and renal cadavar transplantation seem to follow the same immunologic rule. Therefore, it seems reasonable that some of the knowledge gained in kidney transplants could be applied to keratoplasty patients.

Since presensitization against HL-A antigens can be correlated with poor graft outcome, all prospective recipients for corneal transplantation should be screened for lymphocytotoxin when there is a history of pregnancy, blood transfusion, or previous corneal transplant failure. Cross match tests between sensitized patients and cells of the donor should be performed and mismatches of the same specificity as the cytotoxic antibodies of the recipient should be avoided. Recent data from kidney transplants indicate that by following these criteria, graft prognosis can be improved in presensitizations.

Honors and Awards: None

Publications:

Sensitization to Human Lymphocyte Antigens by Corneal Transplantation. Stark, W.J., Opelz, G., Newsome, D., Brown, R.S., Yankee, R., and Terasaki, P. Invest. Ophthal. (In press)

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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

Previous Serial Number: None

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
Other:	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 will be treated for four weeks with n-acetyl-l-cysteine and with a placebo in a double blind, crossover fashion. Parameters which will be measured and evaluated are symptom questionnaires, 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 presently being screened for inclusion in the study.

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: Patients will continue to be screened for inclusion in the project, and therapeutic trials will begin when n-acetyl-l-cysteine and the placebo are available.

Honors and Awards: None

Publications: None

Laboratory of Vision Research

ANNUAL REPORT
LABORATORY OF VISION RESEARCH
July 1, 1972 - June 30, 1973

by Jin H. Kinoshita
Chief, Laboratory of Vision Research

The Laboratory of Vision Research has completed its first year in the new quarters in Building 6 on the NIH campus. At this writing, the laboratories are essentially fully operational except for the incomplete renovations of the Biophysics Laboratory.

The four sections of the Laboratory are:

1. Experimental Embryology, headed by Dr. Alfred J. Coulombre

2. Experimental Pathology, headed by Dr. Toichiro Kuwabara

3. Neurophysiology, headed by Dr. Peter Gouras

4. Biochemistry, led by Dr. Jin H. Kinoshita, serving as Acting Head.

The Neurophysiology Section is the only group not housed in Building 6. At the moment, the Laboratory is composed of 26 scientists including principal investigators, fellows, staff and visiting scientists. Secretaries and technicians add another 9 to the Laboratory personnel.

As the individual reports will reveal, the research program has been productive and there is indication that solid progress has been made in many projects. Furthermore, it is encouraging that interactions are occurring between investigators of different disciplines. Hopefully, more cooperation and collaboration between investigators will develop in the future. I believe that interactions between investigators with different talents will lead to the most significant advances in many problems in vision research. One of the objectives of this Laboratory is to promote the development of a multidisciplinary research approach in the investigation of crucial eye problems.

The participation of the various members of the Laboratory of Vision Research in a number of activities outside of Bethesda has brought greater recognition to the Laboratory. The members have been asked to share their expertise in their respective fields by serving as visiting professors and lecturers in various teaching programs, and by presenting seminars in a number of medical schools. At the National Meeting of the Association for Research in Ophthalmology (ARVO) held in Sarasota in May, 1973, the LVR personnel presented 25 papers. At the organizational level of ARVO, Dr. Paul J. O'Brien and Dr. Peter Gouras served on the Program Committees of the Biochemistry Section and Electrophysiology Section, respectively. Two symposia were sponsored by the Laboratory of Vision Research and supported by the National Eye Institute. Dr. Jin H. Kinoshita was Chairman of the International Symposium on the Lens and Aging, held at the Medical University of South Carolina, Charleston, South

Carolina, in September, 1972. Dr. Hitoshi Shichi was Chairman of the Conference on Rhodopsin, Its Chemistry and Function, held at NIH in June, 1973.

Several members have responsibilities on editorial boards of scientific journals. Dr. Gouras serves on the editorial board of Investigative Ophthalmology and Vision Research; Dr. Kuwabara, Investigative Ophthalmology; Dr. Hess, Journal of Neurochemistry; Drs. Coulombre and Kinoshita, Experimental Eye Research.

In closing, I would like to personally express my appreciation to the Deputy Director of Science, NIH, the Director of NEI, Office of the Scientific Director, NEI and the staff of the Laboratory of Vision Research for their generous cooperation and help in circumventing many difficulties during the process of establishing a new laboratory branch.

Serial No. NEI(I)-71 LVR 001
1. Laboratory of Vision Research
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ocular Morphogenesis

Previous Serial Number: Same

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

Other Investigators: Jane L. Coulombre, B.S.
D. Newsome, M.D.
K. Kenyon, M.D. (Guest Worker)
D. Reese, Ph.D. (Guest Worker)

Cooperating Units: National Institute of Child Health and Human Development, IR; the Laboratory of Biochemistry, IR, National Institute of Dental Research; the Dermatology Branch, IR, National Cancer Institute; NIH; and the Laboratories of Pathology and of Bacterial Products, Bureau of Biologics, FDA, Bethesda, Maryland; Eye Pathology Laboratory, Wilmer Institute and the Department of Pathology and the Genetics Clinic, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

Man Years:

Total:	4.92
Professional:	4.92
Other:	0.00

Project Description:

Objectives: We seek to identify and to characterize the tissue interactions that control the orderly growth of the vertebrate eye. Most congenital defects of the eye are attributable to interference with these interactions.

Methods Employed: Routine chemical, histochemical, experimental-embryological, light-microscopical, electron-microscopical, pharmacological, tissue culture and autoradiographical techniques are used to analyze the development of the visual system in newts, in embryos of frogs and domestic fowl, and in embryos, fetuses or adults of the mouse, rabbit, rhesus monkey and man.

Major Findings: During FY73 new information was obtained or published relative to several tissues of the eye.

I. Retina: A. Pigmented epithelium (PE): 1. The PE of the embryonic chick eye induces the sclera. Our results: a) confirm that the chick PE develops the potency to induce the cartilagenous sclera of this species on the fourth day of incubation; b) demonstrate that the induction occurs only when the PE is confronted by cells that are competent to respond to this influence; c) establish that the PE is not inductively potent when transplanted from donor embryos older than 10 days of incubation. 2. Cloned colonies and explants of embryonic chick retinal PE from embryos of different ages were used to show that: a) these cells are intrinsically polarized from an early stage in development; b) they produce basement membrane and striated collagen fibrils as extracellular deposits beneath their basal surfaces; c) there is a spatial and temporal correlation between the production of basement membrane material and cross-striated collagen fibrils; d) increasing donor age is correlated positively with increasing average diameter of the collagen fibrils as well as with a widening of the range of fibril diameters. e) PE cells from donors ranging from 3 to 21 days of development produce collagen with the α_1-α_2 chain composition of typical chick skin collagen, as confirmed biochemically by studies in collaboration with the Laboratory of Biochemistry, NIDR, NIH, using molecular sieve and carboxymethylcellulose chromatography. This study strongly suggests that the collagen-bearing layers associated with the PE in vivo are produced by the PE cells, and opens the way for studies of the significance of this synthetic capacity for the sequential establishment of the outer coats of the eye wall. 3. Collagen-bearing, extracellular deposits beneath clones of PE cells, rendered cell-free by extensive lysis, induce hyaline cartilage from clones of embryonic head mesenchyme or neural crest in 60% of the test cases, whereas no chondrocytes appeared in control cases. This finding raises the possibility that the induction of the sclera by the PE is mediated by cell-free products of the PE.

B. Neural retina: The results of microsurgical experiments indicate that the chick embryonic optic axons grow randomly when they are forced to emerge from the nerve fiber layer and that when they grow within the nerve fiber layer, they do so along pathways that are linearly oriented toward the choroid fissure. When they are experimentally rerouted, these axons grow along these pathways as readily toward the head of the optic nerve as toward the periphery of the retina. This finding sets important restrictions on the types of hypotheses that can now be entertained concerning the nature of axonal guidance.

II. Conjunctiva: A. Xeroderma pigmentosum (XP) is an inherited disease in man. Affected persons tend to form tumors on body surfaces, including the conjunctiva of the eye, exposed to sunlight or to other sources of ultraviolet light (UV). A study done in collaboration with the Dermatology Branch, NCI, demonstrated that cultures of XP conjunctival cells are deficient in the unscheduled synthesis of deoxyribonucleic acid (DNA) for repair of UV damage, when compared with the conjunctival cells of an unaffected subject.

This observation confirms that the clinical eye findings in this disease may arise from an inability of XP cells to repair adequately UV damage to DNA. It also demonstrates that conjunctival epithelium, a tissue technically easier to use than epidermis, is suitable for the study of the repair of DNA in XP.

B. The scleral ossicles form a ring of 13, 14, 15 and 16 overlapping membrane bones just outside the corneal margin in the sclera of domestic fowl. The development of these bones depends on the prior development and short-lived existence of a ring of papillae in the overlying embryonic conjunctiva. 1. The number of ossicles is a function, not only of the number of papillae, but also of the distance between adjacent papillae. 2. There are three regions in the ring of scleral ossicles: nasal, dorsal (in both of which the bones overlap in one direction) and temporal (in which the bones overlap in the other direction). 3. The determinants of the direction of overlap between adjacent bones are extrinsic to the ossicles themselves and are distributed throughout each region, rather than confined to the discrete locations within each region where overlap normally occurs.

III. Lacrimal gland: A study in collaboration with the Laboratories of Pathology and Bacterial Products, Bureau of Biologics, FDA, has partially explained how the protein composition of tears comes to differ from that of serum. Horse-radish peroxidase, injected intravascularly into rhesus monkeys, penetrates the walls of vessels in the lacrimal gland and moves more slowly through epithelium, where its progress is arrested by zonulae occludentes near the apices of the epithelial cells. These tight cell junctions are thus identified as the site of the blood-tear barrier and, together with other mechanisms, help determine the final protein composition of the tear fluid.

IV. Lens: The metaplastic transformation of adult newt iris cells into lens follows a set sequence of molecular events during the first 4 days following lentectomy: 1. initiation of ribosomal RNA (rRNA) synthesis within one day after lentectomy, 2. initiation of DNA replication between 3 and 4 days, and 3. a 3-fold increase in the number of nucleoli per iris epithelial cell nucleus by 4 days after lentectomy. These same events have been obtained in vitro and are qualitatively, quantitatively, and temporally equivalent to those that occur in vivo. Using the in vitro system, the following observations have been made: 1. A high pH requirement (pH 7.9) was found for maximum rate of synthesis of the two rRNA's and of the heterogenous nuclear RNA. 2. The molecular weights of the two rRNA components are 1.4×10^6 daltons and 0.74×10^6 daltons. The larger component is smaller than its counterpart in Xenopus while the smaller component is identical to its counterpart in Xenopus. 3. An unusual species of RNA, induced as a result of lentectomy or culture, is being characterized. It has a molecular weight of ca. 10^7 daltons. It is degraded by both pancreatic RNase and DNase but is resistant to both RNase H (specific for RNA-DNA hybrids) and RNase III (specific for double-stranded RNA). The results to date suggest that this species of RNA may be an RNA-DNA copolymer (RNA covalently linked to DNA). This has been reported previously only in bacterial and viral systems and has been postulated to be a primer for DNA replication.

V. Cornea: A. Stroma: Collagen is deposited in the acellular primary stroma by the embryonic epithelium of the cornea, in a structural pattern that is a miniature of the mature stroma. This pattern contributes to a large number of the functional properties of the cornea (e.g. transparency, tensile strength, refracting power, etc.). The following observations extend previous studies of how this collagen is produced and deposited. 1. Domestic fowl: a) The Golgi apparatuses of the basal cells of the corneal epithelium stain positively for reticular collagen only between the third and tenth days of incubation, when the lamellae of the primary stroma are deposited. b) Intravenous injections into embryos, of L-azetidine-2-carboxylic acid (LACA), an analog of proline that aborts the production and excretion of normal collagen, renders the Golgi apparatuses reticular-collagen-negative within 15 min. of administration and interrupts the deposition of collagen in the primary stroma. The Golgi apparatuses once again stain positively for collagen 18 hrs. following LACA administration, just prior to the resumption of collagen excretion, indicating that the Golgi apparatus is involved in processing the collagen. c) After LACA has ceased to act, normally structured primary stroma is once again constructed outside the collagen-sparse stromal lesion, indicating that previously deposited lamellae of collagen fibrils do not supply the determinants of stromal architecture. d) Stromal lesions produced by LACA heal subsequently in a manner suggesting that some of the determinants of collagen geometry reside in the extracollagenous matrix of the primary stroma. 2. Frog: The fusion, at metamorphosis, of the spectacle and cornea of the frog tadpole occurs in a plane occupied by a well-defined layer of extracellular, colloidal-iron-staining material. It remains to elucidate the origin of this layer, its fate and its role in fusion of the outer and inner portions of the developing cornea.

B. Endothelium: Retrocorneal fibrous membrane, an abnormal deposit leading to swelling and clouding of the cornea in man, has been experimentally reproduced in rabbits in a study done in collaboration with the Eye Pathology Laboratory of the Wilmer Institute, The Johns Hopkins University School of Medicine. The results indicate that this abnormal membrane may be produced exclusively by metaplastically altered endothelial cells and not, as previously thought, by an overgrowth and scarring of the corneal stroma. 2. There is indirect evidence that the corneal endothelium produces the constituents of Descemet's membrane. It is not yet known if the endothelium makes a contribution to the collagenous primary stroma, or if the primary stroma plays any role in the differentiation of the endothelium. To study these possible roles, corneal endothelial cells from chick embryos at stage 25, 26 or 27 were grown in tissue culture for from 2 to 12 weeks and examined by electron microscopy. The cells did not form the continuous monolayers typical of their differentiated state, but grew in multiple layers of spindle-shaped cells which contained extensive endoplasmic reticulum and which formed only rare intercellular junctions. The intercellular spaces contained masses of randomly oriented, non-banded filaments (ca. 90-150Å diam.), similar in shape, but not in structural organization, to those seen during early stages of the formation of Descemet's membrane in the chick embryo, but none of the cross-striated fibrils so characteristic of the primary stroma. Thus,

production of these extracellular filaments does not require the presence of primary stroma, the usual substratum of the endothelial cells. These observations open the way for analyzing the role of the primary stroma in the tissue organization and differentiation of the endothelium and in the development of structural organization in Descemet's membrane.

VI. Tectum: 1. The optic nerve fibers grow across and cover the tectal surface of the chick embryo between 6.5 and 12.5 days of incubation. The growing front of the optic fibers is less orderly than the highly regular bundles of fibers more to the rear. Optic fibers rarely branch. The optic tectum undergoes a 90° rotation between the seventh and the thirteenth days of development so that the growing fiber front curves and meets to form a raphe on the dorsal, rather than on the posterior, surface of the tectum. These findings contribute to our knowledge of the manner in which the retinotectal projection becomes established. 2. An active migration of head mesenchyme toward the dorsal body midline underlies the directed growth, seen in previous experiments, of bundles of optic axons emerging from grafts of embryonic retina on the surface of the optic tectum and calls into serious question our previously held view that such axons can be actively guided to their appropriate terminals through extraneuronal tissues. The temporal and spatial distribution of mesenchyme migration is under active investigation in another laboratory to assess its role in the establishment of blood vessel patterns and in other aspects of the establishment of the architecture of the body.

During FY73 members of the Section gave 16 seminars based on this year's work, in addition to delivering 5 invited lectures and 10 papers from the platform at national scientific meetings and an in-depth presentation before the Board of Scientific Counselors, NEI, a total of 32 presentations.

Significance to Biomedical Research and the Program of the Institute: Identification and characterization of the tissue interactions that control ocular development is indispensable to establishing the etiology of most congenital eye defects. Presently, most of the work of this Section focuses on identifying and characterizing a special group of basement membranes, the initiator layers. These initially-cell-free, collagen-bearing, PAS positive layers are produced by, and deposited beneath, restricted regions of embryonic epithelia during specific periods in development. They channel the differentiation of mesenchymal cells that volunteer within them, determine the three dimensional structure of tissues derived from neural crest and mesoderm, appear to stabilize the state of differentiation of the epithelia that produce them and may be involved in the control of the cell population dynamics both of the epithelia overlying them and of the mesenchymally-derived cells that invade them. It remains to be determined: to what extent their production can be reactivated in adult tissues; the extent to which adult cells can still respond to implanted initiator layers; the implications of this for pathogenesis and for rational therapy; and the degree to which fractions of these layers can be successfully applied to the promotion of healing and other reparative processes.

Proposed Course of Project: The project will be continued.

Honors and Awards:

Invited lecture, Second FASEB Conference, Atlantic City, 18 April 1973.

Publications:

Coulombre, A. and Coulombre, J.: The skeleton of the eye. II. Overlap of the scleral ossicles of the domestic fowl. Devel. Biol. (in press).

Coulombre, A. and Coulombre, J.: Corneal development. IV. Interruption of collagen excretion into the primary stroma of the cornea with L-azetidine-2-carboxylic acid. Devel. Biol. 28: 183-190, 1972.

Goldberg, S. and Coulombre, A.: Topographical development of the ganglion cell fiber layer in the chick retina. A whole mount study. J. Compar. Neurol. 146: 507-517, 1972.

Goldberg, S. and Galin, M.: Response of retinal ganglion cell axons to lesions in the adult mouse retina. Invest. Ophthalmol. 12: 382-385, 1973.

Kenyon, K.: Ocular ultrastructure of inherited metabolic diseases. In Goldberg, M., (Ed.): Genetics Aspects of Ophthalmology. Little Brown and Co., Boston. (in press).

Kenyon, K., Sensenbrenner, A. and Wyllie, R.: Hepatic ultrastructure and histochemistry in mucolipidosis II (I-cell disease). Pediatric Res. (in press, June, 1973).

Michels, R., Kenyon, K. and Maumenee, A.: Retrocorneal fibrous membrane. Invest. Ophthalmol. 11: 822-831, 1972.

Newsome, D.: Cartilage induction by retinal pigmented epithelium of chick embryo. Devel. Biol. 27: 575-579- 1972.

Newsome, D. and Kenyon, K.: Collagen produced in vitro by the retinal pigmented epithelium of the chick embryo. Devel. Biol. (in press, June, 1973).

Shabo, A., Kenyon, K. and Franklin, R.: Electron-microscopic localization of a blood-tear barrier to tracer protein in the primate lacrimal gland. Lab. Invest. 28: 185-193, 1973.

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Anatomic and Pathologic Studies of Ocular Tissue

Previous Serial Number: None

Principal Investigator: Toichiro Kuwabara, M.D.

Other Investigators: Shigekuni Okisaka, M.D.
Gerald Robison, Ph.D.
David G. Cogan, M.D.
Richard M. Robb, M.D.
Paul Sanderson, M.D.
Robert Brown, M.D.

Cooperating Units: None

Man Years:

Total:	1.4
Professional:	0.7
Other:	0.7

Project Description:

Objectives: Study of normal and diseased human materials is the most basic approach to the deeper understanding of the pathogenesis of disease. By utilizing newer techniques and advanced knowledge, available human materials are investigated on a continuing basis.

Methods Employed: Ocular tissues which had been removed from surgical and postmortem cases in the Clinical Center of NIH were used for this study after their diagnostic examinations had been fulfilled. Specimens were studied by transmitting and scanning electron microscopy, histochemistry and by the trypsin digestion technique.

Major Findings: Among several interesting observations, the following problems were specifically investigated. Lens: By the newly developed scanning electron microscopic technique, lens fibers in various age groups and in pathologic conditions were examined. The general structure of the lens fibers, especially of their intercellular relationship, became considerably clearer. Also it was found that the surface of the mature cell consisted of fine ridges which appeared to form the close engagement between cells. Pathologic cells were found to be losing this structure. Sphingolipidosis: Retinas from

patients with Nieman-Pick's disease and Tay-Sachs' disease were studied electron microscopically. The sphingolipid in a case of Tay-Sachs' disease with the normal hexosaminidase level showed diffuse accumulation of the abnormal lipid within ganglion cells instead of forming lamellar inclusion bodies. A case of Nieman-Pick's disease was extensively studied by Dr. Richard Robb, a Special Fellow, Extramural Program. Cystinosis: Electron microscopy revealed that the accumulated cystine crystals were mostly intracellular and that the crystal laden cells were substantially destroyed. Pathologic changes in the pigment epithelium were studied by Dr. Paul Sanderson. Contact lens: Abnormal deposit on contact lenses was analyzed by scanning electron microscopy. Deposits were often made of calcium crystals and were found to originate at the area of minute bulging of the plastic lens material. Retinal blood vessels: Many patients who died after cardiac surgery showed microembolism in the retinal capillary. The embolic substance was found to be of lipidic nature. The origin of the lipid was tentatively speculated to be the heart-lung bypass equipment.

Significance to Biomedical Research and the Program of the Institute: Deeper studies of available human materials often produce direct clues in understanding of disease. Also, accumulated information on normal and diseased tissues will be valuable for future utilization by vision research scientists.

Proposed Course of Project: These studies will be continued actively in conjunction with the NEI Clinical Branch.

Honors and Awards: None

Publications:

Robb, R.M. and Kuwabara, T.: Pathology of Niemann Pick's Disease. Invest. Ophthalmol., Vol. 12, 1973 (in press)

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Effect of Laser on the Retinal Blood Vessels

Previous Serial Number: None

Principal Investigator: Toichiro Kuwabara, M.D.

Other Investigator: Shigekuni Okisaka, M.D.

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	0.8
Other:	0.4

Project Description:

Objectives: Although use of the laser is becoming one of the most common treatments of retinal vascular diseases, especially of diabetes mellitus, its actual cytologic effect on the blood vessels has not been clearly understood. Also, the varying susceptibility of the blood vessels to different wavelengths of the laser has been extensively discussed clinically, but no basic cytologic study has been systematically performed. The present study is the continuation of related investigations which have been carried out by us at the Howe Laboratory of Ophthalmology, Harvard Medical School.

Methods Employed: Retinas of normal rhesus monkeys were treated by argon and ruby lasers following the techniques used clinically for treatment of diabetes. To simulate the clinical condition of neovascularization in proliferating retinopathy, a small amount of blood was injected into the vitreous. The fine streaks of the blood were exposed to the laser beams. Animals were killed after various time intervals and cytological changes were studied electron microscopically. The vascular system was studied by the trypsin digestion technique.

Major Findings: No appreciable difference in the location or nature of cytologic changes was noted between argon and ruby lasers. Both caused identically severe cellular destruction in the photoreceptor cells and the pigment epithelium. No selective burn of the vascular system by the argon laser was noted immediately after the treatment. However, as a delayed reaction, small segments of capillaries became occluded after the retinal tissue had healed

considerably. Because the occlusion of the capillary was found only in small areas and was surrounded by an intact capillary meshwork, it was difficult to consider that these changes caused any hemodynamic effect in the retinal circulation.

The laser beams aimed at the blood streaks in the vitreous caused severe cellular damage in the inner layer of the retina, including the ganglion cells.

Significance to Biomedical Research and the Program of the Institute: These findings are directly related to the evaluation of the basic principles and effectiveness of laser treatment. Also, establishment of a cytologic standard for laser damage in the retinal tissue is absolutely necessary for the safe use of this instrument in the treatment of ophthalmic patients.

Proposed Course of Project: The chronic effect of multiple burns of the retina will be studied similarly. A study of delayed reaction from subthreshold burn is also underway.

Honors and Awards: None

Publications: None

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Light Effect on the Developing Retina

Previous Serial Number: None

Principal Investigator: Toichiro Kuwabara, M.D.

Other Investigator: None

Cooperating Units: None

Man Years:

Total:	0.9
Professional:	0.5
Other:	0.4

Project Description:

Objectives: Recent studies by us and by other investigators have shown that retinas of normal animals are severely damaged by excessive light exposure. A possibility of a direct pathogenetic correlation between light damage and certain human retinal diseases, such as retinitis pigmentosa and macular degeneration, has also been discussed. This study is a part of a series of related investigations which have been carried out over several years. The purpose of this project is to determine the effect of light on developing retinal tissue in order to possibly elucidate the pathogenesis of certain congenital diseases of the photoreceptors.

Methods Employed: Postnatally developing rats were exposed to the continuous brightness of 500 foot-candles. Another litter was kept in total darkness since birth. Animals which were caged in the normal animal room illumination (15 foot-candles during the daytime and total darkness during the night) were used as controls. Animals were sacrificed at various time intervals. Retinas were processed for plastic-embedded sections for both light and electron microscopy.

Major Findings: Retinas of adult animals which had been exposed to the experimental brightness were found to be severely

degenerated. Retinas of two-weeks-old control rats were found to be readily susceptible to light. The whole photoreceptor cells of these retinas completely degenerated within three days, whereas photoreceptor cells of the developing retinas exposed to bright light were found to survive for a longer period of time and keep producing outer segments. Although the newly formed outer segments were destroyed immediately, the cell body itself did not degenerate until the end of the third week. The first appearance of the outer segments in the rats exposed to bright light was noted a full day earlier than the control animals. These experiments showed that the developing photoreceptor cells completed the production of an apparently predetermined amount of outer segments despite its sensitive susceptibility to the bright light.

Animals which were kept in total darkness appeared to develop outer segments normally. Further observations are to be done in these animals.

Significance to Biomedical Research and the Program of the Institute: The present observations suggest that exhaustion of outer segment production is the direct cause of the degeneration of the photoreceptor cell, rather than light exposure itself. This information is valuable in understanding pathogenesis of certain congenital retinal diseases.

Proposed Course of Project: Similar experiments will be carried out using RCS rats to determine the effect of light on the genetically abnormal photoreceptor cells. Dr. Richard Robb is also finding interesting results in studies of hereditarily blind mice. A study on the prolonged effect of darkness is underway.

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, 1972 through June 30, 1973

Project Title: Effect of Intraocular Pressure on the Ciliary Epithelium

Previous Serial Number: None

Principal Investigator: Shigekuni Okisaka, M.D.

Other Investigator: Toichiro Kuwabara, M.D.

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	0.4
Other:	0.6

Project Description:

Objectives: Fine structural study of pathologic changes in the ciliary epithelium following experimental disturbances of the intraocular pressure has been performed. Studies of this tissue are badly needed for a clearer understanding of the pathophysiology of glaucoma.

Methods Employed: Rhesus monkeys were used in this experiment. Elevated intraocular pressure (45 mm Hg) was produced in one eye for various durations and paracentesis was performed on the other eye. The ciliary epithelium was examined electron microscopically at various time intervals.

Major Findings: The pressure control mechanism of the eye appeared to be disturbed severely by repeated elevation of the intraocular pressure (IOP). The eye became markedly soft after three sessions of pressure elevation for 30 minutes daily. The hypotonic condition lasted for about one week before the IOP reverted to normal, whereas repeated paracentesis appeared to be harmless to the ocular pressure control system. Pressure became normal within two hours after each paracentesis.

In these experimental conditions, the cytoplasmic microorganelles of both the pigmented and nonpigmented cells did not show appreciable pathologic changes. However, alteration of the IOP appeared to cause changes in the basal infoldings of the epithelial cells. Profound thickening and duplication of the basement

membrane of the nonpigmented epithelium was demonstrated following the repeated IOP alteration.

Significance to Biomedical Research and the Program of the Institute: Systematic re-evaluation of the cytologic findings in the ciliary epithelium in normal and pathologic conditions is attempted in this study. Information obtained may lead to new understanding of the function of the ciliary epithelium.

Proposed Course of Project: The study will be expanded by using toxicologic agents. A preliminary study using urea and lactamide has shown marked drop of IOP and cytologic damage in the pigmented epithelial cells.

Honors and Awards: None

Publications: None

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Functions of Cell Microtubules and Microfilaments

Previous Serial Number: None

Principal Investigator: W. Gerald Robison, Jr.

Other Investigator: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: Recent investigations have shown that microtubules and microfilaments, minute structures in the cytoplasm of normal cells, play significant yet ill-defined roles in the movement and structural support of essentially all cells. These submicroscopic structures are required for such important physiological processes as intracellular translocation of cell contents, change in cell shape, secretion cycles, contraction, cell division, and cell differentiation.

Experiments were designed to distinguish the specific roles of these cell organelles and to relate them to given cell functions by using precisely controlled conditions.

Methods Employed: Isolated cells which could be stimulated to change shape and to redistribute their contents upon addition of hormone extracts were incubated under chemically and physically controlled conditions. Drugs such as vinblastine sulfate, colchicine, and cytochalasin B which are known to reversibly block the actions of microtubules and microfilaments independently were added to the media of the incubated cells. Cell responses to these drugs and their structural changes with and without hormone stimulation were examined with light and electron microscopy.

Major Findings: Although microtubules are required for cytoplasmic motion in many cells, and have been implicated with pigment movement in the cells studied, we have demonstrated that vigorous movement and cell shape changes can occur even after their destruction with vinblastine sulfate or colchicine. On the other hand, movement can be blocked experimentally with cytochalasin B without altering cell ultrastructure, contrary to many reports which claim destruction of microfilaments. Therefore, microtubules make less contribution to the movement in the cells investigated than previously believed, and further studies are required to define even the general nature of microfilament function.

Significance to Biomedical Research and the Program of the Institute: Cell pathology requires a determination of the range of variations which occur in normal cells and where possible a correlation between environmental insult (pathogens, etc.) and the functional and structural changes that can be observed. Studies of cells incubated under highly controlled conditions contribute significantly to the information needed. Studies of chemical effects on incubated cells are important to determine the proper use of drugs, as well as to define the extent and pathology of the side effects that can be expected from various types of medicinal therapy. Microtubules and microfilaments are important in basic cell functions and serve as good indications of cell vitality. A more thorough and more precise understanding of their functions will permit better diagnostic utilization of alterations in their characteristics to distinguish normal from pathological cells.

Proposed Course of Project: These experiments will be extended to include additional chemicals and to use other cell types which can be isolated and/or cultured. Some preliminary work has been done with cultured pigment epithelium from the chick eye. Experiments will be designed to investigate the action of hormones at the cellular level. Clinical material from cases of heavy chemotherapy will be used to correlate findings with clinical pathology.

Honors and Awards:

Invitational Speaker: 44th Annual Meeting of the Eastern Branch, Entomological Society of America, Atlantic City, New Jersey, October 19, 1972.

Publications:

Robison, W. G., Jr.: Microtubules, microfilaments and pigment movement in the chromatophores of Palaemonetes vulgaris (Crustacea). J. Cell Biol. (in press), 1973.

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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.
Edward Famiglietti, M.D., Ph.D.

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

Cooperating Units: None

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 neurones at 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: A distinct segregation of cell types has been found in different layers of foveal striate cortex. Non-oriented color sensitive cells are the predominant cell type in layer 4B. These cells can be subdivided by the way they respond to different wavelength bands of the visible spectrum. The two largest groups contrast with each other in being sensitive to opposite ends of the spectrum, i.e., one group sensitive to long and the other to short wavelength regions of the spectrum. Within each group there is considerable overlap in the range of spectral lights and in the size of the spots to which these cells optimally respond. Cells showing the same color and spatial

preferences are found close to one another in layer 4B. Action spectra indicate that these two systems are mediated exclusively by the two principal cone mechanisms in primate fovea, one with its peak sensitivity in the orange-red and the other with its peak in the green region of the spectrum. There is a third system which involves the blue-sensitive cone mechanism which appears to have a more varied spectral but a coarser spatial selectivity. This group of cells is less common than the other two but also shows a similar segregation into specific zones within layer 4B.

As one moves away from layer 4B either toward the surface of the brain or the white matter, non-oriented color selective cells diminish rapidly in number and orientation, and direction selective cells become more common. Here one finds color-selective oriented cells intermixed with color-insensitive ones. There is a trend for the former to have less spatial selectivity than the latter.

These results have suggested a hierarchical model of processing of color and shape information in primate foveal system such that non-oriented cells of the same spatial and spectral preference activate the same orientation selective cell, thereby building up orientation selective channels tuned to certain spectral bands and spatial frequencies. Cells of different spectral bands and similar spatial frequencies subsequently converge on higher order cells which consequently lose any wavelength preference but gain in their ability to detect spatial contrast.

Various predictions of this theory are being tested.

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: "Color and Spatial Specificity in Foveal Striate Cortex" delivered at the Symposium on Color Vision at the Association for Research in Vision and Ophthalmology meeting on May 3, 1973 in Sarasota, Florida.

Publications:

Gouras, P.: Color opponency from fovea to striate cortex in Australian-U.S. Symposium on Vision. Invest. Ophthal. 11: 427-434, 1972.

Gouras, P. and Bishop, P.O.: The neural basis of vision. Science 177: 188-189, 1972.

Gouras, P.: Visual neurophysiology. Feature detecting channels. Invest. Ophthalmol. 12: 2-3, 1973.

Dow, B.M. and Gouras, P.: Color and spatial specificity of single units in rhesus monkey foveal striate cortex. J. Neurophysiol. 36: 79-100, 1973.

Serial No. NEI(I)-72 LVR 110
1. Laboratory of Vision Research
2. Section on Neurophysiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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: Beverly West, M.S. (Ph.D. candidate)

Cooperating Units: None

Man Years:

Total:	1.8
Professional:	1.6
Other:	.2

Project Description:

Objectives: Most of the information processing which permits us to recognize visual details in our surroundings occurs not within the eye itself but more centrally in the brain. Our understanding of such processing has progressed dramatically in the past 15 years by means of microelectrode recordings from single nerve cells, but vast areas of visual brain remain largely unexplored. The goal of this project is to record from cells in the foveal projection areas of the rhesus monkey visual cortex to gain insight into the mechanisms of visual information processing in a primate. Special emphasis is being placed on the mechanisms by which shape and color are coded in the firing patterns of the cells.

Methods Employed: These include electrophysiologic techniques for recording extracellular impulses from single neurons in paralyzed, anesthetized and (eventually) awake, behaving animals. Independently variable stimulus parameters include shape, size, orientation, velocity and direction of movement, color, intensity, and background illumination. Histological techniques are used to supplement the electrophysiology, both for identifying the locations of recorded cells and for outlining the pathways between different regions of visual cortex.

Major Findings: In foveal striate cortex (area 17) of rhesus monkey approximately 30% of the cells show color specificity of some kind, the remaining 70% showing no detectable color preference. A substantial proportion of the color cells respond without regard to the size or shape of the stimulus, whereas most of the noncolor cells show very precise requirements for some spatial feature, such as line orientation, line width, direction of movement, or velocity of movement. The cells with the most precise orientation specificity do not generally show a directional preference as do other more loosely oriented cells, suggesting that orientation and direction are being processed as separate features.

To this extent then, color, orientation, and direction of movement are being segregated or abstracted by the brain for separate processing by different groups of cells. On the other hand, there is clearly a small group of cells with specificity for both orientation and color, the optimal stimulus for such cells being, for example, a narrow vertical red slit of light. The presence of these cells with combined spatial and color specificity could imply that the other cells with only spatial or only color specificity were precursors and that the various features were being recombined in striate cortex prior to further processing.

This possibility was tested by making electrode penetrations into a region of prestriate cortex (area 18) receiving direct projections from striate cortex. Some of these prestriate cells showed color specificity without orientation specificity, and others showed orientation specificity without color specificity, suggesting that some of the information about color and orientation is not being combined in striate cortex, but is being relayed by parallel channels to the next higher level in the visual system. The oriented color cells would then constitute a separate parallel channel conveying specific information about colored lines or color borders.

Significance to Biomedical Research and the Program of the Institute: This work contributes to our understanding of vision as a feature detection process. It could be relevant in the development of a prosthesis for the blind.

Proposed Course of Project: Further single cell recordings in striate and prestriate cortex are planned, in hopes of establishing the functional differences between these two areas. In addition, Mrs. Beverly West, a guest worker from Queens College, is beginning a study which will involve making small injections of a radioactive amino acid into a single layer (established

stereotaxically) or physiologic column (established by means of microelectrode recording) within striate cortex. By combining the techniques of autoradiography and electrophysiology, we hope to learn more about the functional architecture of rhesus monkey visual cortex.

Honors and Awards: None

Publications:

Dow, B.M. and Gouras, P. Color and spatial specificity of single units in rhesus monkey foveal striate cortex. J. Neurophysiol. 36: 79-100, 1973.

Leighton, S.B. and Dow, B.M. Servo-controlled moving stimulus generator for single unit studies in vision. Vision Res. (in press)

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electrophysiological Studies of Mammalian Retina

Previous Serial Number: Same

Principal Investigators: Astrid Kafka, M.D. (Guest Worker)
Ralph Nelson, Ph.D. (Postdoctoral Fellow)
Peter Gouras, M.D.

Other Investigators: Helga Kolb, Ph.D.

Cooperating Units: Zvi Vogel, Ph.D., Laboratory of Biochemical Genetics, NHLI
P. Grimes, A.B. and Ludwig von Sallmann, M.D., Laboratory of Neurophysiology, NINDS

Man Years:

Total:	2.5
Professional:	2.5
Other:	0

Project Description:

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

Methods Employed: Recording from single neurones in isolated perfused or superfused mammalian retina; recording of multicellular responses from different retinal layers; examination of the retina by light and electron-microscopy.

Major Findings: Drs. Kafka and Nelson have recorded from and injected with procion yellow single horizontal cells, bipolar cells, ganglion cells and Müller Vikers in the cat retina. The staining obtained is of a quality which allows fine cell processes to be seen in considerable detail. An attempt is being made to determine what type of horizontal cells receive inputs from only rods, only cones or both. A similar approach is being used to link the functional characteristics of bipolar and ganglion cells with their morphological appearance.

Dr. Gouras is collaborating with Dr. Zvi Vogel on determining whether bungarotoxin alters synaptic transmission in the inner plexiform layers of mammalian retina. This relatively specific blocking agent which permanently binds to acetylcholine receptor sites may provide a means of pharmacologically dissecting out the synaptic processes within the inner plexiform layer of the mammalian retina.

Further studies are being carried out on the new genetic variety of retinal degeneration found by Dr. von Sallmann and Miss Grimes in Osborne-Mendel rats. Correlations between light microscopy and electrophysiology suggest that the defect appears to involve the more central regions of the retina before the periphery. It is hoped that this disease can be identified rapidly in the adult organism so that it can be inbred and thereby better understood genetically.

Dr. Gouras, in collaboration with Dr. Helga Kolb, has been studying the light and electronmicroscopy of eyes from a patient with dominantly inherited retinitis pigmentosa. The patient is one member of a family that has been extensively studied electrophysiologically and psychophysically at NIH. The results have shown there is an excessive amount of lipofuscin in the pigment epithelium which implies that this cell layer must phagocytize outer segment material in this disease. The second point is that a totally new type of pigment epithelium is formed in areas where photoreceptors have been destroyed. This latter type of epithelium forms the pigmentary intraretinal deposits which give this disease its name.

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: None

Publications:

Gouras, P.: S-potentials. Chap. 13, In Fuortes, M.G.F. (Ed.): Handbook of Sensory Physiology, New York, N.Y., Springer-Verlag, pp. 513-529, 1972.

Gouras, P.: Light and Dark Adaptation. Chap. 16, In Fuortes, M.G.F. (Ed.): Handbook of Sensory Phsyiology, New York, N.Y., Springer-Verlag, pp. 609-634, 1972.

Niemeyer, G. and Gouras, P.: Rod and cone signals in S-potentials of the isolated perfused cat eye. Vision Res. (in press).

Niemeyer, G. and Gouras, P.: The perfused mammalian eye as a preparation for electrophysiological studies. Doc. Opthalmologica (in press).

Hoff, M. and Gouras, P.: Tolerance of mammalian retina to circulatory arrest. Doc. Ophthalmologica (in press).

Niemeyer, G.: Intracellular recording from the isolated perfused cat eye. Vision Res. (in press).

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Chemistry and Metabolism of the Lens

Previous Serial Number: None

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

Other Investigators: Lorenzo O. Merola
Minerva Lawrence

Cooperating Units: None

Man Years:

Total:	2.9
Professional:	1.4
Other:	1.5

Project Description:

Objectives: The many aspects of the chemistry and the metabolism of the ocular lens will be studied. This would include the carbohydrate metabolism, the chemistry and metabolism of the lens crystallins and glutathione, and the active transport mechanisms in the lens.

Methods Employed: In many of these studies the organ culture technique would be utilized. The lens crystallins will be studied by using the techniques already described to separate the various crystallins. We are interested in the age effects of the lens and also in the changes that occur in the protein and glutathione during cataract development.

Major Findings: The effect of aging on the lens proteins was studied by analyzing the young calf and the older cattle lenses. Upon isolating gamma crystallin, one of the principal lens proteins, it was found that protein patterns obtained on Sephadex G-75 column indicated that aging had a marked effect on lens gamma crystallin. The older cattle lens gamma profile was considerably different from the calf lens gamma. The cattle lens gamma was made up of a gamma identical to the one found in calf lens plus a different gamma crystallin. By dissecting the cortex from the nucleus, it was found that the cattle lens nucleus had contained only the calf lens crystallin while the cattle cortex was made up of a different gamma. As the lens ages a new form of gamma crystallin emerges, probably

synthesized in the cortical region. This is a clear-cut demonstration of a change in the type of protein that occurs in the aging lens.

In another study a comparison was made of the susceptibility to oxidation of the sulfhydryl groups in bovine and human lenses. It was shown that the sulfhydryl groups of the older cattle lens were resistant to mild oxidizing conditions, and oxidation only occurred when 8 M urea was included in the reaction mixture in addition to cupric ions. On the other hand, the sulfhydryl groups of the human lens were extremely susceptible to oxidation. In homogenates, the oxidation occurred spontaneously and did not even require the addition of catalytic amounts of copper ions. These results suggest that disulfide linkages can be readily formed in the human lens. Intermolecular cross-linking may possibly lead to the insolubilization of lens proteins. In the human lens, therefore, the role of glutathione may be to serve to protect the protein sulfhydryl groups and maintain them in the reduced form.

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.

Proposed Course of Project: These studies will be continued.

Honors and Awards: None

Publications:

Jedziniak, J.A., Kinoshita, J.H., Yates, E.M., Hocker, L.O. and Benedek, G.B.: Calcium-Induced Aggregation of Bovine Lens Alpha Crystallin. Invest. Ophthal. 11: 905-915, 1972.

Chylack, L.T. and Kinoshita, J.H.: The interaction of the lens and vitreous. II. The influence of the vitreous on lens trauma, water, electrolyte balance and osmotic stress. Exp. Eye Res. 15: 61-69, 1973.

Jedziniak, J.A., Kinoshita, J.H., Yates, E.M., Hocker, L.O. and Benedek, G.B.: On the presence and mechanism of formation of heavy molecular weight aggregates in human normal and cataractous lenses. Exp. Eye Res. 15: 245-252, 1973.

Fukui, H.N., Epstein, D.L. and Kinoshita, J.H.: Ascorbic acid effects on lens ^{86}rubidium transport. Exp. Eye Res. 15: 309-313, 1973.

Kinoshita, J.H. and Merola, L.O.: Oxidation of sulfhydryl groups of human lens. The Ciba Foundation Symposium on the Human Lens - In Relation to Cataracts. (in press).

Kabasawa, I. and Kinoshita, J.H.: Carbohydrate associated with gamma crystallin of the calf lens. Exp. Eye Res. (in press).

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Sugar Cataracts

Previous Serial Number: None

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

Other Investigators: Lorenzo O. Merola

Cooperating Units: None

Man Years:

Total:	2.9
Professional:	2.4
Other:	0.5

Project Description:

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

Methods Employed: One approach in this study was to induce cataracts in experimental animals by making them diabetic with appropriate chemical agents, or to make them galactosemic or xylosemic by feeding animals a diet enriched with galactose or xylose. The other 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. These types of cataract will be prevented by the use of aldose reductase inhibitors.

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 sugar cataracts are initiated. There is substantial evidence that aldose reductase participates in the mechanism that leads to the diabetic and galactosemic cataract. However, there has been some question whether the development of the xylose cataract involves aldose reductase. It has been difficult to produce xylose cataracts

in rats because of the poor absorption of xylose. Consequently, it is difficult to raise the blood xylose level to a significant degree.

We have been able to demonstrate clearly by organ culture techniques that aldose reductase does appear to initiate the xylose cataracts as well. In fact, it appears that xylose is much more of a cataractogenic sugar than either glucose or galactose. The reason for this is that xylose is a much better substrate for aldose reductase than is either galactose or glucose. Thus, when xylose is exposed to the lens, a large quantity of sugar alcohol in the form of xylitol is formed and creates a hypertonicity. This leads to a lens swelling much more pronounced than found in the other two forms of sugar cataracts. A potent aldose reductase inhibitor has been developed by the Ayerst Laboratories and this inhibitor prevents the formation of xylitol and prevents the early cataract changes that are observed in xylose cataracts. The demonstration that aldose reductase is involved in the xylose cataracts allows the conclusion that all three forms of cataract involve the participation of aldose reductase in the cataractous process.

Studies have been conducted to assess the various factors involved in the metabolism of sugars in the rat lens through the sorbitol pathway in diabetes and galactosemia.

Activity of aldose reductase in diabetic lens was found to be higher than that in the controls. On the other hand, activity of polyol dehydrogenase was decreased. A surprising finding in the diabetic lens was that reduced Triphosphopyridine nucleotide (TPNH) was maintained at normal levels. This occurred despite the greater demand for TPNH required in sorbitol production by aldose reductase. There was a striking rise in reduced Diphosphopyridine nucleotide (DPNH) and a fall in Diphosphopyridine nucleotide (DPN) levels. A similar pattern of changes in pyridine nucleotide levels also was observed in galactosemic lens.

Findings suggest that in diabetes and galactosemia, conditions in the lens are so altered as to favor the accumulation of sugar alcohols.

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, there is apparently a certain number of people who avoid surgery and are debilitated by loss of vision. 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 approaches to prevent the cataract. The terminal stages of these sugar cataracts may have features common to other forms of cataract. 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: None

Publications:

Kalckar, H.M., Kinoshita, J.H. and Donnell, G.N.: Galactosemia: Biochemistry, Genetics, Pathophysiology and Developmental Aspects. In Gaull, G.E. (Ed.): The Biology of Brain Dysfunction. Plenum Press, New York. 1973.

Kinoshita, J.H.: Cataractogenic Effects of Lactose and Galactose. Nutritional Reviews (in Press).

Jedziniak, J.A. and Kinoshita, J.H.: Lens Polyol Dehydrogenase. Exp. Eye Res. (in Press).

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemical Control Mechanisms

Previous Serial Number: None

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

Other Investigators: Richard E. Bensinger, M.D.
R. Theodore Fletcher
Barbara Wiggert, Ph.D. (Guest Worker)

Cooperating Units: David Newsome, M.D.
Section on Experimental Embryology
Laboratory of Vision Research, NEI

Man Years:

Total:	3.1
Professional:	2.1
Other:	1.0

Project Description:

Objectives: Relatively little is known about the control of enzyme activity in the retina and about the factors that influence the development of enzyme patterns in the embryonic retina and pigmented epithelium. The present study was designed: (a) to assess the possible role of intracellular hormones (cyclic nucleotides) in the reception of the photic stimulus in the outer segments of the retinal photoreceptor cell; (b) to investigate the effects of hormones on the enzymic development of the retina; and (c) to investigate the effects of cyclic nucleotide hormones on the pigmented epithelium in culture.

Methods Employed: (a) Rod outer segments of the bovine retina were isolated by density gradient centrifugation and the activity of enzymes which synthesize or catabolize cyclic nucleotide hormones were determined under varying conditions of illumination. (b) Radioactive glucocorticoid hormone was incubated with embryonic retinas and the uptake of the steroid hormone into the tissue was determined in relation to steroid-increased enzyme activity. (c) Pigmented epithelium cells were grown under sterile culture conditions and the in vitro effects of cyclic nucleotide hormones were adjudged by microscopy and biochemical determination of enzyme activity, melanin deposition, etc.

Major Findings: (a) High levels of the enzymes that synthesize and catabolize cyclic nucleotide hormones (e.g., cyclic GMP) have been found in the bovine rod outer segment. Cyclic GMP synthetic capacity is greater in the dark than in the light. Cyclic GMP metabolism is affected by ions such as Ca^{++} which are known to mimic the electrophysiological effects of light on the retina. (b) The embryonic retina contains specific glucocorticoid-binding proteins which act as intracellular "receptors" and mediate the effects of the corticoid hormones on enzyme induction and biochemical differentiation of the tissue. (c) The hormone cyclic AMP affects growth rate, membrane properties and pigmentation of cultured pigmented epithelium cells. It also dramatically affects cellular morphology, promoting a more differentiated cell type.

Significance to Biomedical Research and the Program of the Institute: (a) The high levels of the enzymes involved in cyclic GMP metabolism found in rod outer segments indicate an active role of the hormone in the normal functioning of the photoreceptor cell. Altered levels of the hormone could lead to degeneration of the retina ("retinal dystrophy"). (b) The normal differentiation of the retina and pigmented epithelium is essential to subsequent normal visual function. Defective hormone uptake by the retina or action in the retina and in the pigmented epithelium could subsequently lead to any one of a number of visual problems.

Proposed Course of Project: (a) To assess the role of cyclic nucleotide hormones in the photoreceptor cell (both cyclic AMP and cyclic GMP) and continue to study the enzymes involved in synthesis and metabolism of these hormones. (b) to further characterize the effects of hormones in the normal development of the retina and pigmented epithelium *in vivo* and in tissue culture and to attempt to pinpoint their specific site(s) of action.

Honors and Awards: None

Publications:

Chader, G.J., Rust, N., Burton, R.M. and Westphal, U.: Steroid-Protein Interactions. XXVI. Studies on the polymeric nature of the corticosteroid-binding globulin of the rabbit. J. Biol. Chem. 247: 6581-6588, 1972.

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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:	0.6
Professional:	0.4
Other:	0.2

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: Newly hatched chicks raised for a period of 7 weeks on a nutritionally adequate purified diet supplemented with 6% glycine, 8% gelatin and 10 mg% nicotinic acid exhibit an enlargement of the eyeball (buphthalmia) but no suppression in growth. Such birds appear to be essentially free of glaucomatous symptoms, i.e., increased intraocular pressure and decreased facility of aqueous humor outflow. The buphthalmia observed in these chicks is not photo-induced because removal of incandescent lighting from the cage or its environs during the feeding period does not inhibit the eye enlargement. The change in vitreous volume observed in male chicks is due primarily to an overgrowth of connective tissue which results from an increase in total soluble hexuronic acid and hydroxyproline in the vitreous. Addition of 1% serine to the glycine-rich diet increases the severity of the buphthalmia

suggesting that the extent of biosynthesis of hyaluronic acid and/or its peptide may determine the size of the vitreous. Replacement of 6% glycine in the feed with equimolar concentrations of γ-aminobutyric acid and L-glutamic acid respectively, failed to produce the buphthalmic condition in these birds.

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 the 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. In addition, the vitreous of the glycine-fed birds will be fractionated on glass-bead chromatographic columns in order to determine if a particular peak of hyaluronic acid and/or soluble protein is altered in concentration as compared to that seen in control animals.

Honors and Awards: None

Publications:

Helmsen, R.J., Gaasterland, D.E. and Rubin, M.: Induction of buphthalmos in chicks fed an excess of glycine. Invest. Ophthal. 12: 348-353, 1973.

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Chemistry of the Cornea

Previous Serial Number: Same

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

Other Investigators: Donald Henson, M.D. (NCI)
Merrill Lynn, Ph.D. (Corning Glass Works)

Cooperating Units: Laboratory of Pathology (NCI) and Technical Staff Division, Corning Glass Works

Man Years:

Total:	1.4
Professional:	0.6
Other:	0.8

Project Description:

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

Methods Employed: Distinct proteins will be 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 ion-exchange chromatography and/or preparative gel electrophoresis. Purity of individual fractions will be determined by the number of bands obtained by staining following polyacrylamide gel electrophoresis and isoelectric focusing.

Major Findings: Soluble corneal epithelial proteins of the calf have been quantitatively separated into two nucleoprotein peaks by a newly developed chromatographic procedure. The method involves the use of molecular sieving through glass beads with strictly controlled-pore diameters in which the pore channels have been previously coated with an appropriate quaternary amine. This procedure supplants the use of Sephadex G-150 or G-200 for large scale preparation of such corneal proteins since a total chromatographic profile could be obtained for 10-15 mg of material in approximately 20 minutes. In addition a procedure has been devised for good release of soluble proteins from stored calf epithelial cells and rabbit corneal cells derived from

tissue culture without homogenization by gentle stirring in cold 0.01 M pyrophosphate buffer saturated with n-octanol.

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 suggest an approach to control of corneal graft reaction and/or viral invasion (e.g., herpes) of the tissue in humans.

Proposed Course of Project: The second nucleoprotein peak obtained from corneal epithelium by fractionation on glass bead columns will be further separated on amine-derivatized glass beads specially prepared by Corning Glass Works in a manner parallel to that used in conventional anion exchange chromatography. If a successful separation of individual proteins is achieved, the same procedure will be applied to soluble proteins derived from calf stroma and rabbit corneal epithelial cells derived from tissue culture.

Honors and Awards: None

Publications: None

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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

Previous Serial Number: None

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:	2.83
Professional:	2.83
Other:	0.0

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) as quantitative indices of certain histological entities or metabolic routes; (3) to use indices in conjunction with other chemical constituents 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: At the present time, analyses are being 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; and light microscopy (ordinary and polarizing).

Major Findings: I. Improved spectrophotometric micromethods for assay of proteins and lipids: (a) A linear assay was developed for microgram amounts of insoluble as well as soluble proteins without heating. The method eliminates an artefact in protein assay caused by lipid interference from autooxidized

fatty acids produced when heat is used to solubilize proteins in alkaline solution. (b) An ultramicroassay for 0-2 nanomoles of phosphorus was worked out for analysis of phospholipids and other P containing compounds, organic and inorganic.

II. Thin layer chromatographic studies of neutral and glycolipid fractions of frog retina and rod outer segments, and rat retina: The phospholipid fraction of retina and outer segment membranes has been well studied by many investigators in several species, but the neutral and glycolipid fractions have not. Retina is unusual in being a part of the central nervous system that is devoid of oligodendrocytes and their product myelin, whose most characteristic lipid component (or chemical marker substance) is cerebroside (including its sulfate ester, sulfatide). The glial cell of the retina is the Müller cell, an astrocyte, a type of cell we have shown to have negligible amounts of cerebroside and sulfatide. In accord with these chemoanatomical principles, our TLC studies revealed that retinal lipid extracts contain little or no cerebroside and sulfatide, and that the water-insoluble glycolipids present migrate like ceramide hexoside compounds that have more than one hexose moiety. We have developed two dimensional TLC systems that separate these compounds, as well as the neutral lipid fraction (and phospholipid fraction, as a by-product) and will proceed with their identification.

III. Sensitivity of intact retinal rod outer segments of frogs to small decrements in ionic strength (Na and Ca salts): Frog outer segments isolated from dark adapted retinas by a magnetic stirring technique in a sodium phosphate buffered sucrose medium (ionic strength, 0.024, pH 6.4) are intact, birefringent organelles that are stable to light and room temperature. However, they immediately show loss of birefringence and undergo form changes consisting of bending, curling, elongation and disruption when transferred into analogous media differing in ionic strength by decrements over 0.003. Addition of Ca ions at a concentration of 1.5 mM minimized the form changes. The form changes were lessened in outer segments from retinas of frogs dark adapted 12-24 hrs. and increased in those from retinas of light adapted frogs. Because the results suggest that influx of water is more rapid than efflux of Na ions during the transfer to lower ionic strength media and that Ca^{++} influences the phenomenon, we are studying the ratios of Ca, Mg, K and Na in rod outer segments prepared from retinas subjected to different light and dark exposures.

IV. Properties of artificial membranes (liposomes) similar in composition to retinal rod outer segment membranes: Liposomes are liquid crystals of lipids that can be formed in aqueous media, generally in the presence of a salt. They commonly form spherulites of concentric bimolecular layers of lipid each separated by aqueous compartment. We have prepared, from commercial lipids, liposomes that are similar in composition to the lipids of rod outer segments. Liposomes have also been prepared from lipids isolated from frog retinal rod outer segments. By polarizing microscopy the liposomes are birefringent; by microelectrophoresis their migration is intermediate between that of single component liposomes of phosphatidyl choline (no mobility) and of phosphatidyl serine (greatest mobility). Liposomes formed by reaction of the lipids with different types of proteins have been studied similarly to examine whether

ionic or hydrophobic interactions occur, and studies are being made of the permeability of the liposomes to ions introduced at the time of their formation.

Significance to Biomedical Research and the Program of the Institute: Data on the characteristic biochemical composition of normal photoreceptor, neuronal and glial membranes will contribute to an understanding of their function in retina and brain. Present work is focused on lipids and biologically significant metals of rod outer segments and retina. 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 (rat and human) is a relatively unexplored area.

Proposed Course of Project: The project is new to the Laboratory of Vision Research. Normal biochemical architecture and pathology of photoreceptor cells will be emphasized. Normal materials to be studied include frog and rat retina and human autopsy retina. When our microbalance room and microtome cryostat are working together, in the future, microtechniques of frozen section sampling, microdissection of pigment epithelium and photoreceptor cell layers from bipolar-ganglion cell layers of retina can be performed and the microsamples weighed and analyzed. Pathological materials will include an animal model of retinitis pigmentosa (heritable rat retinal degeneration, RCS rats), and specimens of human disease in which photoreceptors primarily degenerate (retinitis pigmentosa).

Honors and Awards: None

Publications: None

Serial No. NEI(I)-71 LVR 008(c)
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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:	0.6
Professional:	0.5
Other:	0.1

Project Description:

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

Methods Employed: The self-association of the detergent, Triton X-100, and the association of the detergent with rhodopsin was studied by sedimentation-equilibrium techniques in the analytical ultracentrifuge. Studies on the extractability of rhodopsin as a function of detergent concentration were performed by combining aliquots of purified rod outer segment suspensions with appropriate volumes of detergent of known concentration, allowing these to stand for varying lengths of time, filtering rapidly through a millipore filter, and determining the rhodopsin concentration by measuring the optical density at 500 nm.

Major Findings: Triton X-100 was chosen for these studies since it is frequently used for the extraction of rhodopsin and its exact chemical structure is known, while the exact structure of Emulphogene BC-720, another commonly used detergent, is less well defined. Analysis of the ultracentrifugal data as a monomer-n-mer type association, typical of micelle-forming detergents, indicated a micellar size of 105 monomer units, a change of standard free energy of -3.7 kcal per mole of monomer, and a critical micelle concentration of 0.00134 moles per liter.

This value for the change of standard free energy of association is quite characteristic of hydrophobic bonds.

In order to relate this to rhodopsin extraction, the concentration dependence of extraction was studied in order to find a concentration of detergent which would give zero extraction of rhodopsin from the rod outer segments, a point analogous to the critical micelle concentration. The optical density at 500 nm. of the extracted and filtered solutions was plotted as a function of the logarithm of the detergent concentration. It was found that zero rhodopsin extraction occurred at a detergent concentration of 0.00014 moles per liter, that this concentration was not dependent on the concentration of rod outer segments, and that the ratio of extracted rhodopsin to extractable rhodopsin also appeared to be independent of rod outer segment concentration. This very strongly indicated that the amount of rhodopsin released was a function of the amount of detergent bound, which would be a function of only the free detergent concentration and not the rod outer segment concentration. Accordingly, the apparent intrinsic association constant was calculated from plots of 1/OD vs. 1/C for the detergent and the change of the standard free energy was calculated. This was found to be -3.0 kcal per mole of detergent, a value within the range associated with hydrophobic bond formation. This implies that hydrophobic bonds of comparable magnitude are involved in the maintenance of the integrity of the rod outer segment membrane.

The finding that it was possible to extract rhodopsin from the rod outer segment at detergent concentrations significantly below the critical micelle concentration has two important implications. The first is that there are many binding sites available for the detergent and that this accounts for the significant levels of binding to the membrane at concentrations where the detergent does not undergo self-association. The other is that it is possible to study the binding of detergent to rhodopsin at detergent concentrations where there are no detergent micelles to complicate an already difficult analysis. Because the detergent and the rhodopsin have different partial specific volumes and specific refractive indices, the values of these parameters for the rhodopsin-detergent complex is a function of the amount of the detergent bound, and knowledge of the amount of detergent bound requires knowing the values of these parameters. However, this problem can be solved by using a suitable reiterative computer analysis which alternatively adjusts the parameters until convergence is obtained. Such a program had been written and the ultracentrifugal analyses are in progress.

Significance to Biomedical Research and the Program of the Institute: It has been postulated that hydrophobic bonding between the lipoprotein rhodopsin and the various lipids of the

rod outer segment membrane was of major importance for the maintenance of the structure of the membrane and for the behavior of the rhodopsin in the membrane when exposed to light. The studies described here are quantitative support for the first of these concepts. It is anticipated that the current studies on detergent binding at low detergent concentrations may offer additional support for both postulates. 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 the pathological aspects of scotopic vision.

Proposed Course of Project: Continued studies will be directed toward further elucidation of the thermodynamic aspects of detergent and phospholipid binding to rhodopsin and the significance of these parameters to the function of rhodopsin in the rod outer segment membrane.

Honors and Awards: None

Publications: None

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Physical Chemistry of Model Gel Systems

Previous Serial Number: Same

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

Other Investigators: Dr. Jules Gladner, LBC, NIAMDD

Cooperating Units: None

Man Years:

Total:	0.7
Professional:	0.5
Other:	0.2

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 principal method used in these studies has been analytical ultracentrifugation, since it has been demonstrated to be the most effective technique for studying systems of interacting macromolecules. Considerable emphasis has also been given to the development of sophisticated computer techniques for data reduction and analysis of systems of this type.

Major Findings: Work on the chemistry of succinylated proteins has been resumed since this is relevant to studies on succinylated rhodopsin. Studies on succinyl fibrin and fibrinogen have demonstrated that the entire A-chain of these proteins is rapidly destroyed by thrombin. This is in marked contrast to the usual effect of thrombin which normally releases only four small peptides from fibrinogen and has no effect on fibrin. Succinylation of hemoglobin was found to have a very marked effect on its dissociation, with dissociation occurring at much higher concentrations than for normal hemoglobin. Much effort has been given to computer studies on problems involving self-association and binding of proteins. Simulation studies have

led to a realistic assessment of the accuracy which may be expected in the analysis of such systems. When more than four macromolecular species are present, it becomes very difficult to obtain meaningful values for the association constants and to differentiate such a system from an indefinite association. Equations have been developed for studying the binding of a ligand to a protein under circumstances when the concentration distribution of only one of the two species is observed. This promises to be particularly useful in binding studies involving rhodopsin or retinol binding protein.

Significance to Biomedical Research and the Program of the Institute: The studies on succinyl modified proteins are of particular significance for the relationships which they demonstrate between the effects of charge density and enzyme-substrate specificity and the effects of charge density on the association-dissociation behavior of certain proteins. This information is of value in the evaluation of the results obtained in studies on succinyl rhodopsin. The results of the computer studies are of general value for studies on associating systems and on protein-ligand interactions, and of particular value for current studies on rhodopsin-detergent interactions. 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 the pathological aspects of scotopic vision.

Proposed Course of Project: The studies on succinyl proteins, which were interrupted by Dr. Gladner's prolonged illness, will be resumed and carried forward to the extent that they appear relevant to problems in visual biochemistry. The computer studies will be carried forward and applied as needed, particularly in binding studies involving rhodopsin and retinol binding protein.

Honors and Awards: None

Publications:

Allen, G.S., Lewis, M.S. and Tower, D.B.: Acidic Proteins in Cerebral and Hepatic Microsomes. Problems in Brain Biochemistry. 7: 55-68 (Russian), 69-78 (English), 1972.

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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
Others:	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. This project was designed to elucidate some of the biochemical events involved in this process, in particular the control of rhodopsin transport to the outer segment and the site of the addition of retinal to the rhodopsin polypeptide.

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: Although rhodopsin transport to the outer segment is sensitive to agents that interfere with microtubule function, the extent of inhibition is never great. Moreover, neither the incorporation of choline into outer segment phosphatidyl choline nor incorporation of retinal into rhodopsin was sensitive to these agents which inhibit axon transport, particularly lipid transport.

In addition, many protein components of the outer segments appear to be labeled with radioactive leucine in vitro when examined by column chromatography. However, only one major component, opsin, appears on gel electrophoresis under disaggregating conditions. Hence, a group of precursors may accumulate in the outer segment before being converted to fully functional rhodopsin molecules.

Significance to Biomedical Research and the Program of the Institute: It appears that a mechanism distinct from the microtubule-linked axon transport system is responsible for the transport of rhodopsin to the outer segment. In addition, a variety of rhodopsin precursors in the outer segments would suggest a variety of biochemical events that must take place there, a level of complexity not heretofore attributed to the outer segments.

Proposed Course of Project: Efforts will continue on the identification of precursors of rhodopsin and the biochemical changes they undergo as well as possible mechanisms controlling rhodopsin transport and the assembly of disc membranes.

Honors and Awards:

Invited speaker at the American Society for Photobiology Symposium on the Visual Pigments, Sarasota, Fla., June 11-15, 1973. Elected to ARVO Biochemistry Program Committee. Elected Member, American Society of Biological Chemists.

Publications: None

Serial No. NEI(I)-71 LVR 015 (c)
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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
Others:	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, detergent extraction and purification of rhodopsin by column chromatography.

Major Findings: Glucosamine was shown to be an integral component of rhodopsin. However, radioactive glucosamine appears in rhodopsin before labeled amino acids. Therefore, at least some glucosamine residues are added after the protein has been synthesized and is being transported to the outer segment. The final addition may take place in the outer segment as the membranes are being assembled.

Significance to Biomedical Research and the Program of the Institute: The sequential addition of sugars in the complete synthesis of glycoproteins is thought to be a possible mechanism for both transport of glycoproteins and interaction of membranes containing glycoproteins. The growth of the carbohydrate component of rhodopsin could, therefore, be essential for its transport to the outer segment and its proper insertion into new disc membranes. Failure of these sugar transfers could seriously interfere with normal photoreceptor outer segment renewal.

Proposed Course of Project: Efforts will be directed toward the demonstration of mannose incorporation into rhodopsin and its temporal relationship to polypeptide synthesis. In addition, attempts will be made to determine whether an apparent precursor to rhodopsin is actually an acceptor for the transfer of sugars, giving rise to rhodopsin.

Honors and Awards:

Invited speaker at the American Society for Photobiology Symposium on the Fisual Pigments, Sarasota, Fla. June 11-15, 1973. Elected to ARVO Biochemistry Program Committee. Elected Member, American Society of Biological Chemists.

Publications:

O'Brien, P.J. and Muellenberg, C.G., Incorporation of Glucosamine into Rhodopsin in Isolated Bovine Retina, Arch. Biochem. Biophys., in press.

Serial No. NEI(I)-71 LVR 012
1. Laboratory of Vision Research
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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 elucidate the chemical structure of the rod pigment rhodopsin for an understanding of the molecular mechanism of rod (dim light) vision. (2) To continue investigations of a possible function of lipid in the structure of rhodopsin. (3) To follow the fate of rhodopsin after phagocytosis of rod outer segments by pigment epithelial phagosomes.

Methods Employed: Such biochemical methods as centrifugation, column and thin-layer chromatography and spectroscopic analysis. High voltage electrophoresis and amino acid analysis.

Major Findings: (i) The peptide (MW= 2,400) previously isolated after cyanogen bromide (CNBr) cleavage of deplipidated opsin was found to have serine as the N-terminal residue and methionine as the C-terminus. It contained no carbohydrate. A method was established to isolate the peptide by direct CNBr cleavage of delipidated rod membranes.

(ii) a. Evidence has been obtained indicating that phospholipid and 11-cis retinal associated with rhodopsin contribute to stabilization of opsin conformation independently. Namely, removal of retinal from opsin while phospholipid is still associated does not affect opsin conformation. If phospholipid is also removed, opsin conformation is immediately altered. How-

ever, if retinal isomers are added back to the opsin molecule to regenerate rhodopsin or iso-rhodopsin and phospholipid is then removed, opsin does not undergo conformational change.
b. In regard to phospholipid requirements for regeneration of rhodopsin, recent evidence suggests that phospholipid is involved in the isomerization reaction of all-trans retinal to 11-cis retinal.

(iii) Two photosensitive pigments with absorption maxima at 440 nm and 415 nm, respectively, were detected in the partially purified "lysosomal" fraction of bovine retinal pigment epithelium. Whether or not these pigments are indeed degradation products of rhodopsin is yet to be determined.

Significance to Biomedical Research and the Program of the Institute: The results obtained indicate that phospholipid is essential not only for maintaining a preferred (native) conformation of opsin but also for the isomerization of all-trans retinal to 11-cis retinal.

Abnormal rod function observed under certain pathological conditions, e.g., retinal dystrophy, may be related to a decrease in stability as well as in regenerability of rhodopsin.

Proposed Course of Project: (1) Further characterzation of the nature of interaction between rhodopsin and phospholipid. (2) Elucidation of the chemical structure of rhodopsin.

Honors and Awards: None

Publications:

Shichi, H.: Modified rhodopsin in the pigment epithelium? Vision Res. 13: 477-480, 1973.

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

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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

Other Investigators: None

Cooperating Units: NIAMDD
National Bureau of Standards

Man Years:

Total:	2.0
Professional:	2.0
Other:	None

Project Description:

Objectives: It has become increasingly apparent in recent years that knowledge of how cellular membranes control ionic and material fluxes in and out of cells is essential to our understanding of life processes. This is particularly true in the study of visual systems whether one is concerned with the control of incoming radiation or its detection and interpretation.

The role of the regulation of ionic flux across visual cell plasma membranes in the process of the detection of light and the initiation of neurophysiological visual signals has been well established by us. The control of the ionic flux is mediated by the release of excitatory transmitters by light. We are directing our efforts to answer the questions of how photoexcited visual pigments control the passage of transmitters through membranes and how the transmitters in turn interact with the plasma membrane and thereby regulate membrane ionic flux which initiates the neurophysiological response of the visual system.

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

To determine the ionic contents of the visual cell in response to the application of 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 in a newly designed and developed system which utilizes radionuclide and fluorescent probe measurements on very rapidly isolated receptor cells.
cells.

Major Findings: (1) We have continued to bring forth new evidence which supports our hypothesis that the excitatory transmitter for rod and cone visual cells is calcium ion. Four different classes of experimental results predicted by our calcium model have been verified. These involve the effects of a) calcium deprivation on the photo-sensitivity of the cell and on the kinetics of the photocurrent, b) calcium ionophore, X-537A, on the dark current and photocurrent, c) calcium and light on the fast ionic photocurrent, and d) a determination of the intracellular Ca^{2+}.

(2) The metabolism of the visual cell: We find that the large visual cell ionic currents are tightly coupled to the metabolic energy production of the cell and hence, they influence one another strongly. Studies are being conducted on the effects of the perturbation of the energy producing system by various means on the dark current, photocurrent and ionic pumps of the cell.

(3) Ionic flux analysis: The 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.

Significance to Biomedical Research and the Program of the Institute: The understanding of how certain 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. Revelations 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

every area of biomedical science for it will fulfill the demand for a rapid and sensitive method of resolving the ionic composition of a cell 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.

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

Particular attention will be devoted to determine the connection between the photoexcitation of visual pigment and the generation of the fast ionic photocurrent.

Recently we have found indications that in retinas deprived of calcium for prolonged periods, the photochemical kinetics of the visual pigment seems to be altered. Attention will be directed to this new finding.

Honors and Awards: None

Publication:

Yoshikami, S. and W.A. Hagins: Control of the dark current in vertebrate rods and cones. Langer, H. (Ed.) Symposium on Visual Mechanism. Berlin, Springer-Berlin 1972 (in press).

OFFICE OF BIOMETRY AND EPIDEMIOLOGY

ANNUAL REPORT

OFFICE OF BIOMETRY AND EPIDEMIOLOGY
July 1, 1972 - June 30, 1973

During the past year the planned shift in OBE activities, indicated in last year's report, became a reality. Having lessened the emphasis on statistics relating to the registered blind, the Office is now principally concerned with clinical trials, searching for risk factors in eye disease and other statistical and epidemiologic studies aimed at preventing blindness and at improving diagnosis and treatment of visual disorders.

Major activities were:

- initiation of the Framingham Eye Study
- provision of statistical and epidemiologic consultation to the National Cooperative Diabetic Retinopathy Study
- preparation of several publications on the low heritability of the intraocular pressure response to steroids as estimated from twin studies
- a critical evaluation of methodology and biases related to estimates of heritability based on twin studies
- provision of statistical consultation to NEI intramural scientists
- preparation of the 1969-1970 Report on Blindness Statistics from the Model Reporting Area.

Other activities included:

- training and testing lay persons in reading fundus photographs
- collecting, collating and evaluating data on prevalence, incidence and economic cost of blindness
- continuation of a clinical trial of treatment for myopia among monozygous twins
- continued collaboration with a large diabetes clinic in developing a case-control study for diabetic retinopathy
- continued collaboration with foreign scientists in a cataract etiology study in India
- continued editing and coding of the ophthalmic portion of the Health and Nutrition Examination Survey.

Miscellaneous activities and publications (not listed with individual projects or elsewhere in this report)

Ederer, F.: Serum cholesterol changes: Effect of diet and regression toward the mean. J. Chronic Dis. 25:277-289, 1972.

Ederer, F.: Shall we count numbers of eyes or numbers of subjects? Arch. Ophthalmol. 89:1-2, 1973.

Ganley, J.P., Smith, R.E., Knox, D.L. et al: Presumed ocular histoplasmosis. III. Epidemiologic characteristics of people with peripheral atrophic scars. Arch. Ophthalmol. 89:116-119, 1973.

Ganley, J.P.: Epidemiologic characteristics of presumed ocular histoplasmosis. Acta Ophthalmol. (suppl. 119)1-63, 1973.

Kahn, H.A.: The prevalence of chronic simple glaucoma in the U.S. Am. J. Ophthalmol. 74:355-359, 1973.

Two publications of Harold Kahn's are listed in the NHLI report.

James P. Ganley lectured at: Boston University Department of Ophthalmology
Johns Hopkins School of Hygiene & Public Health

Roy C. Milton consulted with: Dr. Douglas Gaasterland and Karyn Ross, Clinical Branch, NEI, on studies of parameters of intraocular pressure, NEI (I)-71 CB 030(c).

Dr. Mitchel Wolf and Dr. Walter Stark, Clinical Branch, NEI, on a study of the utility of ^{125}I uptake in diagnosis of ocular melanomas.

Dr. Paul Bach-y-Rita, Smith Kettlewell Institute of Visual Sciences, on testing and evaluation of a tactile vision substitution system.

Harold A. Kahn was an invited discussant at the Ciba Seminar on the Human Lens in London, England.

Serial No. NEI (BE) 72-100

1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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

Previous Serial Number: Same

Principal Investigator: Harold A. Kahn

Other Investigators: Helen Moorhead
Rita Hiller

Cooperating Units: None

Man Years:

Total:	1.6
Professional:	.2
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.

Major Findings: The data obtained from the MRA study constitute the major source of blindness statistics in this country over the past decade and consistently show glaucoma, diabetic retinopathy, macular degeneration, and cataract to be the main causes of adult blindness in the U.S.

Significance to Biomedical Research and the Program of the Institute: Blindness statistics obtained from this study have in the past provided the data for the setting of priorities in eye research and a potential instrument for evaluating how well these priorities have been met.

Proposed Course: In order to divert OBE staff to higher priority epidemiological and statistical activities, OBE began to phase out its administration of the MRA in October 1971. NEI offered a contract for administering and improving the MRA to two of the voluntary organizationswhich are interested in blindness statistics; however, these organizations have not as of this date submitted any proposals for taking over the MRA activity. The final MRA statistical report which NEI will publish is for the years 1969 and 1970. In addition to types of data previously published, the 1969-1970 report includes age-standardized and age-specific rates by sex, color, and cause for register incidence and register prevalence.

Because we judge the increment in knowledge about the scope of blindness in the U.S. likely to result from continuation of MRA activities to be quite modest, we shall discontinue this activity for the indefinite future and allocate staff and other resources elsewhere. We expect to keep in touch with current data on blindness through reports from those few states we consider to have reasonably adequate data.

Honors and Awards: None

Publications:

Kahn, H.A. and Moorhead, H.B.: Statistics on Blindness in the Model Reporting Area, 1969-1970. U.S. Dept of HEW, Publication No. (NIH)73-427. National Eye Institute, NIH, Washington, D.C., U.S.Government Printing Office, 1973.

Serial No. NEI (BE) 72-101
1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: National Health and Nutrition Survey

Previous Serial Number: Same

Principal Investigators: Harold A. Kahn

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

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

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 examined over a four-year period according to a standard protocol. 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 was gathered on medical history, dietary history, physical examination, hematologic studies, blood chemistries, and urine chemistries.

Major Findings: Data are being edited and coded.

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 will complete editing the ophthalmology examinations and coding the history and diagnoses obtained from them. Analysis of data will be done by NEI and DHES in subsequent years.

Honors and Awards: None

Publications: None

Serial No. NEI (BE) 72-102
1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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:	1.2
Professional:	1.0
Other:	0.2

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 almost entirely developed by OBE staff with replications to control observer error by an OBE ophthalmologist 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: None

Significance to Biomedical Research and the Program of the Institute: The four eye diseases under consideration are the most frequent causes of adult blindness in this country today. As a guide to prevention of these

eye diseases, it will be very helpful to identify risk factors associated with them. The study has been designed with this objective in mind. Prevalence data for this age group (53-83) in this community will be a useful by-product.

Proposed Course of Project: 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. Necessary revisionsin forms and related protocols were made in April 1973 on the basis of the first two months experience.

Honors and Awards: None

Publications:

Kahn, H.A.: An extraordinary opportunity. Am. J. Ophthalmol. (in press).

Serial No. NEI (BE) 73-121
1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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

Previous Serial Number: None

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

Other Investigators: Fred Ederer
Rita Hiller

Cooperating Units: Department of Statistics, George Washington University

Man Years:

Total:	0.2
Professional:	0.2
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 to study in depth the paper, "The Bayesian Outlook and Its Applications," by Jerome Cornfield (Biometrics 25:617-657, 1969).

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 of Project: The study will continue into the next year, during which time the possible future directions of the study will be determined.

Honors and Awards: None

Publications: None

Serial No. NEI (BE) 73-116
1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Systemic and Ocular Onchocerciasis

Previous Serial Number: None

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:	0.4
Professional:	0.2
Other:	0.2

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 pretreatment 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: Partial analysis of the data collected on 1,202 individuals from six villages in the Upper Region reveal that 75 percent of the total population have systemic onchocerciasis as manifested by positive skin snips. Forty-six percent of the population have subcutaneous onchocercoma nodules indicative of chronic infestation.

Over nine percent of the population examined had severely impaired vision (visual acuity in both eyes worse than 20/200), and over six percent were blind (able to count fingers at one meter or worse in the better eye).

Preliminary analysis of the data on 807 of the examined villagers reveals that about 20 percent had gross evidence of ocular involvement, and six percent had socioeconomic blindness (20/200 or worse in the better eye) resulting from the disease.

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 of Project: Additional data that have been collected need to be coded and analyzed. These data relate to gross and biomicroscopic examination of the eye for presence of onchocerciasis.

Honors and Awards: None

Publications:

Ganley, J.P. and Biles, J.E.: Prevalence of onchocerciasis, visual impairment and blindness in six villages in the Upper Region of Ghana. World Health Organization Technical Report PD/73.2, 1972.

Serial No. NEI (BE) 73-117
1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Lymphocyte Transformation Response in Presumed Ocular Histoplasmosis

Previous Serial Number: None

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 are to be stimulated by a battery of specific and nonspecific antigens. The data from these cases will be compared to matched controls without ocular histoplasmosis (age, race, sex) and to individuals with peripheral scars only.

Major Findings: Data being collected.

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 of Project: The antigens have been obtained and standardized, trial runs completed, and the patients and controls selected. The data will now be collected and analyzed.

Honors and Awards: None

Publications: None

Serial No. NEI (BE) 73-118

1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Prevalence of Choroidal Nevi and Incidence of Malignant Melanoma in a Defined Population

Previous Serial Number: None

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

Other Investigators: George W. Comstock, M.D., Dr.P.H.

Cooperating Units: The Johns Hopkins University School of Hygiene and Public Health

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To determine the relationship between prevalence of choroidal nevi and incidence of malignant melanoma in a defined population.

Methods Employed: A random sample of individuals from Washington County, Maryland were given funduscopic examination for presence of choroidal nevi in 1970. The Washington County Cancer Registry records and medical records from area and referral hospitals were reviewed for individuals having malignant melanoma of the eye diagnosed from 1956 through 1965.

Major Findings: Three percent of the 287 individuals examined were found to have choroidal nevi. The incidence of malignant melanoma of the choroid was found to be 0.66 cases/100,000 population/year. If malignant melanomas arise only from choroidal nevi the average annual incidence rate among those with nevi would be 21 cases of melanoma per 100,000.

Significance to Biomedical Research and the Program of the Institute: This study suggests that if malignant melanoma of the choroid do develop from choroidal nevi, it must be a rare occurrence: approximately 1:5,000. It would not be rewarding to follow all individuals with choroidal nevi for evidence of malignant degeneration. Characteristics of a high risk group of

nevi need to be developed to identify those individuals likely to develop malignant melanoma.

Proposed Course: Completed.

Honors and Awards: None

Publications:

Ganley, J.P., and Comstock, G.W.: Benign nevi and malignant melanoma of the choroid: An epidemiologic study. Am. J. Ophthalmol. (in press).

Serial No. NEI (BE) 73-119

1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Accuracy and Repeatability of Reading Fundus Photographs

Previous Serial Number: None

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:	0.6
Professional:	0.5
Other:	0.1

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 inter-observer 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 intra-observer 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 continuing, in application to this study. The analysis of inter-observer variability and comparison with the standard readings will continue into next year.

Honors and Awards: None

Publications: None

Serial No. NEI (BE) 73-124

1.

2. Office of Biometry & Epidemiology

3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

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

Previous Serial Number: None

Principal Investigator: Rita Hiller

Other Investigators: Helen Moorhead
Norma Naftaly

Man Years:

Total:	1.1
Professional:	0.0
Other:	1.1

Project Description:

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

Methods Employed: Critical review and summarization of available published and unpublished data relating to eye disease, including study of reports from state agencies for the blind for usable information and evaluation of the data potentially available from the Commission on Professional and Hospital Activities.

Major Findings: Eye disease is a major cause of disability and hospitalization; details are continually being revised and extended.

Significance to Biomedical Research and the Program of the Institute: The information reviewed and summarized serves as basic source material in NEI program planning, and is useful in responding to Congressional and other inquiries for data on eye disease.

Proposed Course: This project will continue into the next FY.

Honors and Awards: None

Publications: None

Serial No. NEI (BE) 73-125

1.

2. Office of Biometry & Epidemiology

3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Estimation of Total and Additional Cost for Professional Services on NEI Research Grants (codes RO-1, PO-1, P-15) for FY 1973

Previous Serial Number: None

Principal Investigators: Rita Hiller
Fred Ederer

Other Investigators: None

Cooperating Units: Contract and Grants Branch, NEI

Man Years:

Total:	0.1
Professional:	0.0
Other:	0.1

Project Description:

Objective: To estimate from a sample survey of NEI research grants, (a) the present professional support cost and (b) the future support costs (for RO-1, PO-1, and P-15 research grants) to NEI if all future professional support were paid by NEI through the grant mechanism. At the time of the survey, not all professional support costs were borne by NEI.

Methods Employed: Two estimating procedures were used: (1) method derived by NEI, which essentially was based on estimating separately the present and future cost for each type of grant. This method considered professional time and cost separately for RO-1, PO-1, and P-15 grants; (2) method derived by Dr. Kulwich of NIAID-EP in his "Analysis of Manpower Aspects for FY 1971 NIAID Research Grants" of May 10, 1972. This method estimated cost per grant assuming uniform costs for all three types of grants.

Major Findings: Based on a random sample of grants active in March 1972, we estimated that NEI grant professional support costs would increase from about $7,000,000 to more than $9,000,000 if all such costs were to be paid by the grants.

The estimates were derived from 49 RO-1, 12 PO-1, and 11 P-15 grants. The RO-1 grants were a 12% random sample and PO-1 and P-15 grants were 100% samples. The estimates based on the NEI method included limits for sampling error.

Significance to Biomedical Research and the Program of the Institute: This project supported the planning and budgeting functions of the NEI by providing required estimates of possible future increased cost of professional services on research grants.

Proposed Course: This project is completed. A report was submitted to the Associate Director for Extramural and Collaborative Programs, NEI through the Chief, Office of Biometry and Epidemiology, NEI.

Honors and Awards: Written commendation was received from the Associate Director for Extramural and Collaborative Programs, NEI

Publications: None

Serial No. NEI (BE) 73-126

1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Critical Review of Literature, Foreign and Domestic, in Regard to Prevalence and Incidence Studies of Diabetic Retinopathy

Previous Serial Number: None

Principal Investigator: Rita Hiller

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.0
Other:	0.1

Project Description:

Objectives: A great many papers have been published on diabetic retinopathy. Most of them, however, concentrate on the effect of various treatments on this disease. The objective of this study is to identify those papers which deal partially or wholly with the frequency of occurrence of diabetic retinopathy and evaluate them.

Methods Employed: Analytical statistical appraisal of published studies with respect to basic methodology, criteria for retinopathy, sample size, etc.

Major Findings: Work is continuing.

Significance to Biomedical Research and the Program of the Institute: Although diabetic retinopathy is known to be a major cause of blindness in the U.S., research efforts against this disease are hampered by lack of knowledge as to its frequency in various population groups.

Honors and Awards: None

Publications: None

Serial No. NEI (BE) 73-120

1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 19/2 through June 30, 19/3

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

Previous Serial Number: None

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

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

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.2
Other	0.1

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 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. Preliminary analyses suggest that these data show a decrease in retinal sensitivity as age increases, and further that subjects with tapeto retinal degeneration and other degeneration exhibit reduced sensitivity.

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 of Project: Final analysis will be completed next year. Emphasis will be given to summaries with clinical utility. Consideration will be given to problems of establishing "normal" range of values for various age and diagnostic groups.

Honors and Awards: None

Publications: None

Serial No. NEI (BE) 73-122

1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Reports
July 1, 1972 through June 30, 1973

Project Title: Computer Evaluation of the Multivariate Normal Integral

Previous Serial Number: None

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

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.05
Professional:	0.05
Other:	0.00

Project Description:

This project is the completion of a research effort begun prior to joining NEI. A method was developed for the evaluation by computer of the multivariate normal integral, for arbitrary mean vector, covariance matrix, and region of integration. Evaluation is by means of a modification of a multidimensional adaptive Simpson's quadrature with error control, applied to the iterated integral.

This method has been implemented on a variety of computers, including the NIH-DCRT computer. Copies of the computer program are being distributed to requesters in the U.S. and abroad. The significance of this project to NEI is mainly in the benefits derived from basic contributions to general statistical methodology and literature. The project is being terminated at the end of this year.

Honors and Awards: None

Publication:

Milton, R.C.: Computer evaluation of the multivariate normal integral. Technometrics 14: 881-889, 1972.

Serial No. NEI (BE) 73-123

1.
2. Office of Biometry & Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Bayesian Confidence Limits for the Ratio of Poisson Parameters

Previous Serial Number: None

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

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

This study is a comparison of some Bayesian and classical procedures for determining confidence limits for the ratio of two Poisson parameters. This ratio may arise, for example, in the problem of comparing two groups on the basis of prevalence of some disease or pathological condition, a common problem in epidemiologic investigations. Early results suggest that some Bayesian limits are narrower, with similar confidence, than the classical limits. This study is scheduled for completion during the next year.

Honors and Awards: None

Publications: None

CONTRACT NARRATIVE
Office of Biometry & Epidemiology

Title: Etiology of Senile Cataract

In cooperation with Dr. A. Chatterjee of Ludhiana, India, Dr. S. Franken of Groningen, Netherlands, both ophthalmologists with experience in conducting ophthalmic surveys in India, and Dr. A. Pirie of Oxford, England, a biochemist who has done extensive work on lens protein, a study of etiologic factors in senile cataract has been developed. A detailed study protocol has been submitted to the All India Council for Medical Research for approval under the PL 480 program. The study plan is to verify the very high prevalence of cataract reported from the Punjab and to determine whether diet, sunlight, mineral content of drinking water or other factors under investigation are related to risk of cataract.

CONTRACT NARRATIVE
Office of Biometry & Epidemiology

Title: Etiology of Diabetic Retinopathy

Progress continued toward a case-control study aimed at discovering factors related to retinopathy given the presence of diabetes of long duration. A small contract for a tabulation of the Joslin Clinic patient population with respect to duration of diabetes and presence of retinopathy was awarded. The NEI has designated a random sample of about 1,000 of the recent clinic attendees for study of their clinic records. If a suitable number of the needed categories of patients can be found, in particular if there are enough patients with diabetes of long duration but without retinopathy, we expect to proceed to develop in detail contract plans for a case-control study.

Section on Clinical Trials and Natural History Studies

Office of Biometry and Epidemiology

A major effort was exerted during the past year to insure the feasibility of the national cooperative Diabetic Retinopathy Study. Two of the original ten clinics dropped out, and the remaining clinics encountered serious difficulties in recruiting patients. Eight new clinics were added, several of which met with internal management problems. A site monitoring team visited each clinic to investigate recruitment problems and facilitate their resolution. A national publicity campaign was organized, consisting of a national press release, an address at a science writer's seminar, a letter and brochure mailed to 13,000 physicians, and editorials for several journals. As of the middle of April, there was an upswing in recruitment.

The statistical center of the Cooperative Glaucoma Study at George Washington University was awarded a contract to hire a statistician to analyze the study's data collected for 13 years. The Section will maintain liaison with the biostatistician in the analysis of the data.

The Section provided biometric consultation to the staff of the statistical center of the Cooperative Retrolental Fibroplasia Study. Data collection has been completed and a report is in preparation.

The Section continued biometric collaboration and consultation with investigators in the NEI Clinical Branch. Drs. Robert Brown and Walter Stark studied soft lens treatment of bullous keratopathy and found that corneal neovascularization increased with soft lens wear; a manuscript has been prepared for publication. Dr. William Sullivan is developing a trial of N-acetyl-L-cysteine in the treatment of keratoconjunctivitis sicca.

Fred Ederer served on the Retinal Task Force and on contract review committees for studies of glaucoma and nerve bundle fibre defect.

Serial No. NEI (BE) 72-105

1.
2. Section on Clinical Trials and Natural History Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Statistical Consultation, Diabetic Retinopathy Study

Previous Serial Number: Same

Principal Investigator: Fred Ederer (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: Seventeen medical centers in the United States

Man Years:

Total:	0.6
Professional:	0.6
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; insure uniformity of terminology and definitions and standardization of methodology; monitor 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, as ex officio member of the Executive Committee, voting member of the Data Monitoring Committee, Executive Secretary of the Policy Advisory Group, and

member of the site monitoring team.

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. Through the end of March, 192 patients had entered the study, an average of only 2.3 patients per month since the start of recruitment. However, there were large increases in patients recruited during March and the first half of April 1973, giving promise that the minimum quota of five patients per month would soon be reached by most Centers. This increase followed several national publicity efforts and site monitoring visits to all 16 Clinical Centers from members of the Executive Committee in early 1973.

Critical to the success of the study, after an adequate number of patients is recruited, is the prevention of dropouts, which include patients who fail to return for periodic examinations and those who receive treatment in the control eye. This aspect 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, it is uncertain what the value of the treatment is.

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

Honors and Awards: None

Publications: None

Serial No. NEI (BE) 73-128

1.
2. Section on Clinical Trials and Natural History Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Clinical Trials, Diabetic Retinopathy and Photocoagulation

Previous Serial Number: None

Principal Investigator: Fred Ederer

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.1
Other:	0.2

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.

Only four controlled studies of photocoagulation in the treatment of proliferative diabetic retinopathy have been reported. In these studies, one eye was treated and the fellow eye observed as a control. In none of these studies was the eye to be treated selected randomly, and none of the reports specified a defined procedure for allocating eyes to treatment or control groups. Treated and control eyes were not necessarily similar before treatment. However, three of the reports present visual acuity data before treatment and at last follow-up on individual patients. This makes it possible to analyze the data in like subgroups according to pretreatment visual acuity, which corrects for dissimilarity between treated and control eyes in visual acuity, but not for other differences that could influence outcome, such as fundus appearance. While

there is no substitute for randomization, this approach corrects in part a major defect of the published results.

Methods Employed: A punch card has been prepared for each patient in the three studies. Computer analyses have been generated which divide the data into like subgroups according to pretreatment visual acuity. Visual acuity is treated as a quantitative variable scaled in several different ways. Results were then averaged across subgroups using common weights for the treated and untreated eyes. This method is similar to that used by biostatisticians and demographers to standardize death rates according to age.

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 carefully re-evaluate currently available information to achieve the best possible scientific assessment of the value of photocoagulation in diabetic retinopathy -- and that is the objective of this project.

Proposed Course: The results will be analyzed with visual acuity as the response variable and a report for publication will be prepared.

Honors and Awards: None

Publications: None

CONTRACT NARRATIVE
Office of Biometry and Epidemiology
Section on Clinical Trials and Natural History Studies

Title: Prevalence of Visual Field Loss, Kaiser Research Foundation

Principal Investigator: Fred Ederer

Other Investigators: Harold A. Kahn
Gary Friedman, M.D.
Abe Siegelaub

Cooperating Units: Kaiser Research Foundation

Man Years:

Total:	.20
Professional:	.10
Other:	.10

Groundwork has been laid for a possible study of visual field loss in collaboration with the Kaiser Research Foundation in Oakland, California. The persons to be surveyed were aged 40-59 when they participated in the 1964-65 Kaiser Health Plan San Francisco Bay Area multiphasic screening examination, which included tonometry. A sample of some four thousand persons would be called back for perimetry. The objective is to study the relationship between field loss development and baseline intraocular pressure (IOP), drug usage, glucose tolerance, blood pressure, etc., in an attempt to identify factors prognostic of field loss.

Before deciding whether or not to proceed with the main study, a 2-stage feasibility study is planned. If the results of either stage are unsatisfactory, the main study will be abandoned. The first feasibility stage calls for a classification of IOP by age and sex to determine whether adequate numbers in various IOP groups are available for study. The second feasibility stage is to call back a probability sample of 100 persons for perimetry to evaluate the dropout and cooperation rate. Even if the main study is found to be not feasible, the data obtained in the first stage, IOP distributions by age and sex and left-right IOP correlation coefficients, will in themselves be valuable.

Proposed Course: The Kaiser Research Foundation has submitted the data required for the first stage. These data will be analyzed and prepared for publication. The feasibility of proceeding to the second stage will be determined.

Section on Ophthalmic Field and Developmental Research

Office of Biometry and Epidemiology

During the past year, the main emphasis of this Section was devoted to twin studies. Our local Twin Register for Eye Examinations was maintained through continued interaction with all members.

Active Investigations:

A three-year twin study on the effect of treatment on the progression of myopia has now reached the midway point. In this study, the progress of myopia among individual twins receiving special treatment will be compared with the progress among their cotwins who wear standard spectacles.

Primary findings from the twin study on heritability of the influence of topically applied corticosteroid drugs on intraocular pressure are now being published. This investigation was designed to examine a popular hypothesis that the ocular hypertensive response to topical steroids 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, which were at variance with a widely accepted genetic hypothesis, were subjected to widely solicited expert review during the past year and detailed manuscripts were prepared for publication.

A review of the modern world literature on methodology of twin heritability studies was undertaken in preparing an interpretive description of theory, applications and limitations of thisinvestigative model, based on fundamental principles of population study design. The findings of this project will appear as an invited chapter in a forthcoming textbook on Genetic Aspects of Ophthalmology.

Collaborative twin studies presently underway include a study of finger and palm prints in relation to twin zygosity (HD-CD8(c)) and a study of X_g^a blood group incompatibility in fetal loss (HD-CD35(c)) both being undertaken in collaboration with the Children's Diagnostic and Study Branch of the NICHD and a study of the heritability of cardiovascular disease risk factors in cooperation with the Epidemiology Branch, NHLI.

The final report on a contract to support a feasibility study of television ophthalmoscopy was received and extensively reviewed during the past year. This project was undertaken to assess the usefulness of current methodology in providing an electronic image of the ocular fundus in a format amenable to direct computer analysis for use in epidemiologic studies and in basic research.

A review of the risk of retinal detachment occurring among individuals having myopia was undertaken, in response to the needs of the Division of Commissioned Officer Personnel, U.S. Public Health Service. The relative risk of retinal detachment with increasing gradations of myopia was defined, based on available literature. The conclusions of this investigation were found to lend a valuable

perspective for our internal program planning.

Preliminary Investigations

Preliminary studies were undertaken in project areas under consideration as full scale investigations. Data regarding the ocular hypertensive response to topically applied corticosteroids which were collected by the Allergan Pharmaceutical Corporation were analyzed in collaboration with that corporation. Although originally collected for another purpose, these data may provide a useful assessment of reproducibility of the steroid response among individuals, a question having direct bearing on the inheritance of this phenomenon.

Twin data collected earlier by this Section, on measurements of cup/disc ratio were reviewed. Cup/disc ratio is a measurement of features of the optic nerve head which are important in the diagnosis and management of chronic simple glaucoma. Preliminary data analysis was undertaken to provide an assessment of heritability of the cup/disc ratio.

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, HSMHA, PHS, 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 National Institute of Dental Research, and Department of Ophthalmology, University of Illinois.

Serial No. NEI (CF) - 70 E 001

1.
2. Section on Ophthalmic Field and Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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:	0.5
Professional:	0.1
Other:	0.4

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 by newsletter and telephone. Re-examinations were provided for some members.

Major Findings: This register has provided a source of subjects for numerous studies described in earlier reports.

The following investigations are currently being undertaken in collaboration with other Institutes: (1) a study of fetal loss associated with X_g^a blood type incompatibility between mother and offspring, undertaken in collaboration with the Children's Diagnostic and Study Branch, National Institute of Child Health and Human Development, (2) a study of the heritability of cardiovascular disease risk factors in cooperation with the Molecular Disease Branch, National Heart and Lung Institute, (3) a study of the criteria for determining the zygosity of twins on the basis of fingerprint data, being undertaken in collaboration with the Children's Diagnostic and Study Branch, NICHD.

Studies currently underway by this Section are: (1) the effect of treatment on the progression of myopia and (2) heritability of the effect of corticosteroids on intraocular pressure.

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.

Honors and Awards: None

Publications: These appear under individual project reports.

Serial No. NEI (CF) - 70 E 004

1.
2. Section on Ophthalmic Field and Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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:	0.6
Professional:	0.2
Other:	0.4

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 cotwin 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 cotwin 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 re-examined at 6 month intervals. Participants havenow been followed for 1-1/2 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 day-time 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 be re-examined at least twice per year for three years or until such earlier time that statistically significant treatment effect might be demonstrated.

Honors and Awards: None

Publications: None

Serial No. NEI (BE) 73-127

1.
2. Section on Ophthalmic Field and Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Methodology of Twin Heritability Studies

Previous Serial Number: None

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:	0.25
Professional:	0.10
Other:	0.15

Project Description:

Objective: 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.

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, 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 Aspects of Ophthalmology. Boston, Mass., Little, Brown (in press).

Feinleib, M.: The Twin Heritability Study. Part II. Analysis and Interpretation of Data. In Goldberg, M.F. (ed.): Genetic Aspects of Ophthalmology. Boston, Mass., Little, Brown (in press).

Serial No. NEI (BE) 73-146

1.

2. Section on Ophthalmic Field and Developmental Research

3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Association between Myopia and Retinal Detachment

Previous Serial Number: None

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

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.05
Professional:	.05
Other:	.00

Project Description:

Objective: To assess the risk of retinal detachment occurring among individuals having various gradations of myopia.

Methods Employed: A critical analysis of the literature pertinent to this subject was undertaken.

Major Findings: The risk of retinal detachment is greater among myopic individuals than among non-myopes. Approximately one-third to two-thirds of all spontaneous retinal detachments are reported to occur among myopes. Differences in the reported prevalence of myopia among subjects with detachment appear to be due in part to an inconsistent definition of "myopia" used by various investigators. Further, many studies were not based on a defined denominator population "at risk." Reports which give a relative risk of detachment in association with various gradations of myopia described a strong and relatively linear increase in the risk of detachment up to approximately 10 diopters of myopia. Among individuals having myopia in an amount of 10 diopters, the relative risk of detachment appears to be more than 10 times as high as it is among subjects with no refractive error. Beyond 10 diopters of myopia the relative risk of detachment increases even more rapidly.

Significance to Biomedical Research and the Program of the Institute: The findings of this assessment were of immediate use in the formulation of policy regarding physical qualifications for commissioning in the United

States Public Health Service. They have also been useful to the Institute in assessing the value of further research on refractive errors.

Proposed Course: Completed. A report was prepared for the Division of Commissioned Officer Personnel, USPHS.

Honors and Awards: The principal investigator received a letter of commendation for this work from Assistant Surgeon General USPHS, Director of Commissioned Personnel.

Publications: None

Serial No. NEI (CF) - 70 E 002

1.
2. Section on Ophthalmic Field and Developmental Research
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

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 (FERS 1)

Man Years:

Total:	0.55
Professional:	0.30
Other:	0.25

Project Description:

Objectives: To assess the role of genetic factors in determining the intraocular pressure response caused by topical application of corticosteroid eye drops. Humoral and metabolic factors which may correlate with the steroid response are also being studied.

Methods Employed: A sample of 79 pairs of monozygotic and like-sex dizygotic twins over 15 years of age 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 as cited in the last annual report. 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, the findings of this study were widely circulated for expert critical review and detailed manuscripts were prepared for publication.

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: The primary findings of this project have been published or are in press. The relationship between steroid response and other clinical laboratory measures which relate to the monogenic theory of inheritance will be assessed.

Honors and Awards: None

Publications:

Schwartz, J.T., Reuling, F.H., Feinleib, M., Garrison, R.J., and Collie, D.J.: Twin heritability study of the effect of corticosteroids on intraocular pressure. J. Med. Genet. 9: 137-143, 1972.

Schwartz, J.T., Reuling, F.H., Feinleib, M., Garrison, R.J., and Collie, D.J.: Twin heritability study of the corticosteroid response. Trans. Am. Acad. Ophthalmol. Otolaryngol. 77: 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 I: Frequency distribution of pressure response. Am. J. Ophthalmol. (in press).

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. AMA Arch. Ophthalmol. (in press).

CONTRACT NARRATIVE
Office of Biometry and Epidemiology
Section on Ophthalmic Field and Developmental Research

JEFFERSON MEDICAL COLLEGE OF PHILADELPHIA (HSM 110-69-185)

Title: Television Ophthalmoscopy Development: Feasibility Testing for Geometric and Temporal Studies

Contractor's Project Director: Thomas Behrendt, M.D.

Amount Outstanding and Conveyed in FY 1973: $23,000

Objectives: This is a development project undertaken to assess the usefulness of current methodology in providing an electronic image of the ocular fundus in a format amenable to direct computer analysis for use in epidemiologic studies and in basic research.

Method: In the past, an extensive review by the National Center for Health Services Research and Development of overall system requirements was sponsored by this Section, following which a contract was placed with the Department of Ophthalmology, Jefferson Medical College, to support a feasibility study of electronic scanning of the optic nerve head for the purpose of measuring cup/disc ratio. This particular measurement, an assessment of geometric characteristics of the optic nerve head, is important in the diagnosis and management of chronic simple glaucoma. Requirements of the feasibility study contract included an assessment of hardware requirements, hardware system design, hardware system fabrication, clinical trials and final recommendations of technical requirements and specific applications.

Findings: The final report of the contractor was received and reviewed and an extensive report on the project was prepared by the Section Head during this past year. The contractor submitted specifications which would be required of hardware systems in order to accomplish the desired clinical measurements. He also assembled hardware components, designed and fabricated necessary interfacing and conducted clinical trials for a system of geometric measurements. It was concluded that image quality was presently inadequate to accomplish the test measurements under the condition of clinically acceptable light levels, even though the optimum transducer component presently available was selected for this system. The contractor recommended future employment of improved transducer components which are coming available. The remainder of the currently available hardware system was found satisfactory both for the intended purpose and for alternative applications.

Significance to Biomedical Research and the Program of the Institute:

In this first project ever undertaken to assess feasibility of fundus scanning devices in a clinical context, the contractor started essentially from "ground zero," accordingly the observations made comprise extensive new information heretofore unavailable. This new information should be most useful to the NEI in its planning in the area of instrumentation development. Successful

instrumentation of this type would have immediate and direct application in clinical research, clinical management and population screening.

Proposed Course: Completed. Further work towards this objective will be considered when improved components are available.

OFFICE OF INFORMATION

ANNUAL REPORT
OFFICE OF INFORMATION
NATIONAL EYE INSTITUTE
July 1, 1971 - June 30, 1972

A series of new publications and a number of cooperative projects involving other Institute programs accounted for a large proportion of the Office's activities during the year. A closer tie-in of Information Office objectives with those of the Institute as a whole resulted from the development of an NEI Communications Plan which was submitted to the Office of the Assistant Secretary for Public Affairs, HEW.

PUBLICATIONS

A booklet on cataract and a series of short fact sheets on various eye diseases and conditions were prepared to answer the large amount of requests from the public for information of this type. Quantities of these publications were also distributed to a small group of local and national health organizations. The fact sheets, covering glaucoma, diabetic retinopathy, corneal disease, retinal detachment, macular degeneration, retinitis pigmentosa, and refractive errors, will be used until more detailed booklets on these subjects can be prepared in coming years.

The Office provided assistance to the NEI Extramural and Collaborative Programs (ECP) in publishing a series of fact sheets on NEI grant programs, and to the NEI Office of Biometry and Epidemiology (OBE) for Statistics on Blindness in the Model Reporting Area, 1969-1970.

Working with Dr. Matthew Davis, Chairman of the Executive Committee of the NEI-supported Cooperative Diabetic Retinopathy Study, and Fred Ederer of OBE, the Office helped in the preparation and distribution of material to aid patient recruitment for the DRS. Mailing lists for a letter about the Study addressed to practicing ophthalmologists and internists specializing in diabetes were obtained. The Office also coordinated the preparation of a manuscript for a booklet describing the Study in more detail and arranged for its design and layout. Camera-ready copy was then turned over to the DRS Coordinating Center for printing and distribution.

A newsletter, 20/20, was inaugurated in cooperation with ECP staff. Its purpose is to improve communication between NEI and the vision research community, particularly NIH grantees and contractees.

At the request of the Office of Consumer Services, HEW, the Office planned and had drafted under contract, a manuscript for a consumer booklet on eye care. The booklet is to be one of a series prepared by the Department on health topics. By the end of the fiscal year, the final draft was ready for review by NEI staff.

Supplies of two publications inherited from NINDS, "Eye Research" and "Vision and Its Disorders", were exhausted during the year. Distribution of remaining copies of "Security is an Eye Patch" and the NINDB Monograph,

"Refractive Anomalies of the Eye", continued in answer to a steady number of requests. The following number of publications were distributed:

Cataract	53,000	Refractive Errors	750
Diabetic Retinopathy	1,225	Corneal Disease	1,150
Retinitis Pigmentosa	850	Retinal Detachment	900
Glaucoma	1,325	Macular Degeneration	950
Security is an Eye Patch----------			59,600
Statistics on Blindness in the Model Reporting Area, 1969-1970----------			2,445
Refractive Anomalies of the Eye----------			25

The volume of inquiries from the public received by the Office doubled over the previous year, and telephone inquiries more than doubled. Approximately 650 letters from private citizens were received which required an individually-written response. Among the most commonly asked questions were those concerning the phacoemulsification technique for removing cataracts, the use of the laser in treating diabetic retinopathy and glaucoma, the role of nutrition in eye diseases, news reports concerning marijuana's effect in lowering intraocular pressure, use of the visually evoked response (VER) in diagnosing children's eye disorders, the soft contact lens, vision substitution systems, and the NEI budget.

The Office replied to 33 controlled, written Congressional inquiries. Approximately 1800 telephone inquiries were handled during the year by the Information Office staff.

PRESS RELATIONS

Seven press releases were prepared: the enrollment of patients in the Cooperative Diabetic Retinopathy Study, the beginning of the Framingham study of eye disease, the appointment of new members to the National Advisory Eye Council, Dr. Kupfer's receipt of the Secretary's Special Citation, Dr. Ballintine's appointment as Clinical Director, announcement of the cataract booklet, and announcement of the disease fact sheets. The release on the Diabetic Retinopathy Study was part of a cooperative press relations effort between the Office, the NIH-OD Office of Information, and the information offices of the institutions involved in the Study to publicize this project and to aid in the recruitment of patients. As a result newspapers and radio stations across the country carried the story. A news tape carrying the voice of Dr. Robert Frank, NEI Clinical Branch, describing the objectives of the Diabetic Retinopathy Study was used by some of the radio stations.

The Office assisted press representatives from the New York Times, Washington Post, U.S. News and World Report, Better Homes and Gardens, the Blue Sheet, Toledo Blade, Changing Times, Mutual Broadcasting Co., Associated Press, Popular Science, Environmental Health Newsletter, Woman's Day, Family Health, and Video Methods in preparing stories on vision and visual disorders. A news conference held by the visiting Soviet ophthalmologist, Dr. M.M. Krasnov, to discuss his "laseropuncture" technique for treating chronic glaucoma resulted in a number of press inquiries to the Eye Institute. The

resulting news stories carried across the country quoted Dr. Kupfer's views on the new procedure.

Four radio spot announcements on Institute programs were prepared for the NIH health features for radio service and four columns were written for the NIH Search for Health weekly newspaper service.

MISCELLANEOUS

In cooperation with OBE, we complied with a request from the National Health Education Committee (Lasker Foundation) to update the eye disease portion of their fact book, "Facts on the Major Killing and Crippling Diseases in the United States". With the assistance of ECP, the Office prepared a special listing of NEI grants for inclusion in Blindness, the Annual Report of the American Association of Workers for the Blind. Together with NEI administrative management, the Office helped prepare various budget and program planning documents, including Blueprints, 1975-1979 Forward Plan, and the Director's Opening Statements to the House and Senate appropriations hearings. The original draft of the report of the Institute-convened Cornea Task Force was edited by the Office and submitted for publication in the journal, Investigative Ophthalmology. Research Highlights of 1972 were prepared in collaboration with ECP. The annual Save Your Vision Week and White Cane Safety Day Presidential Proclamations were prepared. In addition, two draft Presidential messages were submitted and a message for the Secretary and one for the Assistant Secretary for Health was drafted. An editorial on glaucoma for Secretary Weinberger was prepared at the Department's request. NEI's contributions to the NIH Almanac, HEW Annual Report, NIH Brochure, and NIH Current Clinical Studies booklet 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 speeches and presentations before the Association of University Professors of Ophthalmology, Association for Research in Vision and Ophthalmology, National Society for the Prevention of Blindness, and the Secretary of HEW's staff meeting. The Office also helped in the preparation of an article by Dr. Kupfer on the NEI which was published in the American Journal of Ophthalmology.

EXTRAMURAL AND COLLABORATIVE PROGRAMS

STATEMENT OF THE ASSOCIATE DIRECTOR FOR
EXTRAMURAL AND COLLABORATIVE PROGRAMS

A. Funding of Research and Training Grants

1. Training Programs

Consistent with the Administration's policy to rely on general resources for aid to students, the training programs of the Institute are being phased-out. No new fellowship awards have been made that were not committed prior to January 29, 1973. Under the phase-out of training grants, all on-going programs and continuing commitments will be funded through the balance of the current commitment to trainees, but no new grants will be made.

2. Research Grants

With regard to funding of research grants, the Institute is not negotiating awards on an across-the-board percentage basis. It is, however, seeking to support the largest possible number of quality research projects through prudent management of available resources. In addition, funding of the Cooperative Diabetic Retinopathy Study has been transferred from research grants to utilization of funds appropriated for research contracts. This decision is not only consistent with the NIH position on funding of fixed protocol collaborative studies, but has also resulted in freeing up additional funds to regular research grants.

The following table indicates the funding distribution of the total extramural research budget for FY 1973:

EXTRAMURAL RESEARCH FUNDS DISTRIBUTION: FY 1973*
(Dollars in Thousands)

	Number of Awards	Amount
A. Research Grant Funds		
- prior year commitments	287	$16,619
- competing renewals	44	2,678
- new	74	2,991
- supplementary awards	(10)	145
subtotal	405	$22,433
B. Contract Funds		
- Cooperative Diabetic Retinopathy Study	12	965
- Glaucoma and Retinal Research Program	9	1,235
subtotal	21	$ 2,200
TOTALS	426	$24,633

* Estimate: May 25, 1973

B. Program Development

The Institute has continued to emphasize support for individual research projects through the traditional project grant and center grant mechanism. In addition, efforts have been initiated to further encourage the application of scientific knowledge to specific disease problems. In pursuit of this objective, Institute staff, with the advice and assistance of the Vision Research and Training Committee, is developing guidelines oriented to strengthening the clinical research center program. Although awards for clinical research centers must necessarily be limited in number, the Institute hopes through this program to: focus research resources, facilities and manpower on particular disease problems; encourage interaction and collaboration between clinical and laboratory investigators; and facilitate the application of laboratory research findings to clinical problems.

C. Communications

With the assistance of the NEI Information Office, extramural program staff have attempted to increase the availability of information regarding NEI research programs, and solicitation, review and award procedures through publication of program brochures, articles and announcements in scientific journals, and the NEI newsletter. Communications have also been enhanced through staff visits to grantee institutions, discussions with grantees at NIH, and through staff availability at the Association for Research in Vision and Ophthalmology meetings.

In addition, extramural program staff have represented the Institute at national meetings of the Association of University Professors of Ophthalmology, the American Academy of Optometry, the Association of Schools and Colleges of Optometry, and the National Optometric Association.

D. Staff Changes

During the past year, Mr. James G. Culp was appointed Chief of the Contracts and Grants Branch of the extramural programs. Mr. Culp was formerly Budget Officer for the Institute, and prior to joining the NEI, gained extensive administrative experience while an NIH management intern and as an administrative officer in the National Cancer Institute.

George T. Brooks, Ph. D.

ANNUAL REPORT
July 1, 1972 through June 30, 1973
EXTRAMURAL PROGRAMS

PROGRAM REPORT

Objectives of the National Eye Institute extramural research programs are to improve the diagnosis and treatment of eye diseases and disorders of the visual system and to develop the knowledge required for prevention of eye diseases and rehabilitation of the blind and partially sighted. In seeking to achieve these objectives, the Institute supports a broad array of clinical and laboratory research projects. Investigations range elucidating basic biological phenomena underlying disease processes and visual function to development of specific diagnostic or therapeutic techniques. Although not every project supported is represented in this report, this section summarizes the overall progress of extramural research supported by the NEI during the past year. The report is organized in the following manner:

I. CORNEAL DISEASES

II. INFECTIOUS AND AUTOIMMUNE DISEASE

III. AQUEOUS HUMOR DYNAMICS AND DISORDERS

IV. LENS DISORDERS

V. LOW VISION DISORDERS, CARE AND REHABILITATION

VI. TUMORS AND SYSTEMIC DISEASES

VII. LIGHT AND RADIATION EFFECTS

VIII. MACULAR DEGENERATIVE DISEASES

IX. RETROLENTAL FIBROPLASIA

X. PHOTORECEPTOR DEGENERATIVE DISEASE

XI. DIABETIC RETINOPATHY

XII. NEURO-SENSORY DISORDERS

CORNEAL DISEASES

EDEMA

PROBLEM

Corneal edema is usually seen clinically as a clouding of vision and is often painful. Though accompanied by swelling of the stroma, these symptoms are mainly due to the accumulation of fluid in the epithelial layer of the cornea. In general, this edema is though to result from loss of function of the endothelium and not from metabolic defects of the epithelium. The normal cornea maintains a fairly constant thickness with a water content of about 75 percent. However, the corneal stroma has a marked affinity for water, and when immersed in solutions, it absorbs and holds large quantities of water. This turgescence is always accompanied by loss of transparency. The mechanism which keeps the cornea deturgesced was first thought to be an osmotic one, but later evidence indicated that metabolic processes are involved. Many investigators in this field believe that the major role in controlling corneal hydration is played by an active pump in the endothelium and that the epithelium is merely a passive barrier, while another school believes that the epithelium is the layer that controls hydration.

Current therapy is directed at replacing the faulty endothelium with a healthy layer, by means of a full thickness corneal transplant. In cases where a transplant is unsuccessful, attempts have been made to replace the barrier function of the endothelium by grafting a plastic membrane into the rear surface of the stroma. Factors such as evaporation from the corneal surface presumably must then be operating to reduce the corneal edema. Such membranes have proved unsuccessful in the long run probably because they interfere with the movement of nutrients from the aqueous humor to the tissues lying anterior to the membrane. In the absence of means for radical improvement of the condition at the endothelial level, palliative measures may be tried in order to restore the epithelium to its normal physiological state. One such measure that currently shows promise is the fitting of a soft contact lens to the affected eye.

TRANSPORT STUDIES

Investigators at Stanford University have developed methods for the study of fluid transport across the cornea and have shown that this transport can be supported by a very simple medium containing only glutathoine and adenosine in a balanced salt solution. They are further clarifying the metabolic requirements of the pump by systematic substitution of other substances for adenosine and they are developing a method for studying the endothelium in the absence of the metabolic contribution of the stromal cells. Fluid is presumed to move across the endothelium by means of actual mechanical pumping through intercellular channels. At the electron microscopic level, studies are being made of the morphological changes that may be observable when the endothelial layer is pumping normally, and when its metabolism is inhibited by low temperature or by metabolic poisons. The definition of the metabolic requirements of the pump could have profound clinical significance, because at least some forms of corneal edema must be related to abnormal endothelial function.

These studies are also helping to elucidate the mechanism of the coupling between energy production and physiological work.

A group at Yale University, interested in the mechanism for maintenance of transparency, hydration, and sensitivity of the cornea is studying the electrophysiology of various animal corneas. Ultramicroelectrodes are used to measure the electrical potentials of various membranes which are then related to the associated ion fluxes. Their most recent work concerns the chloride pump in the frog cornea, and they now have evidence that this choloride transport is activated by epinephrine and adenosine 3',5'-monophosphate (cyclic-AMP). Currently they are: (1) determining the effect of cyclic-AMP on the short circuit current in the rabbit corneal epithelium with concurrent measurements of corneal hydration; (2) measuring ion fluxes across the epithelium of rabbit cornea to learn if there is a primary activation of the chloride pump or a change in permeability to chloride; (3) studying the fate of implants of opaque connective tissue in cornea and of implants of cornea in opaque connective tissue by electron microscopy and observations of transparency; and (4) studying the effect of a number of pharmacological agents on corneal hydration and transport.

Characteristic features of the endothelial pump are of interest in many laboratories. For example, at Columbia University investigators have found an electrical potential difference of 0.5mV across rabbit corneal endothelium in addition to the reported transport of fluid. Inhibitors such as ouabain, cyanide and iodoacetate are known to suppress fluid transport and were found to abolish the potential difference. Both fluid transport and potential difference are depressed reversibly either in the absence of bicarbonate, sodium or potassium ions or in the presence of cytochalasin B. The suggestion is made that in this system the potential difference is associated with fluid transport, and some possible mechanisms for this association are being studied.

While epithelial metabolism is clearly important in causing these cells to swell, its significance in the control of stromal swelling has been disputed. Investigators at Oakland University have established that active transport of sodium ions across the epithelium is not critical in maintaining normal stromal hydration in the intact cornea and that the primary role of the epithelium is a passive one, as a barrier preventing access of fluid to the stroma from the tear film. A heat labile macromolecule synthesized in the endothelial cells has been purified and shown to be a negatively charged compound, most probably glycopeptide in nature. It binds cations which suggests its possible role in fluid transport. Its yield is increased when ouabain, which promotes swelling of the cornea, is included in the incubation medium.

Amino acids have been shown to be concentrated in the cornea by active transport from the aqueous humor and glycolysis in the cornea was shown to contribute significantly to the lactate level in the aqueous humor.

An understanding of the ways in which fluid is transported across the endothelium and in which appropriate permeabilities of the epithelium and endothelium are maintained is significant for the management of corneal problems. With knowledge of the processes which determine normal function (and hence transparency) appropriate surgical and medical treatments can be selectively

and effectively applied. A particular example would be the importance of knowing the nutritional sources and reserves in corneas treated by means of impermeable prostheses which could interrupt the flow of metabolites. Therapy could be improved by appropriate delivery of additional metabolites beyond the limiting implant, e.g., of oxygen to the aqueous in the case of glued on contact lenses or of amino acids and glucose in the case of artificial endothelia.

TRANSPARENCY STUDIES

The application of physical optics to the diagnosis of early structural changes in corneal tissue provides an accurate and quantitative method of determining the effectiveness of treatment for corneal disease. The difference in refractive index between corneal epithelium and air provides the major refractive power of the cornea. The imaging quality of the eye can therefore be seriously affected by optical irregularities which occur with epithelial edema.

The stroma is composed of layers or lamellae of collagen fibers which are the major structural element of the cornea. It has been shown that if the collagen fibrils scattered light independently of each other, the transmission of the human stroma would be only about six percent. Investigators at Massachusetts Institute of Technology and at Stanford and Johns Hopkins Universities have found that the transparency of the stroma can be explained if the correlation of the scattered light from the individual fibers is considered. They have shown that in the normal cornea, most of the light scattered from the individual fibrils is minimized because of destructive interference so there is only a small net amount of light scattered from the individual fibrils at angles of greater than a few degrees from the incident direction. Structural changes in the stroma or epithelium can cause the loss of this correlation in the positions of the fibrils and result in light being scattered at large angles from the incident direction so that the cornea appears cloudy. Electron micrographs of edematous corneas have shown the presence of lakes or small fluid filled regions distributed throughout the stroma. The presence of these lakes, which are comparable in size to the wavelength of the visible light, is sufficient to destroy the correlation between the relative positions of the fibrils. This correlation is required in order to maintain corneal transparency. At Johns Hopkins University swelling of corneal tissue was induced by storage in a cold solution. As it swelled, the orderly arrangement of the fibrils was disrupted and the cornea assumed a milky appearance. When the fibrils were examined under the electron microscope, areas were found completely devoid of fibrils (the so-called lakes). This was confirmed by measuring the scatter of light through such swollen corneas at different wavelengths. In the swollen cornea, the light scattering is much more intense than it is in the normal cornea, but it is less dependent on wavelengths. These investigators calculated how light at different wavelengths would be transmitted through an arrangement of "rods" in a swollen cornea and found that these calculations agreed with the observed values they could measure. These results seem to indicate that it is the disordered arrangement of the "rods" that is responsible for the aberration in light transmission. These findings are seen as a possible tool for predicting the onset of corneal disease and as a guide for determining the basis for clinically

significant losses in corneal transparency that may occur in corneal disease.

INJURY AND WOUND HEALING PROBLEM

Since many corneal diseases and postoperative complications of corneal surgery are characterized by destruction of stromal collagen with resultant ulceration, the fact that enzymes released from the corneal epithelium may cause these ulcerations opens up exciting possibilities of therapy because inhibitors of the enzymes may be available.

COLLAGENASE STUDIES

Groups at the Retina Foundation and at Cornell University have demonstrated a collagenolytic enzyme in burned and otherwise diseased corneas and have shown that corneal healing can be hastened by the use of inhibitors of the collagenase. This work has been done in tissue culture, in experimental animals, and data are also available on clinical studies. In vitro studies indicate that cysteine is the best collagenase inhibitor available.

According to investigators at Cornell University the enzymatic breakdown of the non-alkali burned cornea probably involves enzymes other than collagenase. Lysosomal lysates of polymorphonuclear leucocytes can also degrade corneal proteoglycan as shown by a reduction in its specific viscosity. It is suggested that the elaboration of a proteoglycanolytic enzyme, initiates a preliminary step in the process of selective enzymatic tissue destruction. The last step then would be collagenase production and destruction of the unprotected collagen.

Preliminary investigations have shown that, if dexamethasone is added to serum-free tissue culture media containing human skin and rabbit cornea fibroblasts, a collagenase is elaborated into the media. This evidence directly implicates the fibroblast as a collagenase producer and also shows that this production is under the influence of corticosteroids. Further studies can now be done on the effect of proteolytic enzymes in wound healing and inflammation using this system as a model.

Clinical studies with collagenase inhibitors for the treatment of the alkali burned cornea have been rewarding. The results of treatment of ulcers of the non-alkali burned eye are more difficult to evaluate. Results of a double blind study at the Retina Foundation, Boston, indicated that calcium EDTA (another inhibitor) has some effect in preventing corneal ulcerations resulting from causes other than alkali burns from progressing. These studies are continuing in an effort to find more potent and perhaps synergistic inhibitors which will be more effective in preventing corneal ulcers.

CELLULAR CONTROL STUDIES

Basic studies of the mechanism and control of wound healing in the cornea are underway at the University of Oregon. An organ culture system for whole rabbit corneas in chemically defined, synthetic media has been developed which enables corneas to be maintained in culture for several days under completely

controlled environmental conditions with maintenance of the normal thickness and transparency. Several standardized models of corneal injury have been developed for the study of difference aspects of corneal wound healing. Results obtained with these models show: (1) that blood monocytes serve as an important source of new connective tissue cells in the healing wound; (2) that injury to the epithelium stimulates both protein and RNA synthesis in the epithelium within four hours after injury; (3) that low concentrations of proteolytic enzymes (trypsin and pronase, but not a chymotrypsin) stimulate the activation and transformation of corneal stromal cells to fibroblasts; both trypsin and pronase, but not chymotrypsin, at slightly higher concentrations cause total opacification of the cornea. Growth factors which profoundly stimulate the growth of all cell layers of the cornea have been isolated and partially purified from other tissues. Two of these growth factors are highly potent in the specific stimulation of endothelial and corneal stromal cell growth.

UVEITIS

PROBLEM

Endogenous uveitis is the term used to describe a nonpurulent inflammation of the eye. Uveitis implies that the primary site of the inflammatory reaction is in the uveal tract but in many instances this is not the case; the causative organisms or antigenic stimulus may be located in the retina, the lens, or the vitreous. In still other conditions the primary site of the inflammation may be in the perivascular tissues, and finally many layers of the eye may be involved simultaneously. It is obvious that uveitis is not a single entity, but an inflammation of the ocular tissues that can be caused by many agents and factors and the clinical pictures are not always clear cut.

EPIDEMIOLOGY

The epidemiology of uveitis has not been thoroughly studied in this country and the importance of uveitis as a socioeconomic factor is not known at the present time; for in some instances it may cause a loss of one eye or a partial impairment of vision in both eyes, rather than legal blindness. There are many cases where the uveal inflammation is the secondary cause of blindness, for example, following cataract extraction, operation for glaucoma or a corneal infection. A group at the University of California, San Francisco, is in a good position to provide information on many of the unanswered questions concerning uveitis since they are bringing a comprehensive multidisciplinary approach which includes ophthalmology, virology, epidemiology, and immunology to the problems of external ocular disease. This institution has become an important referral center for cases of uveitis, and probably deals with more cases of uveitis than most other American ophthalmic clinics. Investigators there are observing the natural course of the disease and are assessing the long term value of drug therapy. They are studying the biologic and immunological features of etiologic agents, the clinical and immunological aspects of the host response in man and experimental animals, controlled therapeutic trials in man, and those epidemiological features which may permit improved control of infectious disease.

ETIOLOGY

Much progress has been made in recent decades on the etiologic classification of inflammatory reactions of the posterior uveal tract. These usually take the form of choroiditis or chorioretinitis, most frequently accompanied by the formation of granulomas and a greater or lesser degree of accompanying anterior uveal inflammatory disease. Such posterior lesions are generally accepted as being caused, in the main, by infection by a variety of pathogens, with allergic reactions and the ocular components of systemic disease contributing to the overall picture. In some instances, not only the etiologic agent but also the major aspects of the pathogenesis of the disease process have been described.

In nongranulomatous uveitis of the anterior segment of the eye, however, the factors involved in both etiology and pathogenesis generally remain obscure. As would be expected, when so little is known about the pathogenesis and etiology of a condition, treatment of uveitis is usually empirical. Corticosteroid therapy appears to suppress the uveal inflammation at least in most cases. However, since it is not curative, the eye condition frequently worsens unless the condition is self-limiting.

The objectives of a project at Johns Hopkins University are to study the manner in which the uveal tract is able to support various immunopathologic processes that result in inflammatory disease. It is postulated that in addition to immediate and delayed hypersensitivity mechanisms, the act of antibody formation within the eye may represent an important pathogenetic factor in the development of recurrent anterior uveitis.

In connection with their interest in the molecular nature of the antibodies formed locally in the eye in association with uveitis, the investigators have devoted appreciable time recently toward the development of experimental models involving the implantation into the anterior chamber of various types of secretory tissue. In one instance, autologous lacrimal gland tissue is implanted into the anterior chamber of the rabbit, where it takes up residence on the anterior iris and apparently continues to function. In another instance, autologous terminal ileum is taken during a gut resection in the fetal lamb and implanted into its own anterior chamber, the fetus being employed to assure sterility of donor material. Both of these approaches appear now to offer reasonable hopes of success, the rationale behind the experiment being that while the intraocular tissues normally appear to support the formation primarily of γG and γM antibodies, it is widely established that γA antibodies have a predilection for secretory tissues of the type mentioned above. It is hoped, therefore, that following the induction of immunogenic uveitis leading to local intraocular antibody formation, specific fluorescent antibody techniques will help permit establishment of the immunoblobulin classes of the antibody formed in and around these intraocular implants, thus permitting a study of the conditions controlling the differentiation for immunoglobulin class during antibody formation, and perhaps even a study of the origin of the cells involved in local antibody formation within the eye.

Investigators at Mt. Sinai School of Medicine are currently exploring cellular immunity factors that may be responsible for the initiation or propagation of chronic uveitis. They have demonstrated that leucocytes of patients with uveitis are inhibited from migration when exposed to uveal antigen and in some of these patients when exposed to corneal antigen. In light of present knowledge, this indicates a state of cell-mediated immunity to one or more constituents of normal uvea in uveitis patients and not in normal patients. This finding, in addition to definitely implicating autodirected cellular immunity in the pathogenesis of uveitis, may offer methods of study for the diagnosis and evaluation of therapy in this disease.

At the University of California, Los Angeles, the role of retinal antigens in experimental allergic uveitis is being explored in the following ways: (a) purification of retinal antigens by physicochemical methods; (b) the

characterization of these antigens immunologically; (c) determination of which of these antigens will induce a reproducible model of uveitis in guinea pigs with disease occurring in over 80 percent of the animals; (d) localization of the tissue antigens within the involved eyes using fluorescent microscopy; (e) transfer of the autoimmune disease to normal recipients with sensitized donor cells; and (f) study of the cytotoxic properties of sensitized cells from animals with uveitis.

Because the ocular response produced in this experimental model of uveitis closely resembles the clinical condition, investigators at the Institute of Ophthalmology, London, England, examined aqueous humor taken from patients with acute uveitis for the presence of prostaglandin-like substances. High amounts of such substances were found in samples from the severe cases. These results suggest that raised levels of prostaglandins, possibly released by white blood cells which enter the inflamed eye, may contribute to many of the clinical signs in acute uveitis. It follows that substances which inhibit the action or synthesis of prostaglandins might be of value in treating this disease.

These selected examples of studies on the immunopathogentic mechanisms contributing to uveitis illustrate the kinds of approaches which may lead to newer methods of therapy.

HERPES SIMPLEX

PROBLEM

Of the virus infections of the cornea, herpes simplex keratitis is one of the major causes of corneal disease. While bacterial infections can frequently be controlled by one of several antibiotics, therapeutic agents for viral infection are extremely limited both in number and effectiveness. The course of this disease varies from a relatively mild primary epithelial infection to severe ulceration of the cornea, both of which tend to recur. Disciform keratitis often causes permanent scarring or opacity.

The epidemiology of herpes simplex virus infections points to a long lasting association between the virus and man. Approximately one percent of the population initially infected, manifests a clinical illness and this occurs almost entirely in early childhood. In the remaining population, the infection is inapparent, evidenced only by the presence of circulating antibody. Three quarters of those with antibody develop recurrent eruptions during their lives, elicited by a variety of factors. Between recurrences the virus is latent. The striking feature of this latent infection is its recurrence in individuals who are serologically immune, i.e., those who have circulating antibodies to the infecting virus. Recurrent herpes simplex is one of the major problems in ophthalmology in terms of visual disability. Present evidence indicates that once someone has had an initial attack of herpes, the odds are approximately 25 percent of having another attack within two years. If there has been more than one attack of corneal herpes, the odds are approximately 43 percent of having another attack within two years.

DIAGNOSIS

Diagnostic tests for epithelial herpes simplex include standard tissue culture methods and fluorescent antibody techniques but there is a need for simpler, more rapid techniques which can be used for routine laboratory diagnosis and attempts are being made to develop them.

ETIOLOGY

Herpes keratitis, like all other infectious diseases, is influenced by the balance of virulence of the agent and the resistance of the host. Host defenses that have been studied are serum antibody, secretory antibody, interferon, and the inflammatory reaction. Several of these factors are being investigated at the University of Florida. It has been found that there is immunoglobulin A antibody in tears which is a neutralizing antibody for herpes virus. This antibody can be stimulated in rabbits and man by the topical instillation of dead virus, and the stimulated eye responds more than the non-stimulated. The eyes also vary in their production of herpes antibodies from time to time. Such local antibodies might explain why individuals who get recurrences of herpes have high, stable circulating titers of neutralizing antibody but unknown quantities of locally protective antibody.

Another host mechanism of current interest is prevention of virus disease through interferon. Interferon is a protein made by infected cells which

induces other cells to develop a broad protection against virus disease. Different viruses produce it at different levels and are susceptible to it in different ways. Unfortunately, herpes virus and adenovirus are poor stimulators of interferon, and quite resistant to interferon action. Nevertheless compounds have been found which induce the host to produce interferon. One type is a long chain synthetic molecule which is chemically similar to the genetic material of an infecting virus and can fool the cell into producing interferon. This synthetic RNA has very low therapeutic activity and protects against infection for a limited period of time. Present evidence suggests that this double stranded RNA is much more effective in lower animals than in primates and man.

However, topically applied human leucocyte interferon was found to prevent infection by herpes virus in the owl monkey. There is reason to think that a partially immune person treated with homologous human interferon would demonstrate an even greater interferon effect than the highly susceptible monkey and a clinical trial to test this hypothesis is now being organized.

Another host defense mechanism being explored at the University of Florida in the pathogenesis of stromal herpes is cellular immunity. Measurement of macrophage inhibitory factor, blast transformation, lymphocytotoxicity, and other factors are being studied in guinea pigs infected with herpes. Once the techniques have been worked out, these same measurements will be applied to patients with herpes in order to correlate possible levels of cellular immunity with recurrence of the disease and with the different types of stromal disease.

At the University of California, Los Angeles, elucidation of the immunologic basis for hypersensitivity in herpes simplex keratitis is being sought. It is apparent from observations of investigators there that the hypersensitivity reaction in herpes keratitis involves both cell-mediated immunity and virus antiviral antibody complexes which contribute to the pathogenesis of recurrent ocular infection.

Another broad attack on the basic mechanisms of recurrent herpes simplex keratitis is being made by investigators at New York Medical College who are concerned with the most probable factors involved in recurrence of herpes simplex, e.g., viral replication, interferon mechanisms, the role of circulating local antibody, the role of delayed hypersensitivity, and the role of non-specific types of inflammation.

At Columbia University, work is continuing on the antiviral activity of colchicine and related alkaloids. Preliminary investigation shows that subtoxic levels of some antimitotic agents do have suppresive effects on herpetic corneal ulcers. Because ultraviolet inactivated herpes virus has been shown to produce disciform keratitis in rabbits, there is probably an active toxic component associated with the virus. This component is presumably antigenic and an attempt is being made to fractionate the antigens of herpes simplex virus in order to identify and characterize the toxic factors. This work may provide information which will indicate ways of altering the reactivity of the cornea to the virus, toxic effects. The investigators know that corneal damage secondary to herpetic infection might be due to a combination of toxic

component and the host's immune response, and they are, therefore, including a study of the effect on corneal cells of sensitized lymphocytes.

THERAPY

The anti-viral drug, idoxuridine (IDU) appears to be effective in superficial herpetic keratitis, but usually the response in the case of stromal keratitis is poor. A number of other agents have been tested in animals, specifically trifluorothymidine and adenine arabinoside. Adenine arabinoside is a unique type of antimetabolite. Unlike most, it has little tendency to produce bone marrow deficiency and hematologic abnormalities. In rabbits, it has been shown to be active against herpetic iritis induced by virus placed directly into the anterior chamber. It is active when given either systemically or subconjunctivally, and this activity is seen with no apparent systemic toxicity. IDU and trifluorothymidine which are active topically present relatively little systemic hazard, in part because they are rapidly metabolized. This very property, however, and the rapid breakdown in vascularized tissues makes these compounds relatively unsatisfactory for the treatment of deeper infection. Adenine arabinoside is not only more slowly metabolized, but its major metabolic product, hypoxanthine arabinoside, is also an effective antiviral compound. This substance has been used subconjunctivally in patients at the Massachusetts Eye & Ear Infirmary and although there may be some effect on the disease, the effect is uncertain. Further trial certainly seems justified, however, and even if compounds such as adenine arabinoside and isoprinosine in themselves are not sufficiently active, it is possible combinations of relatively non-toxic substances may permit effective therapy.

AQUEOUS HUMOR DYNAMICS AND DISORDERS

GLAUCOMA

PROBLEM

Glaucoma is a disease of the eye characterized initially by an abnormal elevation of the intraocular pressure. This is followed by an associated loss in the visual field which may lead to 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 diseases, such as diabetes, rheumatoid arthritis and certain inflammatory conditions.

Studies of glaucoma may be divided into two categories, viz.: (a) those which aim to elucidate the mechanism by which intraocular pressure becomes elevated, and (b) those aimed at understanding the mechanism by which intraocular pressure exerts its damaging effects upon the eye. A central issue in most, if not all, glaucoma is adequacy of the outflow channels. 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 and is drained principally from the trabecular meshwork, where it enters Schlemm's canal on its way to the venous circulation. In primary open angle glaucoma, there is an abnormal resistance to outflow but the defect which leads to the abnormal increase is unknown. A second aspect of glaucoma is the damage that it causes in the form of optic nerve fiber loss and the resultant visual field loss. The nerve fibers atrophy in the retina, disc and optic nerve. In addition, the ganglion cells of the retina disappear and 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.

STEROID EFFECTS

The increased intraocular pressure, caused by topical corticosteroids is of considerable importance because steroid glaucoma may be an experimental model of open angle glaucoma and because pressure response to topical corticosteroids appears to be genetically determined. A systematic evaluation of the ocular effects of topical corticosteroids is underway at the New York Medical College where over 600 normal patients are involved. The aim of this study is to learn whether the responsiveness of the aqueous outflow system of individuals to corticosteroids is constant for the individual, to obtain additional knowledge of the responsiveness of corticosteroid glaucoma to antiglaucoma medications as compared to primary open angle glaucoma, and to obtain additional evidence concerning the genetic aspects of corticosteroid responsiveness of aqueous outflow system.

It has been demonstrated by investigators at New England Medical Center and at the University of Alabama that open angle glaucoma patients with and without field loss differ significantly from normals in frequency distribution of levels of initial and final plasma cortisol in response to orally administered

dexamethasone. Thus, a systematic endocrine marker separates patients with glaucoma, especially those with field loss, from normal patients.

Recently, research teams at Washington University and at the University of Uppsala reported preliminary studies which demonstrated that it is possible to detect differences in glucocorticoid sensitivity in systemic cells between normal and primary open angle glaucoma patients. Since ocular tissues are difficult to obtain, these investigators have gone to the white cells of the blood and fibroblasts obtained from biopsies at surgery. Examination of these tissues from the standpoint of inhibition of lymphocyte transformation and fibroblast tissue culture growth, reveals differences between normal and glaucoma patients. These observations are of great interest in that they indicate that we may now have a "biochemical handle" on this disease.

HYPERTENSIVE EFFECTS

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 extraocular vessels. Investigators at Washington University are studying the components of the aqueous flow system in detail in the following types of patients: normal; ocular hypertensive without field loss; ocular hypertensive with early field loss; and patients with normal IOP but visual fields suggestive of glaucoma. Results of the testing at Washington University are being correlated with: (a) the progress of the changes in the visual field; (b) the appearance of the optic disc; and (c) the effect of treatment with drugs. These studies are increasing the understanding of glaucoma, and a critical clinical trial of these techniques is establishing a firm basis for evaluation of effects of drugs and treatments by others.

There has been some evidence from investigators at the Bascom Palmer Eye Institute to suggest that the initial location of glaucomatous damage was the ganglion cell layer of the retina but most of the evidence points to the optic disc. Based on the demonstrations that intraocular pressure affects the blood flow in the region of the optic nerve head, it has been concluded that the mechanism for damage is probably ischemia secondary to interference with blood flow. 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. These changes may result from the increased pressure, from a disorder of the vessels themselves, or a combination of both factors. In any case, 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 has resulted from various histopathologic studies which indicated that at elevated levels of intraocular pressure the capillaries which supply a portion of the nerves fill inadequately. These findings have been confirmed in monkeys using fluorescein angiography, a technique which employs a fluorescent dye to visualize the small vessels around the optic nerve head, combined with a method for inducing a rise in intraocular pressure. So far, all lines of

evidence demonstrate defects in the optic disc or Peripapillary choroidal vasculature at acutely elevated intraocular pressures. However, the relevance of the defects to the pathogenesis of optic nerve damage in glaucoma in man is not clear because the investigations have not been done under conditions of chronically elevated intraocular pressure. With high resolution fluorescein angiography, investigators at New York Medical College have designed studies to provide information as to the relevance of these defects to the pathogenesis of glaucoma. Angiograms are being obtained in normal subjects, in glaucoma patients with irreversible field defects, and in high responders to steroid testing. It is hoped that the detailed knowledge of the relationship between intraocular pressure and the flow of blood through ocular vessels may make possible the treatment of glaucoma based on changes in the circulatory system before there is damage to the optic nerve. Insight into the changes in circulation which precede a deterioration in vision is also being gained by investigators at the University of Chicago.

NEUROLOGICAL EFFECTS

Investigators at the Bascom Palmer Eye Institute at the University of Miami have done considerable work on the normal structure of human and monkey nerves in an attempt to develop an experimental model for glaucoma and optic nerve atrophy and are studying them by the combined methods of ophthalmoscopy, fundus photography, fluorescein angiography, and light and electron microscopy. Recent studies are concerned with acute effects of intraocular pressure on the optic nerve. Intraocular pressure is elevated by means of a manometric system through a small needle into the anterior chamber, and various parameters of optic disc function are being studied. In one experiment, 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. In another series of experiments, intraocular pressure was maintained at certain levels, (correlated with mean ophthalmic blood pressure), for 8 hours, and the animals allowed to recover. The type of permanent changes induced (i.e., irreversible ischemic changes) was determined histologically several weeks later. It appears that nerve fibers, as well as many retinal elements, are unable to survive for 8 hours if the intraocular pressure is as high as mean ophthalmic artery pressure, but the glia do survive at even higher pressures. The implications of these findings is that in glaucoma the glia may be affected by something other than ischemia, and this would be a new concept in current thinking about glaucoma.

NEUROPHARMACOLOGICAL STUDIES

Efforts to improve the adequacy of the outflow channels make use of drugs and chemically mediated-surgical procedures which promote aqueous outflow or reduce formation of aqueous humor; the aim is to compensate for whatever is obstructing the aqueous outflow and causing the pressure to rise. Medications which act predominantly in increasing outflow facility may be classified as parasympathetic (cholinergic) agents, such as pilocarpine and anticholinesterase drugs such as isofluorophate (DFP). An example of a drug which suppresses secretion is the carbonic anhydrase inhibitor, Diamox (acetazolamide).

Investigators at Uppsala University are studying aqueous humor dynamics and its pharmacologic alterations in several species of monkeys. An improved rapid method for perfusing monkey eyes at different pressures and obtaining quickly the coefficient of outflow and rate of production of aqueous humor has been developed. The variations in coefficient that occur with various perfusion pressures and during perfusion with various drugs is correlated with the anatomic features of the filtration sites of the various species. Trained monkeys are being used as subjects for prolonged perfusion experiments so that the factors responsible for the diurnal variation in intraocular pressure and its alteration by autonomic drugs can be investigated without the distortions introduced by anesthesia.

Other studies at Uppsala University include investigations in monkeys of the long term effects of epinephrine and pilocarpine in increasing the coefficient of outflow. Results demonstrate that epinephrine but not norepinephrine increases outflow facility. In order to investigate whether epinephrine occurs physiologically in the eye, a technique has been developed for determination of small amounts of epinephrine in the presence of a large excess of other catecholamines.

Although the clinical value of epinephrine has been recognized for many years, the biochemical mechanism by which catecholamines lower intraocular pressure is unknown. However, 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 is presented from the Department of Opthalmology at Yale University that cyclic-AMP may play a central role in mediating the action of catecholamines on aqueous humor dynamics. 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.

Other studies at Yale University are concerned with a systematic search for adrenergic drugs which have potential use in the treatment of open angle glaucoma. Experimental evidence has suggested that compounds interfering with the re-uptake and binding of norepinephrine and epinephrine may enhance their activity. Compounds interfering with the enzymatic degradation of norepinephrine appear to prolong the action of the drugs. A systematic synthesis and screening program to determine whether the above classes of inhibitors can cause augmented ocular responses to norepinephrine is being conducted.

Because evidence increasingly implicates relative ischemia of the optic nerve as a major factor in field loss, it is important to identify pharmacologic agents which can restore or preserve optic nerve function despite decreased blood supply. Animal studies *in vitro* and *in vivo* have shown that diphenylhydantoin (DPH) can partially reverse the effects of anoxia on heart and nerve cells, and preliminary work at Washington University demonstrated that DPH confers partial protection, *in vitro*, to the rubidium transport system of the rabbit optic nerve subject to anoxia. With this background, a trial was conducted with DPH therapy being given to a small group of patients with

glaucomatous field loss. These clinical results with DPH were encouraging and are now being followed by a carefully controlled study involving 50 patients. A double blind crossover study is being carried out consisting of six months of treatment with diphenylhydantion and six months treatment with a control drug (phenobarbital). A clinical protocol has been designed to include studies of visual fields and other tests of retinal function.

Basic physiology and pharmacology of aqueous humor formation and flow is being investigated at Tulane University by producing a chemical sympathectomy with 6-hydroxydopamine applied topically to the eyes of animals. Intraocular pressure is lowered and outflow facility is elevated. In addition, increased sensitivity to epinephrine follows this sympathetic denervation. These findings suggest that patients with glaucoma might be aided by combining chemical sympathectomy and topical epinephrine therapy. To date, 85 patients who have had chronic recalcitrant or uncontrolled glaucoma, and who were both medical and surgical failures, have been treated by chemical sympathectomy in combination with epinephrine. Results indicate that this treatment may offer an alternative to ocular surgery as a method of treating unresponsive cases.

SURGICAL STUDIES

Surgery is extremely important for relief of pressure in cases of glaucoma which do not respond to chemotherapy. Surgical procedures to facilitate the outflow of aqueous humor must be performed when medical treatment is not effective. 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, investigators 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. By perfusion of intact globes, this research group has discovered several factors in microsurgery of Schlemm's canal that have not previously been recognized, viz.: (1) in the current surgical procedure of probe trabeculotomy ab externo there is strong tendency for channels opened by the surgery to close again, apparently as the disrupted trabecular meshwork tissues go back into place; and (2) 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. They have carried out morphologic studies of Schlemm's canal utilizing thin-section techniques and scanning electron microscopy, and have established that standard histologic fixation techniques themselves significantly change the resistance to flow through the aqueous outflow system. This work has provided evidence calling for new interpretations of the effects of microsurgery of Schlemm's canal in patients and that new views must be taken of: (1) the structural intricacies of Schlemm's canal; (2) the influence of intraocular pressure on the outflow channels; and (3) of the one-way valve mechanism for control of blood in Schlemm's canal, which seems to be analogous to the arachnoid villi of the central nervous system.

Results of surgery have been inconsistent and frequently there are debilitating post-operative complications. Investigators at George Washington University are systematically evaluating aqueous humor dynamics in order to

determine the long term effects of trabeculotomy and the effects of hyaluronidase perfusions; monkeys are the experimental animal model. Information obtained from these studies may make it possible to develop surgical techniques to maximize successful fluid movement out of the eye in patients undergoing surgery for glaucoma.

CATARACT

PROBLEM

Any opacity of the lens is a cataract. The beginning of a cataract may be very insidious, consisting of molecular aggregation of protein at a level of organization where such changes may have little visible result. Another type of cataract involves destruction of fibers occurring in certain regions of the lens as in the cortex or subcapsular region. The alterations in protein during cataract formation may present many variations in morphology and be the result of many kinds of physical and biochemical disturbances. Cataracts, then, are symptomatic and are the end products of a diversity of insults to the lens. If something of the etiology is known, the entity may be distinguished by a specific name, e.g., radiation cataract. Other cataracts of unknown etiology whose occurrence appears to attend aging, are called senile cataracts. In this latter group are the great majority of human cataracts. Senile cataract is one of the leading causes of legal blindness in the United States and account for about 14% of the total number of blind persons. An additional 6.5% are blind because of cataracts with other etiologies.

TREATMENT

At present, surgery is the only effective therapy for cataracts once they have formed. In some cases, prophylactic measures are helpful. Cataract formation can sometimes be prevented or delayed: in diabetics, early diagnosis of the disease and control with insulin; in congential galactosemia, by withdrawal of lactose or milk from the diet; in radiation cataracts, by proper shielding. Immunization or innoculation with rubella virus of all girls prior to childbearing age, is believed to reduce the number of infants born with cataracts caused by rubella.

ETIOLOGY

Most disease-oriented research on the lens is concerned with discovering the chemical and physical changes associated with development of cataracts in eyes of experimental animals, because a fresh human lens that has not become completely opaque is rarely obtainable from an individual with a specific ocular disease.

There are many similarities in the metabolism, antigenic properties and mode of growth of lenses from different species. Thus, experimental cataracts probably are of value in assessing the mechanism of their formation in man.

A broad range of approaches and many disciplines are being used in the study of experimental cataracts. The objective of an interdisciplinary research activity at the Massachusetts Institute of Technology and the Harvard Medical School is to identify the biochemical and physical structure of the light scattering elements in the cataractous lens.

A recent theoretical understanding of the physical factors which produce transparency or opacity of the ocular tissue makes it clear that the aggregation of lens proteins into high molecular weight agglomerates can produce sufficient light scattering to render the lens opaque. These investigators are attempting to identify such large macromolecules in normal and cataractous lenses by careful biochemical processing of the lens homogenate and by light scattering studies *in situ*.

Calcium has been found to induce aggregation of calf lens soluble protein, and estimation of the molecular weight and refractive index of such aggregates indicates that they could serve as light-scattering elements *in vivo*. Also, the isolation by investigators at Columbia University of a very high molecular weight fraction of alpha-crystallin from the nucleus of the mature bovine lens lends credence to this hypothesis. Evidence is being sought for the presence of such aggregates in human cataracts, experimental sugar cataracts, and hereditary cataracts in mice.

By measuring the angular distribution of scattered light, the size of the scattering elements may be estimated. Data obtained from processing 30 normal lenses shows that between ages 28 and 75 relatively little change occurs in the heavy molecular weight protein region. After age 76, however, the concentration of protein having molecular weights greater than 150×10^6 gm/mole was roughly 10%, a value twice that observed for normal younger lenses. Similar data obtained from 38 cataractous lenses below 72 years of age showed that the heavy molecular weight component of the total soluble lens proteins increases by a factor of 2 or 3 in cataract versus the normal lens of the same age. The molecular weights, the biochemical composition and the biochemical origin of these aggregates from both the older normal and cataractous lenses are being identified. By working with the precise macromolecular element which is responsible for the opacification of lens and by understanding the structure and method of formation of this macromolecular entity, the biochemical basis for cataract may eventually be elucidated.

MORPHOLOGY

A morphological approach to the study of the alterations that occur in the lens during aging and cataractogenesis is being taken by investigators at the University of Colorado. Their objectives are to further elucidate the structural organization and some of the histochemical properties of the young mammalian lens, of the aging lens, and of the lens during cataract formation and, in some cases, during recovery from cataract.

Cataracts being studied include senile and hereditary cataracts in humans and animals, and those induced experimentally in animals. The histochemical experiments are aimed at further exploration of transport and metabolism in the lens including such determinations as acid phosphatase location and amino acid uptake. In addition, the intracellular and extracellular location of capsular substances in abnormal lenses is being investigated. Currently, the studies concern changes that occur in the lens of the "normal" rat during aging, and ocular changes in a strain of rats with hereditary cataract.

Vacuolization of the area of the lens cortex has occurred to varying degrees in nearly all the eyes of the aging "normal" rats. Concurrently, the ocular tissues of these animals are being utilized, at various stages of development of lens opacity, for histological and ultrastructural observations, and for histochemical localization of mucosubstances, structural proteins, lysosomal enzymes, etc.

The progress of cataract has been followed clinically and histologically, beginning at 3 weeks of age in over 60 rats of a mutant strain with hereditary cataract. By 3 weeks, extensive opacities existed in only a few lenses, the remainder being in an incipient stage of cataractogenesis. The latter lenses were relatively clear, but had certain characteristic abnormalities, including anterior polar, subcapsular cataract, anterior location of lens nucleus, and cortical vacuoles associated with the posterior suture. Subsequently, vacuolization and cloudiness extended to most of the posterior and lateral portions of the lens cortex. There were two (2) patterns of cataract maturation in these rats. In one the posterior lens capsule ruptured. The other, a more common pattern, occurred at a later age without capsular rupture. By 17 weeks of age, mature or hypermature cataracts had developed in all lenses. Characteristic of all lenses studied were regions of overproduction of epithelium and capsule, and focal interruptions of lens epithelium. In the cortex, a general disorganization of the bow region, fiber swelling and extensive vacuolization preceded and was associated with cataract maturation. Associated with the anterior lens surface were blood vessels emanating from the iris. These vessels probably represented remnants of pupillary membrane, or, alternatively, posterior synechiae resulting from a previous inflammation of the anterior segment of the eye. It is believed that excessive lens epithelial proliferation and migration in this strain is stimulated by the abnormal lens-associated vasculature. The resulting lack of epithelial organization probably produces the cortical changes leading to cataract maturation.

At Northwestern University, there are studies in progress designed to show the defects in the normal migratory and differentiative patterns of the epithelial proliferative zone cells imposed by a penetrating injury of the anterior lens capsule of frogs and mice. Alteration of the tension on the lens capsule is being attempted in the two animal species. This procedure will test the hypothesis that the normal proliferative zone resides at the lens equator because it is the region receiving the greatest tensional force from the pull of the zonular fibers and the elasticity of the capsule. If the hypothesis proves to be correct, increased tension on the lens capsule, from whatever cause (irradiation, chemical, or dietary-induced swelling of the underlying lens fibers, or mechanical deformation of the capsule by trauma) may be implicated as a primary etiological factor in cataractogenesis.

Cell biologists at Oakland University and the University of Vermont are concerned with factors controlling cellular reproduction, growth and migration in the normal and injured ocular lens. The lens is particularly suitable for carrying out this type of study because it is a purely epithelial system lacking nerve or blood supply. It can be conveniently analyzed by cytochemical techniques such as microspectrophotometry. It is one of the few organs whose constituent cells can be observed in the living state when the organ itself is removed from the animal of origin. The entire population of cells can be examined at once in the fixed or living condition upon whole-mounts.

BIOCHEMISTRY, METABOLISM AND TRANSPORT PROCESSES

It is well known that with aging of animal lens the percentage of insoluble protein in the lens steadily increases. Investigators at Columbia University have found evidence for the increase in the molecular weight of the soluble protein, alpha crystallin, with aging. These older, larger molecules also seem to contain sugar and are less soluble than the younger sugar-free protein. One aspect of this work deals with a detailed study of alpha crystallin and attempts are being made to find the mechanisms, enzymatic or other, by which these changes take place. Because it is also known that the rate of protein synthesis in the lens slows with aging, the detailed mechanism of alpha crystallin synthesis in the lens is being studied. By determining the extent of incorporation of radioactive methionine into the N-terminal position, the extent of initiation of alpha crystallin synthesis can be measured. Recently, it has been demonstrated that calcium at concentrations found in the lens (.005 M) is able to cause a transformation of the sub-units of alpha crystallin to high molecular aggregates and that this effect is completely dependent upon the utilization of alpha crystallin sub-units from the central region of the lens. Inhibition of this action can be produced with glutathione at physiological concentrations. It is conceivable that the disappearance of glutathione from the central region of the older lens, together with a decrease in amino acid content and increase in calcium content may be fundamental factors in producing central opacities in older lenses.

The mechanism and kinetics of transport of amino acids, sugars, purines, pyrimidines, and their naturally occurring derivatives in rat and calf lenses is being studied at Albert Einstein College of Medicine. The nature and complexity of the transporting sites in the plasma membrane is examined by kinetic experiments, by means of selective competitors, and by attempting to alter the reactive sites and ionic environment of the transportors.

At Oakland University the relationship of the mechanisms by which diffusible ions are transported into and out of the lens is being studied. Currently, the parameters governing the flux of sodium between the lens and its bathing media are being evaluated in order to arrive at a quantitative interpretation of the effects of alterations in the chemical and physical environment of the lens.

An understanding of the variety of changes in lenticular physiology that result in altered optical properties as well as the maintenance of normal lenticular physiology is being sought at the University of Colorado by studying the details of distribution and movement of sodium, potassium, and chloride in the lens. Ion-water-protein interaction is considered by some investigators to be responsible in part for regulation of cellular water and ion balance. Although there is good evidence for a cation transport mechanism located in the lens epithelium, its role in regulating ion balance in the deep lens cortex is unclear. It may be that ion balance in the lens cortex is in part due to ion-water-protein interaction since the protein-water ratio is quite high. Cataractous changes are closely associated with permeability characteristics. Such changes might be linked in part to an alteration of the physical-chemical characteristics of the lens protein.

At Wills Eye Hospital, cataract produced in rats by feeding a proprionitrile compound (PHPN) has been of recent interest. The morphology of the cataract resembles the nuclear stage of sugar cataract in rats and the cataract produced by feeding triparanol to rats. Like each of these, PHPN cataract is not preceded by marked early hydration and swelling. The first change observed in these lenses is a sudden influx of sodium at about 7 days, followed by gradual loss of glutathione, sodium and potassium. Just before the nuclear cataract appears at about 15 days, there is a sudden increase in size of the free amino acid pool, suggesting a decrease of protein synthesis. This appears to be confirmed by amino acid incorporation data. This increase in amino acid concentrations, which may be osmotically significant, closely resembles events preceding nuclear opacification in galactose-fed rats.

The development of galactosemic and diabetic cataracts has been studied extensively at the Massachusetts Eye and Ear Infirmary. The working hypothesis is that the enzymatic conversion of the sugars to sugar alcohols is the mechanism that triggers the formation of these cataracts. All other changes observed in the early stages of the cataract - swelling, vacuoles, amino acid loss and electrolyte imbalance - are secondary to the accumulation of sugar alcohol. Thus, aldose reductase, the enzyme involved in the synthesis of sugar alcohol, plays a key role in these cataracts. If this hypothesis is correct, an inhibitor of this enzyme should prevent formation of these cataracts. Studies revealed that tetramethylene glutaric acid (TMG) was one compound that was an effective inhibitor against purified lens aldose reductase. It was also shown to be especially effective in preventing sugar cataracts in vitro. Lenses cultured in media containing high concentrations of sugar in the presence of TMG remain as clear as the normal controls while their counterparts incubated in the absence of TMG show characteristic cataractous changes. These observations seem to support the concept that aldose reductase plays a major role in the development of sugar cataracts. Recent studies at the Massachusetts Eye and Ear Infirmary have revealed that intraocular injections of inhibitors in the eye of living animals can also delay the onset of sugar cataract formation. Current studies are directed to finding suitable inhibitors that will be effective when given by mouth or by drops instilled into the eye.

Recently the efforts of a laboratory at the University of Illinois Eye and Ear Infirmary have been directed towards elucidating the role of the lens fiber membranes in the normal process of electrolyte and water transport by the lens and their abnormalities in cataracts. The investigators found that certain surface-active detergents, when injected into the vitreous cavity of the rabbit eye, induced cataracts. Lenses gained water when exposed either in vivo or in vitro to these surface-active agents. Electron microscopy of lenses exposed to cetyl pyridinium chloride revealed disruption of the lens fibers membranes. They believe the results of their experiments support the theory that continuing fiber membrane damage results in cataract formation.

Senile cataracts are often highly pigmented. Studies at Bowman Gray School of Medicine suggest that tyrosyl residues on proteins may be oxidized, condense with one another to form the pigment, and at the same time, cross-link the proteins into insoluble aggregates. This process may arise by change in the oxidizing capacity of the lens tissue or appearance within the lens of an abnormal protein particularly susceptible to oxidation. Both of these possi-

bilities are being investigated. It has been observed that a yellow pigment is associated with the insoluble protein fraction of human cataractous lenses and it seems to contribute to the insolubility by cross-linking the proteins. This pigment appears to be part of the melanoids which are known to occur in the lens. One or more of the proteins in the highly insoluable fraction contain an unusually high amount of tyrosine, and it is proposed that some of this tyrosine is oxidized to form the melanoids. Studies suggest that one of the oxidized components of the melanoids is 0, 0'bityosine formed by the oxidation of tyrosyl residues of these insoluble proteins. In the potentially cataractous lenses, there may be a slow concentration and close alignment of lens proteins that would bring bityrosine residues together for interaction. This interaction may be a slow cumulative process by either a photochemical or oxidative reaction.

MOLECULAR BIOLOGY

Since the lens is composed of protein and water, it is logical to assume that the initial events of the cataractous process may reside in defects of protein synthesis. For example, the production of a lens protein population which differs from normal in either the types or amounts of certain protein may result in an increased susceptibility to loss of transparency of the lens.

Cataract may follow shortly upon this aberration in protein synthesis. On the other hand, events which occur early in life, such as nutritional deficiencies, viral infections or exposure to toxic substances, may cause a change with delayed effects. In this instance, the defective protein population remains throughout life and results in the onset of cataract perhaps 10-20 years later.

The objective of investigators at the University of Missouri is to determine what changes occur in lens transfer RNA during the differentiation of an epithelial cell into a fiber cell, what causes these changes, what are the mechanisms involved and what effects these changes have on the synthesis of lens-specific protein. Their results support the idea that the tRNA population in the lens is specialized. They have shown that lens phenylalanine-tRNA is different from that in liver tissue, possibly indicating a special function. Also, this tRNA changes during lens cell differentiation. These data suggest that specific tRNA modifying enzyme is present in lens epithelial cells which is lost during differentiation. This is certainly only one of many events affecting lens protein synthesis, but any changes in the activities of these tRNA modifying enzymes, whether induced by drugs or disease, may lead to irreversible changes in lens proteins.

Previous work on tRNA modifications have resulted only in general observations. These include the stimulation of tRNA methylases in tumor cells, or the stimulation of tRNA methylases by polyamines *in vitro*. This work may provide information on what special enzyme is present in lens and what result this modification has on the biological activities of a specific tRNA.

IMMUNOLOGY

Studies at Columbia University of the immunochemistry of lens proteins include: (1) immunological characterization of the types of lens proteins

(alpha, beta, gamma crystallins) present in the insoluble albuminoid in various mammals including man; (2) immunological characterizations of the types of lens proteins present in cryoprecipitable lens proteins; (3) comparison of the antigenicity of the various lens crystallins and their relationship to autoimmune phenomena. This includes quantitation of both humoral and cellular immune responses; (4) investigation of the possible pathologic effects of in vivo anti-lens antibodies (a) for changes in development of various tissues of embryos and fetuses and (b) for changes on the lens proteins themselves; (5) determination of the number of types of lens antigens shared among different tissues within the same species; and (6) immunologic characterization of the types of lens proteins associated with various experimental (e.g., metabolic, x-ray) and human cataracts.

ENVIRONMENTAL FACTORS

In terms of toxic and traumatic influences, it is possible that the human lens is much more likely to be subjected to a variety of insults than to any one cataractogenic agent. This idea is being explored at Emory University using experimental animals (rat, mouse and gerbil). Effects of a 2-component insult to the lens is determined by measuring alterations in lens metabolism and lens fiber permeability as reflected by increased or decreased tracer uptake using labeled substances such as galactose, thiourea, leucine, etc. Cataractogenic factors being explored are photosensitizing agents, long wave ultra-violet light, diabetes, lens toxicants such as steroids, pilocarpine, etc.

At the University of Rochester, it has been observed that near ultra-violet irradiation of lenses or solutions of lens proteins in the presence of phenylalanine, tyrosine, or tryptophan produces dark pigments firmly bound to lens protein. When mice maintained under near ultra-violet light are compared with controls kept in darkness or fluorescent light, cataractous changes have been noted. These animals are sacrificed at intervals and lenses are being examined for alterations in proteins. Recent results have indicated that near ultra-violet light at levels close to those present in sunlight can alter many free aromatic compounds such as tryptophan in the lens so that they become pigmented, bind firmly to the lens and alter the chemistry and function of these proteins adversely.

AMBLYOPIA

PROBLEM

Amblyopia is defined as reduced visual acuity without ophthalmoscopically detectable anomalies of the fundus. Some types of amblyopia are as follows: (1) Strabismic amblyopia. Strabismus is defined as a deviation of the eye which the patient cannot overcome. Strabismic amblyopia is defined as reduced visual acuity in one eye in patients with strabismus, or a history of such, without ophthalmoscopically demonstrable anomalies of the fundus. Reduced visual acuity is only one of the many disturbed sensory and motor functions that adds to the complexity of the amblyopic syndrome. Strabismic amblyopia is thought to be caused by active cortical inhibition of impulses originating in the fovea of the deviated eye of the strabismic patient. Thus, it is considered the consequence rather than the cause of strabismus. Estimates are that from 1% to 4% of the population is affected. By early detection, patching of the good eye and by surgical treatment for strabismus at the proper age, the prognosis for normal vision can be good;

(2) Amblyopia ex anopsia is found in patients whose visual acuity has remained markedly and irreversibly decreased after having been deprived of normal visual stimulation during some critical period in their early lives. It has been shown that at birth the visual system is still developing and requires visual stimulation in order to complete its development. Should the stimulation be denied during a critical period, the visual system does not complete its development and normal vision is no longer possible.

(3) Anisometropic amblyopia is a condition in which there is a difference in the sizes of the images on the retina of the eye due to a large difference in refraction between the two eyes; and amblyopia results.

(4) Ametropic amblyopia occurs in one or both eyes in children and adults who have significant refractive errors and have not previously worn glasses.

INVESTIGATION OF THE CRITICAL PERIOD FOR DEVELOPMENT OF AMBLYOPIA ex ANOPSIA

In order to determine more precisely the critical period for producing amblyopia and for studying the mechanism of the impairment, research has been conducted at the Johns Hopkins Hospital University and at Baylor College of Medicine on animals who have been deprived of visual experience at an early stage of their lives. One eye of rhesus monkeys was sutured shut for a certain period, and then opened. Tests were made for visual performance. With the use of behavioral methods for assessing visual acuity and with improved testing equipment, it has been shown that the age of maximum susceptability to form vision deprivation in rhesus monkey is not, as originally assumed to be, between birth and 12 weeks, but between birth and six weeks. Lid closure after the age of 6 weeks may cause mild amblyopia; however, lid closure after 3 months has no effect on the development of visual acuity.

Effects of intermittant lid closure were studied in 5 monkeys. Preliminary results reveal that intermittant lid closure of one eye for a period ranging from 2 to 4 weeks may cause irreversible amblyopia, if lid closure is performed before the animal is 8 weeks old. In older animals intermittent lid closure has no effect on the development of visual acuity. These behavioral studies have shown that the rhesus monkey is a good animal model for amblyopia studies, since the clinical characteristics of experimental amblyopia in rhesus monkeys is extraordinarily similar to amblyopia as it occurs in man. Further experimentation in that direction will eventually provide the clinician with guidelines at which age to operate congenital cataracts, perform corneal transplants, or do strabismus surgery in order to preserve visual acuity in young children.

Similar studies on the critical period for amblyopia are being conducted on monkeys at Harvard University. Previously it had been shown by the Harvard group that for kittens, the effects of eye closure begins suddenly near the start of the fourth week, remains high until some time between the sixth and eighth weeks, and then declines, disappearing finally around the end of the third month.

EFFECT OF VISUAL PERCEPTION ON THE CORTEX

A group of investigators at Harvard University is conducting neurophysiological and behavioral studies on the effects of visual deprivation on the cortex using the cat as the model animal. In the adult cat the visual cortex has a highly ordered functional architecture and cells are highly specific in their responses to natural stimuli. Previous studies have indicated that innate mechanisms were crucial for the normal development. In view of findings in other laboratories this question was re-examined with a series of experiments in normal and binocularly deprived kittens at various ages after birth. In the normal kitten physiological experiments indicated that the maturation process seemed to involve an increase in the specificity to visual stimulation; at 3 weeks a cortical cell would respond to a line moved across its receptive field but the orientation of the line was often not important. In the 4-5th week all cortical cells would be selective in terms of line orientation but with a rather wide angle of tolerance. Finally in the 6-8th week cells would have the precision of normal adult cells. Kittens raised with both eyes occluded showed a similar pattern of development but had in addition a substantial fraction of abnormal cells. These findings indicate that cortical specificity is provided for by innate mechanisms but visual experience is important early in life for normal development.

At Johns Hopkins University, an attempt has been made to see whether it is possible to demonstrate morphological changes in the cortex due to vision deprivation. An electronmicroscopic study of synapses from areas 17 to 18 in the visual cortex of normal and amblyopic monkeys was conducted. Synaptic density and morphological synaptic characteristics were described and compared. No significant difference existed in the number of synapses counted in comparable tissue from both specimens. The data collected for different parameters of synaptic characteristics (length of synaptic contact, distribution, number and shape of pre-synpatic vesicles and mitochondria) also showed that no differences were present in the visual cortex

of an amblyopic and normal animal. These preliminary findings indicate that the severe neurophysiological defect which could be demonstrated in the visual cortex of monkeys with strabismus and stimulus deprivation amblyopia may not have an analogous morphological equivalent.

EFFECT OF VISUAL DEPRIVATION ON RETINAL FUNCTION

At the University of Miami, a study is underway to determine the effects of visual deprivation on the different stations of the visual system. At the retinal level, it has been shown for cats, monkeys and humans that all components of the electroretinogram are smaller in the light deprived eye. This has been shown for the developing as well as the mature retina. The evidence to date indicates that the alteration is in the pigment epithelium. At the lateral geniculate level, it has been shown that there are alterations in the receptive field organization of the some of the lateral geniculate cells.

DIAGNOSIS AND TREATMENT

Amblyopia has long been known to originate in childhood in the majority of adult patients affected by it. Beyond this, however, little is known of its pathogenesis. Partly due to the difficulty of diagnosing young children and partly due to the lack of refined diagnostic tests the condition is usually not detected until it is relatively far advanced.

At Alabama Medical College a research program has as its objective the implementation of a test which is precise enough to allow prediction of strabismic amblyopia before it reaches an extreme, permanently dysfunctional level and, in so doing, to further specify the pathogenic mechanism of strabismic amblyopia.

Small-angle squint is a familiar clinical entity. Recently, however, discussion has arisen of a new classification of "microstrabismus" or "microtropia". While there is not as yet complete agreement on the definition of what comprises the condition, the major investigators of it seem agreed at least that there is a condition of "ultrasmall" or "inconspicuous" strabismus something on the order of 5 degrees or less. There is some evidence that while microtropia may remain constant throughout life it may also progress into a greater angle of squint especially in young children. Hence, there is need for a sensitive test to detect even a small amount of strabismus.

The approach of a group at Albany Medical College for detection of strabismus is based upon the assumption that strabismic amblyopia arises not from some dysfunction in one eye but from an interaction between them both. "Patching" therapy of either the deviating or normal eye is based upon the assumption that the normal eye somehow is interfering with the amblyopic eye's function. Visual acuity and brightness discrimination in the amblyopic eye is reduced in direct proportion to the intensity of stimulation of the normal eye. Even the stray light slipping passed the edge of an occluder will decrease the amblyopic eye performance.

Studies with kittens also at Albany Medical College have shown that

binocular lid suture early in life did not produce as drastic dysfunction as either monocular suturing or monocular translucent occlusion, whether constant or alternated between eyes every other day. A test for early stages of strabismic amblyopia is being devised based upon a sensitive test for stereopsis. The hypothesis is that a stereoacuity decrement will occur before a monocular visual acuity decrement. Recently, an entirely new hybrid computer/photo-optical method of generating random stereograms has been developed. Preliminary tests with several young squint patients have shown that the microtropic amblyopes seem singularly unable to detect the random-dot stereogram targets. Further refinement of the test and more data on normals and known squinters and amblyopes is planned.

Another development in the field of strabismic amblyopia diagnosis has been acomplished at the Rensselaer Polytechnic Institute. Based upon a refinement of the Maxwell spot technique, this equipment measures fixation position within 0.1 degrees. With this equipment, it has been possible to show that for all amblyopic eyes tested, there was some degree of eccentric fixation.

STEREOSCOPIC VISION - DEPTH PERCEPTION

PROBLEM

There are several ways by which a person perceives depth; for example, by perspective, covering of more distant objects by closer objects, shadows, or vergence movements. In the absence of any of these clues, however, depth is apparent to a normal person based upon the fact that the retinal images on the two eyes are slightly different because the eyes view the outside world from two slightly different points of view. The image of the right eye is like that of a photograph taken slightly to the right of the object and the image of the left eye is like a photograph taken in a similar fashion to the left. When these retinal images are fused in the brain, the disparity gives rise to a sense of the third dimension. If one fixates on a point, it is readily apparent that there is an area wherein the position of the object is sensed and the images of the two eyes are fused (Panum's area). Outside of the region there may be a sense of depth but the vision is double. Although lack of stereoscopic vision is not incapacitating, good stereoscopic vision greatly aids the performance of many tasks such as driving an automobile, most industrial jobs, sports, etc. Defects in stereoscopic vision also may be indicative of other visual problems, such as the first stages of amblyopia as will be subsequently discussed.

RESEARCH

At the University of California, Santa Barbara, there is an ongoing study of binocular localization by human observers in a situation where stimulus information is restricted to disparity and convergence. There are five specific goals: (1) to evaluate stereoacuity as a function of distance from a fixation point; (2) to determine the effect of eye movements on the relation between depth and disparity. Although the contribution of eye movements to stereoacuity has been disputed, the investigator believes that eye movements do play a role in the appreciation of large depth intervals. It is

claimed to have been shown that for a constant perceptual criterion, less disparity is required to satisfy the criterion when eye movements are permitted than when they are not. One of the goals of the present project is to examine this phenomenon in detail, (3) to determine the relation between the size/distance ratio and the visual angle, (4) to formulate and test a new geometric model of stereoscopic visual space, and (5) to ascertain the relation between visual judgments and accuracy of pointing.

The principal thrust of the work is concerned with the interrelation between perceived depth, perceived egocentric distance, disparity, and convergence of the eyes. This is a basic research project aimed at understanding stereoscopic vision. However, it could be relevant to the alleviation of the common and serious visual defects of strabismus and amblyopia. Although strabismus is commonly attributed to a defect on the motor side, it could be that at least in some cases the initial defect is in the ability to register disparities for the purpose of controlling fusion.

This research also has application to preventive medicine. It deals with perception under conditions where stimulus information is limited and where illusions occur. This is the same kind of situation which is encountered when one operates an automobile, boat, or airplane in darkness. The better perception under such conditions is understood, the better position we will be in to avoid accidents.

The visual latent period is defined as the time interval between stimulation of the retina by light and stimulation of the cortex by the impulse generated at the retina by the light. At Southern Illinois University, Carbondale, a study is being conducted on the effects of illumination conditions on the visual latent period as measured psychophysically and electrophysiologically (the electroretinogram and the visually evoked cortical potentials). The two classes of data are being collected on the same observers using white and monochromatic stimuli presented foveally and peripherally over a wide range of scotopic and photopic retinal illuminances, under light and dark adaptation, and for various conditions of luminance contrast. One of the main procedures used in this study is the Pulfrich stereophenomenon. This phenomenon can be demonstrated easily with the bob of a pendulum swinging on a fronto-parallel plane before the subject. Observed binocularly with one eye darkened by a filter, the bob appears to move in a more or less elliptical path, nearer the observer when going on one direction and farther from him when going back. When the filter is placed before the other eye, the direction of the motion is reversed, that is, the bob appears to trace the same elliptical path in an opposite direction.

Explanation of the Pulfrich phenomenon has been based on the laws of stereoscopic depth and angular disparity, together with the assumption of a retinal latency period - a lapse of time between retinal stimulus and cortical stimulus - which somehow is inversely related to the illuminance of the retinal image. The less the illuminance of the retinal image, the longer the latent period; and thus the effect is explained specifically in terms of the difference in latency periods for the two eyes. The availability of several alternative methods of measuring the visual latent period provides a unique opportunity to assess the contribution of the sensory versus the

motor components of the total visual response latency as well as to compare the monocular versus the binocular temporal processes of integration occurring in the visual cortex. The wide range of variables used will yield new data that are needed to relate the theories of monocular and binocular space discrimination to those of other basic areas in vision, such as, visual photochemistry, visual electrophysiology, light and dark adaptation, intensity discrimination, retinal interaction, color vision, and the visual latent period. Because the new experimental data will contribute to a better understanding of the underlying neurophysiological processes involved in the temporal response to stimulus illumination, the methods and data of these studies should prove fruitful in their application to clinical ophthalmology for detecting and identifying patients suffering from ocular disease.

A study of binocular vision and its disorders which may have clinical significance is being conducted at the Massachusetts Institute of Technology. Both psychophysical and neurophysiological approaches are being employed. Just prior to the initiation of this project, the principal investigator had discovered that perhaps 30% of the population is, to some extent, stereoblind. There are three categories of stereoblindness: people who cannot discriminate crossed disparities (an object further from the subject than the plane on which he fixates), people who cannot discriminate uncrossed disparities (an object closer to the subject than the plane on which he fixates), and people who cannot discriminate either. Two methods to test the subjects are used. In the first, the subject looks at a sand-blasted plexiglass screen and is presented with a vertical line of various disparities by means of polarizers. He is then asked if the line appears to be in front, behind, or in the plane of the screen. In the second, the subject fixates on a cross, is presented with a bar of various disparities for 80 milliseconds, and is asked to indicate its depth by setting a probe to the same position in space. Both methods reveal the classes of stereoblind people.

This discovery suggests that there are three classes of disparity detectors in the cortex. The principal investigator is attempting to fatigue one class by embedding his test stimulus in a matrix of lines of various amounts of opposite disparity. Viewing a spiral, which leads to the spiral aftereffect, may modify stereoscopic perception. Only some people use convergence as a cue to depth, and the ones who do not may be stereoblind. This research has as one of its objectives a determination of the relationship between stereoscopic depth perception and the control of vergence movement. Neuroanatomic studies in the cat are also being directed towards determining the relationship between vergence eye movements and the presumed substrate for stereopsis: the dorsal lateral geniculate nucleus.

At the California Institute of Technology, a study is being conducted on the mechanism for achieving fusion of the disparate images in the two eyes in the region where there is fusion of the two images and perception of depth (Panum's area). The visual system is able to compensate for quite large disparities in the horizontal position of an object imaged in both eyes but is limited in the vertical direction. Quantitative observation of eye movements have shown that the eyes are not capable of undergoing any appreciable cyclofusional rotation (rotation about the line of sight) in an attempt to achieve fusion of two images with vertical disparity. Fusion is actually achieved, however. Hence, it was concluded that fusion is achieved by a central vision mechanism.

At the University of Vermont, there is a research project dealing with the influence of various factors on stereopsis. The targets may be composed of random dots in three-dimensional space with no distinct contours or composed of lines having distinct contours. The basic knowledge of stereopsis gained from this study may lead to a better test for the acuity of stereovision which will eliminate cues not based on true stereopsis (i.e., retinal disparity).

ANIMAL MODELS

In order to delve deeper into the neurophysiological aspects of stereovision, it is necessary to find a suitable animal model. At Vanderbilt University, it has recently been shown that the cat has stereoscopic vision. The degree of stereovision is still under investigation. Although the binocular visual system of the cat resembles that of primates and extensive neurophysiological research suggests a neural basis for stereopsis in the cat, there was no proof of stereopsis before these psychophysical experiments were conducted. Whether or not the falcon has stereopsis is still under investigation.

Studies at Harvard University have shown conclusively that the Rhesus monkey has stereoscopic vision. This conclusion was reached through two lines of evidence: (1) from recordings of single cells in the visual cortex, cells were found which respond to stimulation of both eyes but not stimulation of either eye suggesting that these cells are involved in stereopsis; (2) from behavioral studies using Julesz random dot patterns. A Julesz pattern is a pair of computer-generated patterns consisting of random dots (e.g., like "snow" on a television screen when there is no station on the channel). When this pair is fused stereoscopically a shape, such as a square, will appear to be floating above or sunk below the remainder of the pattern. The stereoscopic effect is solely due to retinal disparity. The monkey was able to distinguish "in front" versus "behind" versus "no square" proving that it had stereoscopic vision. By providing animal models capable of giving behavioral indicators of binocular visual phenomena such as stereopsis, it is possible to coordinate behavior more closely with underlying neurophysiological mechanisms. Most of the behavioral information concerning the function of the binocular visual system has been based on human psychophysical observers and the advantages of the combined behavioral-neurophysiological paradigm could not be exploited. Now, using animal models such as the cat and the rhesus monkey, it is possible to pose questions about the development of binocularity and to determine the consequences of systematically introducing anomalies such as strabismus into the system.

CARE AND REHABILITATION RESEARCH

At the Johns Hopkins University School of Medicine extensive studies have been conducted for many years of patients with low visual acuity and of the various optical ways in which they can be treated. These include an extensive application of the Tübingen perimeter, using projected achromatic and chromatic test targets presented in both the static and the kinetic mode. Of particular interest are patients with acquired color vision defects (e.g., from disease or drugs). The course of untreated maculopathies, particularly

senile macular degeneration, are documented with various function tests, including central field studies, acuity measures, changes in acuity with luminance, and determination of appropriate reading aids. Objective techniques for estimation of refractive error are being investigated. The Read-Write closed-circuit television system will be evaluated as a visual aid for readers, writers and mathematicians. The TV system will also be evaluated as a aid in group teaching of partially sighted children. Finally, the investigator wishes to develop a Badal refractometer, which will allow measurements of refractive errors in partially sighted patients with nystagmus, lens opacities, and related abnormalities.

At the Institute of Medical Sciences, San Francisco, a lightweight, battery operated, fully portable seeing aid for the blind is being developed which will project in tactile form upon the trunk a facsimile image of the visual scene registered by a television camera. The device is intended for use as a visual substitute system for the blind. Images are transmitted via fiber optics from a special pair of spectacles to a lightweight television camera and then to an array of electrotactile stimulators in contact with the abdomen or back.

During the past year the design of the Model III, consisting of an array of 1024 (32 X 32) stimulators and the electronic package to distribute the pulse-width modulated video signals to each appropriate electrode driver has been completed. The camera and optical system work satisfactorily. The complete system weighs five pounds including two pounds of rechargeable nickel cadimum batteries for eight hours of operation of the system. Power consumption under average conditions is approximately three watts. Progress is being made on fabrication and testing of the various circuits of the Model IV system, which consists of an array of 4,000 stimulators (50 X 80).

It is anticipated that this 80 line wide system would provide a 40 to 50 degree "field of view". The complete visual prosthesis with this field of view may prove useful for more global tasks, such as mobility as well as more sophisticated employment and educational needs. Equipment, such as Model IV, with greater resolution and information capacity is required to answer the questions of how much simultaneous tactile image detail the brain can handle and whether it can selectively attend to some parts of the "picture" while ignoring activity in other parts. The design of electrodes for maximum comfort and effectiveness is under investigation.

At the University of California, Berkeley School of Optometry, a study is directed towards making plastic and tempered glass lenses more fracture-resistant. It is divided into three parts. The first part of the study is concerned with the fundamental fracturing processes of glass and plastic lenses. It involves calculating the stress distribution in a lens for various impact velocities and geometries, using a mathematical model of the process. The model is evaluated against experimental factors such as lens mass, shape, and power, and curvature of the front surface, as well as missile shape, mass, and velocity. Part two is a statistical evaluation and the establishment of norms for the fracture of lenses with various stresses. The third part of the study is experimentation to evaluate various techniques that should improve the fracture resistance of various lenses, such as detecting

inherent weaknesses in the lens during early stages of the manufacturing process, improved surface preparation by heat treatment, the sealing of surface flaws by flame polishing (possibly quenching in an oxygen-free atmosphere), and the novel application of a plastic membrane to the surface of the lens.

TUMORS AND SYSTEMIC DISEASES

MELANOMAS

PROBLEM

Ocular malignant melanomas are the most common primary tumors in the eye and constitute a significant problem. The tumors in the eye include not only those originating there, but those deposited by metastases or growing in from direct extention (as cerebral glioma along the optic nerve).

Research using animal models is being conducted on the etiology, diagnosis and treatment of eye tumors. Studies of etiology are concerned with the pathogenesis and fine structure of pigmented tumors of the eye, the relationship of various normal ocular pigmented cells and pigmented tumor cells and the possible role of virus in the development of ocular tumors. The influence or presence of certain enzymes such as lactate dehydrogenase (LDH) and DNA-polymerase, particularly the DNA-polymerase which is under the influence of RNA ("RNA-directed-DNA-polymerase"), is being investigated.

ETIOLOGY

Investigators at Yale University School of Medicine are using histopathologic techniques in order to determine how normal pigmented ocular cells become cancerous. These cells are being examined with both light and electron microscopy. Particular attention is being paid to the possible role of viruses. Pure cultures of retinal pigment epithelial cells from humans and hamsters have been successfully grown in tissue culture for periods up to six months and cytological features suggestive of dedifferentiations and benign tumor formation have been documented. Whereas previous attempts to establish continuous lines of pigmented cells from human ocular malignant melanomas, normal adult and fetal uveal tissue, and uveal nevi have been unsuccessful, melamoma cells taken from the area of orbital invasion and in another instance from areas of distant metastases in the lungs, liver and peritoneal cavity, have grown vigorously in tissue culture. By light and electron microscopy it appears that these cells have retained their essential morphological characteristics. Because these cultures appear to provide permanent cell lines, this tissue culture approach offers promise as a tool for studying the characteristics of malignant tumors and their formation from normal cells.

This research group has recently begun investigating the role of DNA-polymerases in malignant cells. Previously it has been known that DNA-polymerase which allows the flow of information from RNA to DNA (RNA-directed DNA-polymerase) is present in all RNA oncogenic viruses tested. Preliminary results indicate a distinct polymerase is present in retinoblastoma and possibly ocular melanoma. Future experiments should give additional evidence of the relationship between the polymerases found in normal tissue and those found in the tumors of these tissues.

DIAGNOSIS

There is great need for better diagnostic methods for detection of tumors

and differentiating between malignant tumors and those conditions which do not require enucleation of the eye. Enucleation of an eye that was erroneously thought to contain a malignant melanoma is a tragic loss. The problem is even more serious if dealing with a patient with only one eye. Ultrasonography (i.e., scanning the eye and orbit with an ultrasound beam) is being investigated as a method for detecting pathological structures such as tumors in locations and under conditions where they would not be detected by conventional optical observation. Research is being conducted on an enzymatic method for diagnosis of malignancy based upon the premise that the aqueous humor in the presence of a malignant tumor has a different lactate dehyrogenase isoenzyme pattern from that of a normal eye. Another possible enzymatic method for diagnosis of malignant tumors recently initiated is based upon differences in the DNA polymerase content (RNA-directed DNA-polymerase within the eye.)

A malignant tumor must be removed; however, there is some evidence that in the process of removing the tumor the malignancy may be spread. Whether or not this spreading occurs during surgery and if so what can be done to prevent it is currently under investigation.

High resolution B-scan ultrasonagraphy is a valuable new technique for diagnosis of ocular and orbital pathological conditions. This safe, atraumatic and rapidly performed examination yields unique information about the spacial relationships of the various structures of the eye and the surrounding orbit. The eye of the patient is immersed in a saline solution and an ultrasound beam is directed into the eye. This beam scans the eye, the reflected ultrasound is sensed by a detector, and the resultant signal is displayed on a television-type screen thereby giving a "picture" of the reflected ultrasound. The picture on the display tube is photographed. This type of scanning, known as "B-scanning" was first achieved in 1958. During the past year the accomplishments at the College of Physicians and Surgeons, Columbia University may be summarized as follows: (1) establishment of the diagnosis reliability of the method. Based on three years experience with B-scan on over 1000 patients characteristic scan patterns for various pathologic conditions were observed and rules and schemes for diagnosis formulated. These were tested on 100 ocular cases and 100 orbital cases. Verification of the diagnosis was by pathology and other independent tests or long term follow-up. For ocular diagnosis the ultrasonic method was correct for all cases except 2 out of 14 involving the presence or absence of a foreign body. Examples of conditions diagnosed were: retinal detachment, vitreous hemorrhage foreign body, traumatic injury and tumors. For use in orbital diagnosis, it was found to offer better than 85 percent reliability in diagnosing the presence of an orbital tumor. Rare false-negatives and approximately 12 percent false-positives occur; (2) a detailed study of the characteristic ultrasonic B-scan patterns for various types of tumors in the orbit has been conducted. These tumors were hemangiomas, (the most common of all orbital tumors) lymphomas and neurogenic tumors; (3) inflammatory changesin the orbit around the optic nerve in the case of optic neuritis has been observed by ultrasonography. Other diagnostic techniques have not been able to explicitly demonstrate this condition.

At the Eye Research Foundation, Bethesda, Maryland, investigation of a method for possible preoperative differentiation of malignant and benign

tumors in the eye based upon differences in the electrophoresis patterns and level of lactate dehydrogenase (LDH) isoenzymes in the aqueous humor is in progress. The rationale for this program is based upon previous investigations which reported alterations in the pattern and level of LDH isoenzymes in cancer patients as compared with normal controls. The two recent technical developments by the participants in this project which have made this research possible are: (1) the "Thomas Paracentesis Needle" which permits relatively atraumatic sampling of aqueous humor from man and animals; and (2) ultramicro techniques for determining LDH isoenzymes and protein electophoresis patterns as developed by Nicholas M. Papadopoulas, require less than 0.1 ml of sample. In the clinical studies, preoperative and post-operative samples of aqueous humor of patients who are to have enucleations are analyzed for LDH isoenzymes and protein levels. The enucleated eyes are sent to the Armed Forces Institute of Pathology for histopathological studies which include biochemical analyses of the vitreous humor, uvea, retina and the tumor itself. Isoenzyme assays are also performed on samples of the blood for comparison.

Many analyses have been made of protein and LDH-isoenzyme levels from eyes which do not have tumors. Samples of fluid from 65 eyes were taken prior to cataract surgery. A consistent LDH-isoenzyme pattern was found. This same pattern was observed in eyes blinded by retrobulbar neuritis. Similarly, characteristic LDH patterns were found for tissues of cornea, lens, iris, retina, vitreous and ciliary body from "normal" eyes. Having perfected this technique and having established "normal" aqueous and tissue patterns, the next step was to study aqueous from eyes with tumors.

Although only 6 ocular specimens have been examined, the results indicate the following: (1) an LDH isoenzyme pattern which definitely varies from the "normal" aqueous humor; (2) a variation of the LDH pattern within a specific tumor category is probably due to mitotic activity (i.e., malignant malanoma patterns varying with types of melanoma); and (3) a definite overall aqueous humor and tumor LDH-isoenzyme and protein pattern varying with major tumor categories (i.e., retinoblastoma vs. malignant melanoma). Parallel work is also being conducted on rabbits.

Another approach to diagnosis of malignancy is being investigated at Yale University. As previously discussed, the presence of a DNA-polymerase appears to be characteristic for retinoblastoma and possibly ocular melanoma. If further investigation confirms this conclusion, the detection of this enzyme may lead to another method for diagnosis of malignant tumors.

TREATMENT

An investigation into the question of whether enucleation of an intraocular malignant melanoma may produce showers of tumor cells which can cause the spread of the tumor is in progress at the University of Arkansas. During surgery, these secondary tumor showers are probably most susceptible to chemotherapy treatment while in the blood stream. Other ways of treating intraocular disease surgically, chemotherapeutically, or with radiotherapy are also being studied. During the past year a series was completed in animals in which intraocular pressures were measured continuously during the enucleation

procedure. It was shown that the massaging effect of enucleation caused from 0 to 400 mm Hg rises in intraocular pressure. There is a definite potential of massaging cells out into the blood stream with enucleation techniques currently in use, and therefore, a "no-touch" technique is being developed.

SYSTEMIC DISEASE RESEARCH

At the University of Michigan Medical Center, a study is being made of the etiology of Graves' disease which may lead to a new approach to therapy. This is a disorder occurring in patients with hyperthyroidism which can severely impair vision. It is characterized by an extracellular accumulation of edema fluid and mononuclear inflammatory cells in the extraocular muscles. As a result, severe proptosis and chemosis can occur leading to visual loss from exposure keratitis; alternatively, visual loss can occur from pressure effects on the optic nerve. The hypothesis being tested is that the ophthalmopathy and dermopathy of Graves' disease arise from circulating factors acting on connective tissue to cause an abnormally high production of glycosaminoglycans (acid mucopolysaccharides). The results so far indicate that cultured cells from retrobulbar tissue can be very useful in searching for factors that may stimulate or inhibit glycosaminoglycan productions and in studying the biochemical methods involved.

At the Estelle Doheny Eye Foundation, Los Angeles, research investigators have successfully established pure primary cultures and cell lines of corneal endothelium, lens epithelium and retinal pigment epithelium. They are now using these tissue cultures to construct studies which otherwise would be difficult to conduct such as the toxic response to purulent exudates, erythrocytes or erythrocyte fractions, steroids, nucleic acid analogs and certain growth stimulating factors. They are also using cultured malignant melanomas for studies of immune response to such tissues.

At the University of Illinois, Chicago, a research project is engaged in gathering clinical, histological, and biochemical information about several ocular diseases that cause blindness in animals. The diseases - cataract, sickle cell anemia and primate macular degeneration - have human counterparts, and the investigators feel that study of these animal models will help further the understanding of human ocular diseases.

The current specific objectives are as follows: (1) Cataract - to identify biochemical alterations which take place in the development of genetic cataract in the miniature German schnauzer dog as well as those which take place in the spontaneous dissolution of the cataract in these species; (2) Sickle cell retinopathy - to create and study the retinopathy associated with RBC sickling in vivo. Sickling is induced in deer of proper hemoglobin type in order to study histopathology, retinal vascular changes and systemic alteration in blood chemistry, important to maintenance of reginal physiology; and (3) Heredo-macular degeneration - to establish a colony of baboons with genetically transmitted heredomacular degeneration and to study the clinical, histological and electrophysiological manifestations of the lesion.

LIGHT AND RADIATION EFFECTS

PROBLEM

A basic phenomenon of physics is that light cannot be effective if it is not absorbed by a substance. In the retina, light is absorbed by visual pigments such as rhodopsin. When light is absorbed by rhodopsin, this complex molecule undergoes intramolecular rearrangements as well as possible reorientation in the photoreceptor membrane. It has been known that photosensitizing substances in other organs, such as skin, may also undergo intramolecular rearrangements with resulting toxic reactions, as a result of exposure to light. The toxic effects of prolonged exposure to full sunlight on vision in man were documented at least thirty years ago. However, the mechanisms of action have been explored in man and in animal models during the past decade. The transduction of light into neuronal activity in retina involves the balance between a complex series of physical and electrophysiological reactions. Interruption of the numerous interdependent phenomena may result in a disease state which may lead to a partial or a complete loss of vision.

ENVIRONMENTAL AND HEREDITARY FACTORS

Study of visual pigments in fish may result in the interpretation of differences in spectral sensitivities of photopic and scotopic visual systems. A cooperative study being conducted by investigators at Cornell University and at the University of Oregon emphasizes factors which are definable attributes of vertebrate vision and can be related to function. These investigators demonstrate that accurate measurements of spectral distribution of light are crucial to understanding how visual targets are seen. The overall goals are the relationships between the visual pigments, animal behavior and the photic environment. Underwater light measurements are bringing greater quantification of the relation between visual pigment absorption and available light. The attempts to relate extractable cone pigments to specific cones are important in attempting to focus on how cone pigments differ from rod pigments. Studies on the controlling effects of light and dark, temperature and hormones on rhodopsin-porphyropsin ratios might demonstrate retinal adjustments which produce changes in photosensitivity.

An understanding of the biological significance of visual pigments as evolutionary adaptations to visual requirements of animals involves the patterns of inheritance of visual proteins in different species. In a project at the New York University Medical Center, it has been reported that tadpoles usually have more than 90 percent porphyropsin in their retinas when kept in the light. In complete darkness the system switches to a predominance of rhodopsin. The intensity-response curves to three wavelengths have been measured by illuminating tadpoles by different colored lights at different intensities. The experimental conditions were such that the cone system was not functional and implicated either rods or another receptor involved in the photopigment shift in response to light. These observations are true for Rana but not Xenopus and Amblystoma tadpoles; the latter two do not change visual pigments at metamorphosis. In fish the effects of light and darkness are more complicated; some species react to light and darkness in the opposite manner to that of the tadpole.

Investigations at the University of Texas have revealed that reflecting layers (tapeta lucida) are common structures in fish. They are an integral part of the visual mechanism which regulate the amount of light entering the photoreceptors. Although tapetal reflection does not occur in the human eye, residual reflection from the choroid and pigment epithelium has been used to measure absorption of human visual pigments in studies at the New York University Medical Center and at the Florida State University. The tapeta of animals and fish present an adaptive way of resolving the problem of light utilization and of maintaining visual acuity in fish at great oceanic depths, in turbid waters and by nocturnal animals.

METABOLISM OF PHOTOPIGMENTS

Investigators at Harvard University had observed that the bullfrog tadpole possesses porphyropsin which changes to rhodopsin during metamorphosis. Adult bullfrogs have been found to retain as much as one third of their visual pigment in the form of porphyropsin which is segregated in the dorsal portion of this animal's retina in the winter months. In the summer months the porphyropsin declines to about 5 percent. The pigment epithelium exhibits the same segregation of vitamins A_1 and A_2. They have found that if the bleached retina is dissected out of the eye and laid back upon the pigment epithelium, the retina resynthesizes whatever visual pigment corresponds to the vitamin A which the pigment epithelium offers it.

There is relatively limited information on the relationship of nutrition and the eye. Investigators at the Harvard School of Public Health have explored the consequence of acute and chronic nutritional deficiency of specific nutrients, such as vitamin A, vitamin E and selenium. These substances are reported to be concentrated in the retina and are requirements in vision. Acute vitamin A deficiency in rats produced electroretinographic (ERG) defects in 38 to 40 days, although significant retinal degeneration was not observed by electronmicroscopy until 55 to 60 days. All changes were prevented by keeping the animals in the dark for as long as 6 months. These data are in support of observations reported at the State University of New York at Buffalo, namely that light initiates the damage. Deficiency of vitamin E or selenium alone or in combination had no measurable effect on the retina in terms of altered ERG or retinal morphology. These investigators have raised eleven kittens in an attempt to reproduce an insidious blindness. Six kittens were fed vitamin A deficient diets and 5 kittens received a commercial diet to serve as controls. After 5 months an increase in ERG threshold has become apparent in all 6 kittens on deficient diets, and they then progressed to retinal degeneration. The significance of these studies becomes apparent when nutritional blindness can be used as a model for study of a specific visual pigment and photoreceptor defect.

Research activities of investigators at Yale University have been involved with the study of visual pigments of crustacea through microspectrophotometric examination of single rhabdoms in order to ascertain the number and absorption properties of arthropod visual pigments in single photoreceptor organelles, the molecular orientation of the chromophore in relation to membranes, the reaction of the pigments to light as well as the regenerative phase of the visual cycle. In addition, the analysis of invertebrate color vision is

significant in terms of the general problem of information processing in simpler systems in which details can be analyzed in terms of cellular components.

Investigators at Amherst College are studying the biochemistry of the mammalian visual cycle by examining the distribution and proportions of vitamin A within the retinal tissues during light and dark adaptation. By administration of radioactive vitamin A to rats made vitamin A deficient, these investigators can determine the proportions of vitamin A compounds in the subcellular fractions of the pigment epithelium and retina. The experiments extend similar observations made at Harvard University and at the State University of New York at Buffalo. Under continuous bright illumination, 11-cis retinal disappears and all-trans retinal transiently increases. Retinol is formed and diffuses into the inner segment and pigment epithelium where it is esterified. During dark adaptation, essentially the reverse process occurs. Vitamin A can be demonstrated in the pigment epithelium, in the proportions to be expected from the rate at which rod outer segments are being removed and phagocytized. There is evidence that 11-cis retinal is derived from retinyl ester of the outer segment and pigment epithelium. By combining the vitamin A detection technique with cellular fractionation, this group of investigators should be able to relate different stages of the visual cycle to photopigment metabolism.

TRANSDUCTION

Purification of rhodopsin, studies of the chemistry and physiological function of the proteins and analysis of membrane structure of the outer segments of photoreceptors are necessary for an understanding of visual excitation. The physiology of the events which link the initial photochemical reaction to membrane permeability and potential change are essential to understand underlying mechanisms of transduction. A goal of a project at Yale University is to investigate the effect of intracellularly injected cyclic nucleotides. This group has performed many experiments injecting cyclic nucleotides into retinal cells of the compound eye of the horseshoe crab, Limulus. In some cases the injection of cyclic nucleotides increased the membrane potential and amplitude of the receptor response. Because of the complexity of the response, the pharmacological action of cyclic nucleotides cannot be easily interpreted, but work is in progress to clarify the experimental results by an investigation of the relationship of the response, amplitude, latency and waveform to different conditions such as light adaptation or the concentration of various ions in the bathing medium.

The crayfish photoreceptor unit may provide a simple yet versatile system for studying the neural effects of cyclic nucleotides and their interaction with photoreception. Investigators at the Charles F. Kettering Foundation, Ohio, are exploring the effect of cyclic nucleotide levels on the electrical output of photoreceptor cells. The crayfish photoreceptor units function as pacemakers. They integrate incoming impulses from mechanoreceptors, and their spike frequency can be measured with electrodes placed along the abdominal nerve cord. Perfusion with theophylline completely and reversibly abolishes the light response. It is speculated that the effect of theophylline may be as an enzyme inhibitor (for example, of phosphodiesterase) or that it may influence calcium transport.

Research in progress at the Vanderbilt University School of Medicine will permit a more extensive development of a conductance-change model of visual receptor excitation. The effects of changing external concentrations of sodium and calcium ions as well as internal concentrations of the same ions are being studied in relation to changes in sensitivity to light. This investigation may elucidate details of ionic mechanisms generating the light response in photoreceptor cells.

The high activities of enzymes associated with cyclic nucleotide metabolism in the rod outer segment suggest an important role in visual function. Examination of the specificity of these enzymes in the purified state is necessary to determine their role in retinal receptors in response to a light stimulus. Investigators at the University of Colorado Medical School have demonstrated by enzyme kinetic studies that the same enzyme catalyzes the hydrolysis of several cyclic nucleotides. Inhibitors of phosphodiesterase were found to be poor inhibitors of cyclic nucleotide hydrolysis. They were also able to show light inhibition of guanyl cyclase activity of intact rod outer segments and suggest that ciliary microtubules in rod outer segments may exhibit a higher specific activity of the cyclase than do intact outer segments. This knowledge may explain retinal function at the molecular level, however, it is the hypothesis of this group that cyclic nucleotides are involved in adaptation rather than directly in visual excitation.

The calcium binding system of retinal rod disc membranes is being investigated in terms of its interaction with modification by calcium ions, cyclic nucleotides and illumination. If there is a link between calcium ions and phosphodiesterase, then this would have importance in linking calcium with cyclic nucleotide levels in rod outer segments. Investigators at Yale University have investigated this problem and have concluded that observed calcium binding probably does not involve a calcium sensitive phosphodiesterase.

Phosphodiesterase, the enzyme which breaks down cyclic nucleotides, has been found to be present in large quantities in the vertebrate photoreceptor. Investigators at Case Western Reserve University have found that this enzyme is soluble and not membrane bound. It can be cleared out of the photoreceptor after water washes. The enzyme inhibitor theophylline appears to have little effect, and the activity does not depend upon light. Other phosphatase enzymes found in bovine photoreceptors are normally membrane bound and are not light dependent. The question of cyclic nucleotide as the signal molecule which gives rise to the nerve impulse remains unresolved. The nature of the membrane mechanisms responsible for generation of light-evoked photoreceptor potentials remain to be clarified.

MEMBRANES

The process of vision begins with the absorption of light by visual pigment; however, the exact sequence of events which result in the production of a receptor potential have not been completely described. Several approaches are required to study the process of visual transduction; these studies involve visual pigment structure and conformational changes, photoreceptor membranes and nature of the receptor potential.

Membranes have critical roles in the function of photoreceptors. The rod outer segment membranes may also serve as a simpler model for the study of cell membranes, in general, because they have a limited number of components and are specialized in function. Investigators at the University of California, Santa Cruz, and at Case Western Reserve University, point out that rhodopsin has been thought to be involved in hydrophobic interactions in the disc membrane on the basis of the fact that rhodopsin is insoluble in aqueous buffers but can be solubilized in detergent micelles. Experiments indicate that rhodopsin is located in the hydrophobic interior of the membrane. The disc membranes appear to consist of a phospholipid bilayer with the rhodopsin in the interior. Local disruption of the disc permeability barrier at the site of light absorption with release of a transmitter substance to the plasma membrane may be involved in visual excitation.

A few laboratories are investigating the utilization of light by model rhodopsin compounds and the mechanism of photoisomerization of the visual pigment. Maximum quantum efficiency of photoisomerization is desirable, and therefore, it is useful to know whether certain structural features of rhodopsin are involved. A possible mechanism for the initial event is a permeability change which occurs in the disc membrane by the absorption of light by rhodopsin. A research group at the University of California, Berkeley, have been developing and studying a model system which contains rhodopsin in a phospholipid bilayer. With this system, it is possible to investigate light-activated membrane ion-gating properties of rhodopsin free of other receptor components.

The description of membrane structure and light-induced events of the molecular level in the membrane should lead to fundamental understanding of transduction. By use of X-ray diffraction, investigators at the University of Pennsylvania believe that they have determined the location of rhodopsin within the photoreceptor disc membranes and the forces responsible for this location of rhodopsin within the photoreceptor disc membranes and the forces responsible for this location and the local arrangement of rhodopsin over the surface of the disc membrane. Completion of these analyses will allow a more detailed evaluation of light-induced changes in the membrane structure and the mechanism of energy transfer.

It has long been known that rhodopsin molecules undergo chemical changes, triggered by absorption of light quanta, which eventually lead to nerve signals. One such reaction, the light-stimulated isomerization of 11-cis retinaldehyde, the prosthetic group of the rhodopsin molecule, was discovered nearly twenty years ago. Other reactions of rhodopsin which may lead to neural excitation, to adaptation, or to regeneration of visual excitability, have proved more elusive. Within the past year, investigators at the Johns Hopkins School of Medicine and, at the University of Wisconsin have found that rhodopsin molecules are phosphorylated by the terminal phosphate group of adenosine triphosphate (ATP) in a reaction which is markedly stimulated by light. The reaction requires magnesium but shows no sensitivity to cyclic AMP. Only one site on the rhodopsin molecule, probably a serine residue, is phosphorylated. The reaction occurs slowly (peak phosphorylation requires 10 minutes in the light at 37°C.), and therefore, it may be too slow to play an immediate role in visual excitation. In view of its striking light sensitivity, and the fact that phosphorylation of proteins in other membranes may have physiological

significance, this discovery may be an important advance in our understanding of the biochemistry of the visual process.

MACULAR DEGENERATIVE DISEASES

PROBLEM

The optical structures of the human eye focus light on a distinct area of the retina which is located temporal to the optic disc. This area is called the fovea centralis and possesses the greatest acuity of any portion of the retina by virtue of its high concentration of cone (day-light) photoreceptors. The region at whose center the fovea centralis is located is normally darker in color than the remainder of the retina because it contains a yellow pigment and is referred to as the macula lutea or macular region. The name is derived from the more yellow color which is due to a xanthophyll pigment. Because it has the greatest acuity of any portion of the retina, and because it is used exclusively for most visual tasks except for vision in very dimly lighted surroundings, minute lesions which might not cause visual discomfort in peripheral areas of the retina do cause serious loss of central visual acuity and color discrimination when located in the macular region. This region of the retina appears to be involved in a variety of retinal degenerative conditions. In addition, the macular region is subject to all the pathological alterations which may develop in other areas of the retina. The abnormalities associated with the macular region of the retina are of special concern because of the debilitating consequences of the loss of central visual acuity and depressed color vision. Collectively, these pathological conditions are referred to as macular degenerations. For the most part, these disorders have not been fully studied or explained. Macular degenerations are occasionally found in infancy, adolescence or young adulthood, but their incidence and prevalence increase markedly with aging. Some types of macular degeneration appear to be inherited on a genetic basis. Usually the familial types of macular degeneration are bilateral symmetrical and progressive. In later stages of the disease, it is usually necessary to use low-vision aids which will allow the subject to continue activities such as reading which require high visual acuity. Other macular degenerations may develop as a result of vascular or inflammatory diseases. These are often unilateral or asymmetric and may not be progressive. In general, the genetic defects at the molecular level, the etiology, the natural history and possible therapeutic measures are unknown or poorly known for these diseases. Until more information is obtained, clinical ophthalmologists can do little more than observe the development of macular degenerations, limit therapy to symptomatic measures and localize the disease process on an anatomical basis.

CLASSIFICATION

Investigators at the Johns Hopkins University point out the existing confusion of classifications which have been devised in the absence of adequate knowledge of the etiology and pathogenesis of diseases of the macula. The classification systems in use are based on combinations of descriptions of clinical features, age of onset, functional tests, anatomical location of lesions and hereditary patterns. They suggest that macular diseases be placed in categories which are based on the anatomical localization of the most evident pathological change which can be identified. In part, the confusion persists due to the dearth of studies of pathological material.

VITREORETINAL SURFACE DISORDERS

The formation of new blood vessels may accompany pathological processes. Neovascularization is frequently the site of hemorrhage into the vitreous with the possible formation of degenerative fibrous proliferation. Subsequent retinal detachment with the region of the macula may result. Although photocoagulation may be effective in the destruction of neovascularization at the surface of the retina, it is not effective in destroying blood vessels which form within the fibrous bands in the vitreous. Secondary retinal detachments caused by vitreous strands may be prevented by removal of the strands. Eyes with vitreous opacities also interfere with treatment by other modalities, such as photocoagulation.

The vitreous is a gelatinous material whose structural components consist of hyaluronic acid and collagen fibers. It is possible to replace the vitreous with inert fluids such as saline. However, it is necessary to remove the intravitreal hemorrhages, surface vessels and fibers. Laboratories at the University of Illinois and at the Bascom Palmer Eye Institute are developing instrumentation and are perfecting the technique for surgical removal of diseased vitreous by use of instruments which are introduced into the eye through the pars plana. The operation of the instruments is observed through an operating microscope and a contact lens on the cornea. Among the major differences in the two instruments is the cutting principle. The instrument being developed at the University of Illinois is called the "vitrophage" which chops the vitreous fiber. The instrument being developed at the Bascom Palmer Eye Institute operates using a rotary cutting action, and is called the "vitreous infusion suction cutter (VISC)". At the Bascom Palmer Eye Institute, 130 patients have been operated on and 100 cases have been statistically evaluated. Twenty of these cases had opaque vitreous due to hemorrhage; 29 cases are judged to be successful on the basis of visual acuity improvement. Some cases have gone from detection of hand movements to 20/40 acuity, postoperatively. It is anticipated that these studies will lead to improved techniques for cutting of intravitreal traction bands and removal of hemorrhages.

There are many kinds of fibroproliferative and vasoproliferative lesions which may occur on the retinal surface and in the overlying vitreous body. These lesions may cause loss of visual acuity due to hemorrhage or eventually due to extensive fibrous traction bands and retinal detachment. The availability of surgical and autopsy specimens have provided an opportunity for investigation of the anatomy of the vitreo-retinal junction at the Jules Stein Eye Institute, UCLA. The study has revealed marked topographical differences in the thickness of the inner limiting membrane of the retina and in its attachments to the foot plates of the Müller fibers of the retina, as well as differences in the attachment of the vitreous to the retinal surface. These topographical variations may be important in the development of proliferative vascular lesions on the retinal surface. A study of the surface of the optic disc margin and the peripapillary retina confirmed the presence of a thin basal lamina of adjacent retina. Study of the vitreoretinal membranes indicates that epiretinal membranes are acquired lesions which are possibly related to focal ischemia of the retina.

NEURAL RETINA DISORDERS

An anatomical and physiological defect may be preceded by, as well as accompanied by, chemical events. The molecular pathology may be at the level of blockage of a metabolic pathway, a chemical deficiency or an intramolecular rearrangement. There are clinical syndromes associated with faulty metabolism of glycolipids, glycoproteins, and polysaccharides. Inherited storage diseases, such as the sphinogolipidoses and the mucolipidoses, demonstrate complicated syndromes involving changes in the macula which may be useful in clinical diagnoses. Sophisticated biochemical studies are being conducted at the University of Illinois in order to develop precise diagnosis in anticipation of future treatment. This team of investigators has described a family with characteristics of complex lipid storage disease with macular coloration and B-galactosidase deficiency in conjunctival biopsy.

Investigations concerning the retinal biosynthesis of mannose containing glycoproteins is useful in understanding the molecular derangements in the nervous elements of the macula. Several of the sphingolipidoses involve derangements in the metabolism of sialic acid-containing glycolipids. Investigators at Case Western Reserve University point out that there is a paucity of information available concerning the chemical nature and biosynthesis of sialic acid-containing heteropolymers in retina. In addition, this group has obtained evidence which show the existence and properties of enzymes in the retina which catalyze the biosynthesis of mannose containing lipids and glycoproteins. The subcellular distribution of these enzymes in the neural retina revealed that these enzymes are located in cell nuclei.

Further information with regard to retinal lipids may provide a basis for examination of the role of lipid metabolism in maintaining the metabolic energy in support of the visual process and neural elements. A study at Wayne State University compared the fatty acid composition of the chicken retina with values obtained in ox and human retinas. The overall phospholipid composition of checken retina is different from other retinas principally in that the phosphatidyl-ethanolamine (PE) fraction is greater than phosphatidylcholine (PC) fraction. In rabbit, bovine and frog retinas, the PC fraction is always greater than the PE. Visual cell mitochondria have been isolated and shown to be functional *in vitro*. Lipid structural studies for whole retina and for subcellular components of photoreceptors are still in progress.

Phosphatidic acid holds a unique position in phospholipid metabolism in that it may provide precusors for pholphatidylinositol, lecithin and phosphatidylethanolamine. A project which was conducted at the Mount Sinai School of Medicine, New York, and currently at the George Washington University has observed that retinal phospholipids can be synthesized by both mitochondrial and microsomal fractions, and that the synthesis of lecithin by retinal homogenates is significantly reduced when pigment epithelium is removed prior to homogenization. These data suggest that phospholipids of the photoreceptors can originate from particulates from at least two structures. The relation of metabolic anomalies to structural defects opens possible areas of study of hereditary macular degenerative diseases. In addition, the possibility of control of synthesis of phospholipids by diet and pharmacological agents or the uptake of preformed lipids by retina can be considered.

The question of whether abnormalities in retinal polyunsaturated fatty acid metabolism may be related to retinal degenerations has been considered by investigators at Baylor College of Medicine and at the Jules Stein Eye Institute, UCLA. The phospholipid class composition and fatty acid composition of normal and dystrophic rat retinas are similar. Therefore, the question of whether polyunsaturated fatty acids are necessary for normal retinal structure and function remains unsolved. These investigators have found that in the rat, the level of retinal polyunsaturated fatty acids cannot be lowered by raising rats on fat-free diets. This study appears to weaken arguments for the control of metabolic and of structural defects through diet.

VASCULAR DISORDERS

The outermost part of the retina receives its blood supply from a vascular sheet composed of three layers of blood vessels. The inner most layer is called the choriocapillaris, and consists of a dense network of dilated capillaries which extend from the optic disc to the ora serrata. The foveal region is dependent upon the choriocapillaris for its entire nutrition, since it is the primary blood supply to that region. A common cause of macular degeneration is presumed to be alterations of the blood supply from the choriocapillaris. Reduction in the blood supply due to sclerosis of the choriocapillaris is not evident in its early stages by ophthalmoscopic examination. Because evidence of such macular degeneration may appear late in life, it is labelled as senile macular degeneration.

The elderly now represent approximately 10% of the population in the United States. People over 65 years of age show an increase of senile macular degeneration. A study of more than 1,000 elderly people conducted at the Jewish Home and Hospital for the Aged, New York, indicated that 30% had some macular degeneration. The incidence increased to 38% in the over 80 years age group. The emphasis in this project has been on clinical observations and pathological specimens of patients with macular disease. The efforts will be in the direction of physiological studies and fluorescein angiography of the retinal circulation in the aged. Examination of the choroid in postmortem eyes is in progress in order to study the macular region in detail.

The ability to measure retinal and choroidal blood flow in a quantitative fashion using arterio-venous oxygen differences in the choroidal and retinal vessel should contribute the assessment of the role of the vasculature in chorioretinal diseases. At Boston University Medical Center, patients involved in macular degenerative studies are studied with photographic and scanning eye oximeters which are being developed there. A total of 91 patients with central serous chorioretinopathy have been observed. Twenty-seven of these patients have been followed carefully enough to document the natural history of this disease. The disease is self-limiting and little benefit seemed to have occurred from treatment with photocoagulation. The onset of the condition was observed in the 4th and 5th decade with a minimal to moderate deterioration of vision. Chief complaints are blurring of vision and central scotoma. Macular degenerations of the senile type are considered to have a vascular pathogenesis; however, it is not clear whether retinal lesions are the result of occlusive or exudative phenomena in the choroidal vasculature.

Investigators at the Bascom Palmer Eye Institute remain hopeful that photocoagulation might influence the course of senile maculopathies. However, the data obtained from 53 treated patients remains inconclusive. Branch vein occlusion has resulted in macular edema with associated visual loss and is receiving attention from clinicians who advocate early photocoagulation. Patients who have been examined but untreated at this institution since 1963 are now being called again in an effort to determine the natural history of this disease. Based upon the data to be obtained, a modality of therapy, such as a photocoagulation, may be considered.

PIGMENT EPITHELIAL DISORDERS

Prior to the more sophisticated biochemical studies of the pigment epithelium, assignment of functions to this structure were speculative. Based upon anatomical appearance and location, the pigment epithelium would appear to serve as a support for the photoreceptors and to provide a pigmented absorptive sink for extraneous light energy. By virtue of its position between the photoreceptors and the choriocapillaris, the pigment epithelium may serve in the transport of nutrients and waste products to and from the photoreceptors. Senile macular degeneration is believed to be related to disorders of the pigment epithelium and the choroidal vascular system. The etiology of possible choroidal vascular disease has not been completely identified; nevertheless, changes in the macular region may have their primary source in the choroidal vasculature. It is now believed that the pigment epithelium has additional metabolic and biological functions, such as the synthesis of the interphotoreceptor mucopolysaccharide matrix, removal of discarded rod discs, and vitamin A storage and release for use by the photoreceptors.

These investigations into the complex role of the pigment epithelium seem to strengthen the view that failure of this tissue may be a primary source of some forms of macular degeneration. The problem is being approached by investigators at the University of California, San Francisco. The lysosomal system and phagocytosis by the pigment epithelium are to be studied by cytochemical techniques in order to learn how the system participates in the removal of photoreceptor outer segments. Such studies will involve normal and diseased retinas. Three human and eleven rabbit eyes have been used to study the localization of aryl sulfatase, 5'-nucleotidase and acid phosphatase. Aryl sulfatase has been localized in lysosomes; the Golgi apparatus of pigment epithelial cells does not show the presence of this enzyme. Photocoagulation has been used to produce focal destruction of the retina in order to produce an abundance of rod outer segments and to overload the pigment epithelium. This method stimulated an increase in hydrolytic enzyme activity in phagosomes, lysosomes and vesicles in the vicinity of the Golgi apparatus. These results suggest that lysosomes and vesicular structures participate in the transfer of acid hydrolases from the Golgi complex to the phagosomes.

GENETIC STUDIES

Under the direction of investigators at Duke University, a family with early onset of autosomal-dominant macular degeneration has been traced through 7 generations and 250 individuals. A subgroup of this family with aminoaciduria has been observed. In some cases, macular diseases may be the result

of chronic choroidal ischemia due to changes in vascular caliber. This same group of investigators has been studying the responses of choroidal vessels to oxygen and carbon dioxide breathing.

During the past three years, investigators at Johns Hopkins University have identified 9 families with vitelliform (Best's) macular dystrophy. The pedigrees contain 470 living persons. The mode of inheritance is autosomal dominant, and, therefore, it is possible that as many as one-half of these people carry the abnormal gene. Since 90 percent of the pedigree members live within an accessible distance from this clinic, this population remains available for study. The excellent cooperation of these families provides an opportunity to follow clinical history and pathogenesis. The detection of the genetic history in this macular degenerative condition can be an important contribution to the human genetic map as related to functional testing which can be performed in a clinical setting.

ANIMAL MODELS

A study at Johns Hopkins Hospital has been divided into serous and hemorrhagic detachment of the macula. The group has acquired older monkeys with naturally occurring maculopathies which are similar to those observed in humans. The emphasis is upon the experimental production of macular detachments and subretinal neovascularization. Macular lesions will be followed as they develop in the monkey. The animals are being studied with fluorescein and indocyanin green angiography. The eye from one of these monkeys is being studied by light and electronmicroscopy. Subretinal vascular leakage and neovascularization in the region of the macula has been observed in a few animals. The value of this experimental model is being emphasized by this research team.

Simultaneous recording of the passage of fluorescein and indocyanin green dye would provide information on the relationship of choroidal and retinal blood circulations to the macular area. Both dyes are water soluble and mix well in solution. The pigment epithelium acts as a natural barrier which optically separates the two circulatory systems. In a project at the Johns Hopkins University, a fundus camera has been modified to permit simultaneous photography of the passage of dyes selectively through retinal and choroidal circulations in the same subject using a single dye injection. Studies are being pursued in adult rhesus monkeys and in human subjects.

A type of central retinal degeneration can be found in approximately 30 percent of all Siamese cats at five years of age and older. Some of these cats develop serous detachments in their area centralis which is functionally similar to the macular region in man. Breeding programs are in progress at the Johns Hopkins University and at the Montefiore Hospital and Medical Center, New York. The model is similar to some forms of human macular degeneration. At both institutions, studies of both the genetics and electrophysiological variations are under study. Although angiographic techniques are difficult in the cat, double dye injection studies will be undertaken.

The critical role of the pigment epithelium in maintaining the retina has also been recognized by investigators at the University of Oregon Medical School. In collaboration with investigators at the Hadassah Hospital, Israel, they are utilizing biochemical and morphological techniques to examine a series of developmental stages of laboratory animals to establish that the initial appearance of macromolecules in the interphotoreceptor space is in the form of a polysaccharide-rich cell coat which forms an integral part of the apical surface of the pigment epithelium. The study implies that the pigment epithelium is capable of synthesizing the interphotoreceptor mucopolysaccharide matrix. This project will attempt to assess the role of synthetic and phagocytic activities in human pigment epithelium.

The maculas of rhesus monkeys exposed to the indirect ophthalmoscope for 1 hour show a disruption of the pigment epithelium and choriocapillaris, as well as degeneration of photoreceptors. At the Armed Forces Institute of Pathology, Washington, D.C., investigators find that there is an apparent regeneration of the photoreceptors and the pigment epithelium 3 to 5 months after the exposure to light. Whether the regenerated pigment epithelium will function in a normal manner remains to be determined. The project will define the basic reparative processes. This team of investigators established photic maculopathy in two rhesus monkeys which had undergone behavioral training for evaluation of visual acuity. On the basis of visual acuity and morphological changes, they concluded that the regenerated structures were functional. These experiments help explain the clinical observations of patients who do recover from light induced maculopathies. It is possible that no treatment will be effective for late stages of macular degeneration, but in the early stages the process may be prevented from progressing if the causative factors can be identified.

RETROLENTAL FIBROPLASIA

PROBLEM

Retrolental fibroplasia (RLF) has been referred to as a retinopathy of prematurity because it may be seen in the first month after birth of the premature infant. The earliest changes are clinically evident to careful observers prior to the tenth week of life. RLF became the largest cause of blindness in infants by the early 1950's; however, the problem appeared to be resolved when the relationship of high oxygen tension in incubators where premature infants were kept to the development of RLF was understood. The need for high oxygen for the premature infant with respiratory distress continues to pose a threat to the vision of these infants. There is need to develop careful monitoring programs and training of clinical observers in order to minimize the risk of retinopathy. RLF usually involves both eyes, and may progress to retinal neovascularization, hemorrhage, traction on the retina with serious effects on macular function or detachment of the retina, and in some cases, total blindness. Research is needed in order to determine safe arterial oxygen levels at which premature infants can be maintained with minimum risk of inducing RLF.

ANIMAL MODELS

Correlations between light, blood vessel caliber, ambient and blood oxygen tensions may provide information with regard to cause and early diagnosis of onset. The effects of visible light on retinal blood vessels and receptors as influenced by hyperoxia, hypoxia, hypercapnea and hyperbilirubinemia have been under investigation at Temple University School of Medicine. Newborn piglets and Gunn rats with congenital hyperbilirubinemia have been selected as animal models. Twenty-six piglets and 20 Gunn rats have been exposed to 8, 18, 40 and 100% oxygen with and without light. The animals have been examined by indirect ophthalmoscopy, electroretinography, histopathology of the retina, and determination of blood gases during exposure. Retinal photography and electronmicroscopy was performed on selected tissues. The results have shown that light alone produced destruction of the photoreceptors, edema in the outer retinal layers and neovascularization of the ganglion cell layer. Forty percent oxygen produced some constriction of retinal arteries with slight proliferation of small arteries near the disc; the changes were more pronounced at 100% oxygen. Oxygen at 40% and 100% levels with animals exposed to blue light produced generalized retinal edema, vasoconstriction and deterioration of photoreceptors and cell nuclei in the ganglion layer of the retina. The data indicate that hyperoxia with high intensity light have greater deleterious effects on retina than do either alone. At the State University of New York, Buffalo, further studies of the susceptibility to damaging light induced by temperature, abnormal oxygen environment and drugs affecting general and specific enzymes involved in membrane transport are in progress. These studies have implications for photocoagulation therapy and the management of newborn infants in oxygen.

Investigators at the Johns Hopkins Medical School have located a beagle farm where approximately 900 female dogs are bred yearly. Such a source of

supply has enabled this group to obtain beagle pups with documented birthdates. The beagle pup develops the same retinal vasoconstriction when exposed to high oxygen concentration, as does the kitten. However, after a carefully quantitated study measuring arterial blood oxygen levels and retinal vasoproliferative response in approximately 75 beagles, it was concluded that the proliferative lesion is not as uniform as that observed in kitten hyperoxia experiments. Therefore, the cat may be preferable to the dog for RLF studies. The precise quantitation of the experimental lesion will serve as a background for studies to isolate possible factors liberated by the ischemic retina which stimulates retinal vasoproliferation.

A project at the University of Virginia proposes to provide cytochemical and morphological information on changes which are coincident with retinal development and regeneration in amphibia, chick and mouse. The effect of vascular patterns on the distribution and nature of Müller cells and interaction of pigment epithelium and neural retina are under investigation. These studies will provide a foundation for investigation of pathological variations in the development of the retina, particularly the structural alterations observed upon establishment of the vasulature.

The precise timing of retinal morphogenic events is significant in understanding developmental anomalies, such as those found in retinopathy. A detailed analysis of the ultrastructure of the retina of the Japanese quail is in progress at the Wayne State University. These investigators note that in this model there are several features which indicate considerable fluid transport through the choriocapillaris endothelium. Analysis of short term ferritin studies reveals rapid passage of this tracer molecule from the choriocapillaris through Bruch's membrane and into the basal infoldings of the pigment epithelium. Staining of cell surface coatings in combination with ferritin may determine the importance of surface coatings in endothelial transport.

Information to be obtained from a project in progress at the Bascom Palmer Institute, University of Miami, illustrates that the general problem of health care in eye diseases may rest in the opportunity to study in vivo changes of the relative blood flow under various conditions. The monitoring of oxygen tension in the preretinal vitreous should prove useful in a variety of retinal toxicity and degenerative conditions. These investigators have developed a method for estimation of relative blood flow which is based upon dye dilution curves constructed from densitometry measurements on fluorescing vessels visualized in fundus angiograms. This group has used their techniques to study the effects of hyper-and hypocapnia in monkeys. Increased arterial carbon dioxide tension was found to cause expansion of the vascular volume and shortening of the mean circulation time. The preretinal vitreous oxygen tension was measured at different arterial carbon dioxide tension levels. The amount of oxygen diffusing through capillaries into the retinal and preretinal tissue varied directly with the arterial carbon dioxide tension. The role of retinal oxygen consumption rate and blood flow changes are also under consideration.

The predisposition of the temporal peripheral retina to RLF raises the possibility of a peculiar susceptibility to retinal ischemia in this area. Information obtained on oxygen tension levels and arterial-venous oxygen differences in selected zones of the retina may be useful in understanding the pathogenesis of RLF. Studies have been designed at the Johns Hopkins Medical School in order to elucidate an understanding of the diffusion of oxygen through the layers of the retina and to investigate the effects of hyperbaric oxygen on retinal ischemia.

DIAGNOSIS AND TREATMENT

The information which will be obtained from an ongoing collaborative study of blood gases and eye examinations will have direct application to the safe clinical management of oxygen therapy in the premature infant. This study will provide new information on the natural history of RLF and its earliest ophthalmoscopically demonstrable signs. The results obtained from five participating hospital nurseries have been forwarded to the Coordinating Center at Oakland University, Michigan, for analysis. The participating clinics have submitted 1,050 premature infants to the study. Fifty-five of these infants have developed RLF, and of these, 14 have progressed to the cicatricial form of the disease. Accompanying factors to be analyzed and which are considered to be possibly detrimental to the premature infant and to be possibly involved as contributing agents are arterial oxygen, environmental oxygen, time in oxygen at concentrations above 20%, pH, arterial carbon dioxide, single versus multiple births, sex, birthweight and gestational age. Analyses have been conducted in such a way as to discriminate between level and time spent in an altered gaseous environment. There appears to be an overwhelming inverse relationship between birthweight and incidence of RLF. To date, the only factor, in addition to birthweight, which is positively associated with the development of RLF is the length of time the infant is kept in an oxygen enriched environment. The analyses are preliminary but indicate that a sufficient number of infants have now entered the study to permit statistically valid conclusions.

PHOTORECEPTOR DEGENERATIVE DISEASES

RETINITIS PIGMENTOSA

PROBLEM

Some diseases of the eye show a familial tendency. There is a form of retinal degeneration which progressively worsens throughout life and may result in blindness by middle life in a small percentage of the victims. This group of retinal diseases is referred to as retinitis pigmentosa. The early signs of the retinitis pigmentosa syndrome may appear in the first decade of life in the form of night blindness. A peripheral ring of depressed vision may develop and spread centrally through life until only a narrow central field of vision remains. This condition permits reading vision but the absence of peripheral vision makes movement in unfamiliar surroundings difficult. As more families with this disease present themselves for study, there will be more pedigrees available. There are family histories which demonstrate that the retinitis pigmentosas are inherited in a number of genetically determined fashions: as an autosomal recessive; as an autosomal dominant; and as a sex-linked characteristics. Retinitis pigmentosa usually occurs as a single entity with bilateral loss of vision. However, it may be part of a series of complex syndromes which may involve obesity, mental retardation, hypogenitalism, polydactylism, and hearing loss and can also be accompanied by high myopia, cataract and glaucoma. There are families which show the condition of sector retinitis pigmentosa; as well as those who show the varieties of autosomal and sex-linked inheritance patterns. However, in the sector disease, the visual field loss does not spread uniformly across the retina in a ring form, field loss is found only in patches of the retina. Nevertheless, some vision loss can be detected in the "normal" areas of the retina. It becomes a matter of degree and distribution of retinal degeneration within a given diseased eye. Sector retinitis pigmentosa has been shown to be less progressive and can be distinguished from widespread retinitis pigmentosa which is progressive, on the basis of ophthalmic and clinic tests.

ANIMAL MODELS

The sequence of events which occur as the retina is formed, and as it renews itself once formed, need to be better understood. Investigations which trace the path of biologically active molecules in the retina from synthesis to degradation have as a major objective clarifying the understanding of metabolic activity and function of the specialized cells in the retina. A continuing interest in the function of enzymes in normal and dystrophic retinas and their relationships to visual process have lead a research team at the Boston Biomedical Research Institue to design a study of the uptake and metabolism of glutamate and glutamine under various conditions within the retina. Glutamate is of interest because of its influence on the inner retinal layers after parenteral administration in immature mice, its possible role in neurotransmission and in ammonia detoxification. Particular emphasis has been focused around the induction of the enzyme glutamine synthetase. Fetal human and bovine retinas have not shown an induction of glutamine synthetase activity by steroids, as do avian retinas, in tissue culture. However, both

mature mammalian and avian retinas do have glutamine synthetase activity.

Data from a variety of experimental approaches can extend the knowledge of cellular differentiation, growth, and function in normal and degenerative retina. The C3H mouse has an hereditary retinal dystrophy which may serve as a model which will assist in defining the primary biochemical abnormalities of retinal degenerative disease. The research program at the Veterans Administration Hospital, Sepvlveda, California, has presented evidence that the disruption of glucose and energy metabolism in the C3H mouse retina occurs after the disease is well advanced and that the degenerative condition develops in conjunction with the differentiation of photoreceptor cells. Cyclic-adenosine monophosphate (cyclic-AMP) and cyclic-AMP phosphodiesterase (cyclic-AMP-PDE) enzymes have been implicated in the differentiation and function of vertebrate retina. In the normal mouse retina, cyclic-AMP-PDE increases 8-fold between the 6th and 20th postnatal day. Kenetic studies of the retinal reveal the presence of the enzyme in inner layers of the normal newborn mouse. However, after the 6th postnatal day, a second cylic-AMP-PDE can be demonstrated which coincides with the differentiation and growth of photoreceptor outer segments. In the C3H mice, the postnatal increase in the specific activity of the cyclic-AMP-PDE is substantially lower than in the normal retina. This project is an attempt to quantitate the biochemical events and to devise a rationale by which retinal degenerative disease can be circumvented.

Several hereditary photoreceptor disorders have been reported in a number of purebred dog breeds. A colony of Alaskan malamute dogs are being bred and maintained at the University of Pennsylvania School of Veterinary Medicine for the purpose of correlating the functional and fine structural abnormalities responsible for selective death of the retinal cone photoreceptors. The day-blindness (hemeralopia), reported in the Alaskan malamute, is inherited as a simple recessive disorder, which occurs by 8 weeks of age. In some dogs, the loss of photopic vision can occur as late as 6 months of age. The dogs show a concomitant loss of cone vision and photopic electroretinogram (ERG). Light microscopic examination fails to demonstrate a change in retinal architecture of affected dogs before and after development of day blindness. Quantitative electron microscopic analysis showed a decrease in cone nuclear density of day-blind dogs, which is probably the result of photoreceptor degeneration rather than the cause of photopic dysfunction. Preliminary work has confirmed that day-blind dogs fail to respond to high intensity intermittent light stimulation at both high and low frequency levels. Distinct electrophysiologic abnormality has permitted this research team to identify dogs affected with day-blindness and to correlate fine structural abnormalities with functional deficit. Retinas from 4 affected dogs, 6 months to 4 years old, with the missing photopic component of the ERG have been studied. The 6-month old hemeralopic dog had approximately the same rod/cone ratio as normal age-matched controls. Cone inner and outer segments were normal. The striking abnormality is present in the perinuclear cytoplasm of almost all cone photoreceptors. The progressive nature of this disease is indicated by the complete absence of the cone photoreceptors in dogs 2 years of age and older. In these hereralopia dogs, cone function is normal at first and then deteriorates. Associated with the dysfunction is a neurofibrillar degeneration which appears in the cones. This animal model may prove to be extremely important in relation to the study of photoreceptor (cone) function in humans.

When light is flashed in the eye, there is an electrical response called the early receptor potential (ERP), which is believed to be associated with intramolecular displacements of electrical charge in visual pigment molecules induced by light. The results of a project at the University of Pittsburgh are relevant to the study of retinal degenerative diseases in which there are defects in the adaptational mechanisms. During the past year, an apparatus was built which makes it possible to deliver two flashes of light to the retina. The first flash isomerizes the visual pigment, and therefore produces photoproducts of bleaching. The second flash of light follows after a time interval of milliseconds to minutes and irradiates these photoproducts which may be photoreversed to form intact visual pigment. This research approach is an attempt to elicit electrical responses from the irradiated photoproducts. If the cone photoproduct responses do exists, they have wave forms and time courses almost identical to the ERP generated by the unbleached photopigment. The significance of this research lies in the fact that early receptor potential (ERP) and photoproduct responses make it possible to obtain information about the photopigments and their products. Information about photoproduct decay and photoreversal will provide better understanding about the generation of the ERP and visual excitation. The ERP may be useful in defining defects in visual pigments in subjects with progressive cone degenerations, as opposed to the electroretinogram (ERG) which is generated by later events in the visual process.

The relationship between the layer of cells next to the retina (pigment epithelium) and the photoreceptor cells has been further clarified by ongoing investigations at the Jules Stein Eye Institute, UCLA, and at Harvard University. An hereditary dystrophy is found in the Royal College of Surgeons (RCS) breed of rat. The dystrophy is characterized in its early stages by an abnormal accumulation of rod outer segment-like membranous material at the interface of photoreceptors and pigment epithelium. The hereditary retinal disorder which occurs in the RCS rats has been regarded as a model for retinitis pigmentosa. Although there are parallels between human retinitis pigmentosa and hereditary retinal dystrophies of the rat , the similarity of these diseases is still speculative. However, studies of rats with retinal dystrophy are based upon the assumption that a better understanding of events in the animal condition will shed light on the human disease. Rats with dystrophic retinas have pigment epithelium cells which do not phagocytize debris from the retinal rod cells. This results in the death of these visual cells and blindness. Such a comparison of the normal and pathological changes that can occur in rats with and without retinal dystrophy may increase our understanding of human retinal degeneration. After the dystrophic rats are five weeks old, the production of outer segment material gradually ceases, the rod cells degenerate and the outer segment material is possibly removed by the inner retina. The histologic appearance of dystrophic retinal tissue when examined under an electron microscope is similar to that in the end stage of photoreceptor degeneration in man. Explanations for the failure of the pigment epithelium to phagocytize outer segment discs are speculative. The rod outer segment membranes may be abnormal and prevent the pigment epithelial cells from engulfing them or the phagocytic mechanism of the pigment epithelial cell may be at fault.

By breeding the gene for retinal dystrophy into strains of rats, the disease has a slower progression, and the animal model is more amenable to

laboratory investigation. Furthermore, expression of the gene delaying retinal degeneration is influenced by visible light, and this concept is applicable to man and a fairly accurate model of retinitis pigmentosa. Investigators at Harvard University in collaboration with the team at Children's Hospital Medical Center have found that RCS rats respond to visual stimuli even though their photoreceptors have completely degenerated and disappeared. Precautions have been taken to ensure that these animals are not responding to other stimuli. When the iris is immobilized by drugs, these animals continue to show a comparable behavioral response to light. However, when the eyes are removed, the animals no longer respond. The spectral sensitivity of these animals is under study. The RCS rat pigment epithelial cells seem to have an unusual protein synthetic role and appear to be a source of rhodopsin-containing material, which may contribute to the unusual behavioral responses observed in the RCS rat.

An analysis of the mechanisms by which visible light produces irreversible damage to photoreceptors, the relationship to hereditary visual cell degeneration and to the availability of vitamin A are under investigation at the State University of New York at Buffalo. It has been suggested that in rats with hereditary retinal dystrophy there may be a deficiency of a factor or a carrier which serves in the transport of retinaldehyde between outer segments and pigment epithelium. In addition, the photoreceptors of these animals depend upon a continuous supply of vitamin A for survival. Regeneration of rhodopsin does not occur when the pigment epithelium is not attached to the retina and retinol remains in the retina. The isolated preparation of an intact retina, attached to the pigment epithelium, has been obtained. This retinal preparation retained an almost normal electrical function for at least two hours, and regeneration of rhodopsin occurs after light exposure. This in vitro preparation may be useful for study of vitamin A transport, adenyl cyclase, metabolism and damaging effects of light in retinas from animals with hereditary visual cell degeneration and as a laboratory model for retinitis pigmentosa.

As previously indicated, the rod outer segments, and not the cones, are continually renewed by repeated formation of new membranous discs at the base of the rod outer segment, coupled with intermittent shedding of old discs at the apical end of the photoreceptor. The investigators at UCLA have demonstrated that most of the protein transported from the inner to the outer segment is used in this manner by the photoreceptor to synthesize new discs and visual pigments. The newest aspect of the study has shown that a small portion of the protein spreads among the old discs where it may be replacing molecules, other than visual pigment, which may have been lost or metabolized in some manner. In cones, all the protein delivered to the outer segment of these receptors is delivered to the discs as a diffuse protein renewal. The diffuse protein renewal process is similar in rods and cones but the rate at which it occurs is higher in the cones.

DIAGNOSIS

Families are entering controlled studies in order to establish a base for the differential diagnosis of retinitis pigmentosa. In this manner genetic counseling may be provided. Early diagnosis may enable exploration of thera-

peutic regimes which may relate to the effects of light, nutrition and drugs which have unique affinities and effects on the retina. Reduced scotopic (rod) vision and progressive loss of peripheral fields and blurred vision are the early symptoms of retinitis pigmentosa which the patient is aware of. Examination of the fundus at advanced symptomatic stages will reveal narrowing of blood vessels, a yellowing of the optic disk and deposition of pigment with irregular processes. The pigmentation of retinitis pigmentosa is distinguished from the secondary pigmentary degeneration of other diseases by pigment distribution and location, quantity of pigmentation and scotoma patterns.

Ophthalmic examination of the fundus is practical in the late stages of retinitis pigmentosa; however, early detection requires more sophisticated analyses. Electrophysiological methods have been used in the study of carriers, asymptomatic and minimally symtomatic individuals and siblings. In an effort to understand the early stages of retinitis pigmentosa, the electroretinogram (ERG) may offer a means of clinical differentiation among retinal abnormalities.

Studies of various types of retinitis pigmentosa are being classified mainly on the basis of inheritance and age of onset in close to 200 patients who are enrolled in a program at the University of Chicago. The most common type of retinitis pigmentosa seen by this research group is an autosomal recessive form. Patients with this type show marked retinal function changes at an early stage of development, followed by a rapid progression with a high incidence of ocular complications. In infants, this condition is a common cause of blindness in the first year of life. The consequences may be a decrease in central acuity, marked constriction of visual fields, severe night blindness and a markedly abnormal electroretinogram.

The late autosomal recessive form has its onset usually after 30 years of age. Retinal function impairment is mild compared to the early autosomal recessive form, central vision is frequently spared, and progression is slow. In a few patients, onset of the diseases may be in the fourth or fifth decade of life.

The University of Chicago research team has used fluorescein angiography to confirm previous impressions that a diffuse or blotchy fluorescence throughout most of the posterior and intermediate eyegrounds is typical of all patients, regardless of the stage of the disease. This typical angiographic appearance may be helpful in making a diagnosis in early cases before eyeground changes are evident. The clinical functional abnormalities precede clinical structural abnormalities, as seen by the methods employed at the University of Chicago.

Over 300 patients have been evaluated at the Massachusetts Eye and Ear Infirmary in an effort to detect and evaluate the early stages of hereditary retinal disease. Electrophysiological testing is in progress, and the electroretinographic response (ERG) in early stages continue to provide a basis for differentiating retinal abnormalities in different types of retinitis pigmentosa. Results from this project demonstrate that in patients with night blindness and early stages of retinitis pigmentosa, a cone ERG

and/or cone early receptor potential (ERP) defect exists in every genetic type. Delays in cone ERG b-wave latency time have been observed in many types of retinitis pigmentosa. The reduction in amplitude of the ERG a-wave which, in large part, is generated by the photoreceptor cells, indicates that these degenerations involve the photoreceptors in the early stages. The reduction in amplitude of the ERP which is generated directly by visual pigment molecules indicates that in the earliest stages these degenerations must involve the outer segments of the photoreceptors. The ERG in the early stages of autosomal recessive retinitis pigmentosa is characterized by a reduction in amplitude and a delay in b-wave latency of both cone and rod system responses. The ERG from patients in whom autosomal recessive retinitis pigmentosa is suspected, but no other family members are involved, have shown that the ERG is usually the same as the ERG from families where the recessive inheritance is established. Although the involvement of other syndromes and the complexity of hereditary patterns may complicate diagnostic procedures, it is urgent that current studies of retinitis pigmentosa be continued in order to diagnose the condition when children are minimally symptomatic. At the asymptomatic stage, retinal function is relatively normal and therapeutic methods need to be explored.

Dominant sector retinitis pigmentosa is minimally progressive and can be distinguished from recessive widespread retinitis pigmentosa on the basis of temporal aspects of the ERG. The research group at the Massachusetts Eye and Ear Infirmary has shown that in sector retinitis pigmentosa, cone and rod ERG b-wave latencies are normal, while in widespread recessive retinitis pigmentosa, the b-wave latency is delayed, even at a stage when the amplitudes of the ERG in sector and widespread types are comparably reduced. This investigation supports the concept that ERG amplitudes and latencies are probably independent parameters of normal retinal function.

Investigators at Tulane University School of Medicine have had the unique opportunity to evaluate clinically, genetically, electrophysiologically and psychophysically, a number of known carriers of the gene for a hereditary disease consiting of congenital deafness and retinitis pigmentosa. A large population who belong to an ethnic group in Southwestern Louisiana were proven to be a large genetic isolate; a number of both blind and deaf individuals have been found among them. In this pedigree, 158 individuals have developed retinitis pigmentosa in later life. The deaf-blind syndrome is transmitted as a single autosomal recessive gene, and there is no predictive characteristic ocular abnormality associated with carriers of the gene in this genetic isolate.

There have been reports of patients with unilateral retinitis pigmentosa, the onset of which is more sudden and late in life. This condition is a variant of the more common form of bilateral retinitis pigmentosa and may have its origin in a vascular disorder which could be either an acquired or a genetic characteristic. A long term follow-up of such patients is being performed at the New York University Medical Center. Several patients have been classified as having unilateral retinitis pigmentosa. This variant of photoreceptor degenerative disease has been recognized as a valid entity. Nevertheless, it is difficult to explain: (1) how retinitis pigmentosa can involve only one eye; (2) why the age of onset occurs so late and more precipitously

than does the bilateral form; and (3) why the fundus appearance is unlike the more common form of retinitis pigmentosa. The studies at New York University suggest that the unilateral form is not a genetic variant but an acquired retinopathy which is probably of vascular origin. This hypothesis may be tested by inducing unilateral retinitis pigmentosa in animals by compromising the blood supply to the choroid and retina. Prognosis, treatment, and genetic counselling depend upon correct diagnosis. Animal models and functional studies will help clarify diagnostic problems where incomplete and early forms of retinal degenerative diseases occur.

DIABETIC RETINOPATHY

PROBLEM

Some of the clinical signs of faulty carbohydrate metabolism in diabetes mellitus include hyperglycemia, glycosuria and water loss due to the inability of tissues to metabolize glucose. This disease may affect vision because of complex pathological changes in the retina which include microaneurysms, vascular proliferation and hemorrhages. Degenerative changes in long-term diabetes are a serious threat to vision and longevity in man. The pathogenesis is not well defined in part, due to the lack of a reliable experimentally-produced model retinopathy in diabetic animals. Long-term studies with animal models may involve additional problems such as whether retinopathy develops as a secondary effect of the metabolic disorders of diabetes, or whether vascular disorders are dependent upon the metabolic defects of diabetes but not necessarily coincident with diabetes. Although investigations are in progress, an understanding of the relationship of diabetes mellitus and the pathogenesis of retinopathy remains elusive. There are no reliable means of prevention or treatment. Upon development of retinopathy, the prognosis remains poor in most diabetic patients. The availability of an animal model with hereditary, chemically or surgically produced diabetes which would show reproducible retinal lesions, would do much to further knowledge of retinal pathology. The appearance of occasional reports of spontaneous diabetic retinopathy in animals leaves their laboratory usefulness in doubt. Capillary aneurysms and other retinal lesions have been found to develop in animals which have been made diabetic. Alloxan or growth hormone has been used to produce a diabetic state which must be of some years duration before demonstrating early stages of retinal lesions. Although these animals may offer the opportunity to investigate diabetic retinopathy at its earliest stages, it is necessary to produce a model for the proliferative stage of retinopathy. It is a general impression that the retinopathy depends less upon the severity than upon the duration of the diabetes. If an anatomical similarity between diabetic retinopathy in man and microvascular lesions in animals made diabetic can be demonstrated, a common pathogenesis may be suggested.

ANIMAL MODELS

Pituitary ablation has been performed in humans. Controversy exists whether this procedure stabilizes or improves progressive diabetic retinopathy. Long-term studies in diabetic retinopathy have not resolved the question of prognosis after hypophysectomy. The overall objective of a project at the University of Nebraska is to obtain evidence as to the benefit of hypophysectomy in the prevention of diabetic retinopathy. The evidence is based on the appearance of retinal microaneurysms in the alloxan diabetic monkey. Previous studies have indicated that diabetes of four years duration would provide a satisfactory incidence of microaneurysms. The current goal is to maintain groups of hypophysectomized-diabetic and nonhypophysectomized-diabetic monkeys toward a duration which will permit significant observations of the effects of hypophysectomy. The monkeys in this study have been maintained for 13 to 48 months. These monkeys show normal growth patterns with fasting blood sugar concentrations at an average value of approximately 300 mg% in diabetic animals and total blood lipid levels were 1205 mg% and

852 mg% in the hypophysectomized and non-hypophysectomized diabetic animals.

Ophthalmological examinations have detected cataracts of various degrees in 6 out of 9 monkeys in the hypophysectomized-diabetic group as compared to none in the non-hypophysectomized diabetic group on the same diet. Retinal microaneurysms may not be detected until whole mounts of the retinal capillary network have been studied. Although hypophysectomized-diabetic monkeys had fasting blood sugar at the same level as the non-hypophysectomized-diabetic monkeys, the former were found to excrete more sugar. The high incidence of cataracts in hypophysectomized-diabetic monkeys is an unexpected development which should be explored. The maintenance of the monkeys represents progress toward the goal of providing a significant duration of survival for comparison of retinal microaneurysms in this animal model. Histological observations of other affected organs will be made, and this will permit correlations with retinal microaneurysms.

Efforts to extend knowledge of the retinal vascular system in normal and diabetic subjects are in progress at the Montefiore Hospital and Medical Center, New York City. This project may contribute to a broad understanding of clinico-pathologic correlations due to the access to both diseased and normal retinas of a wide variety of animals obtained from the New York City zoological gardens. The approach is to find or produce experimental models which will enhance knowledge of retinal vasculature. This project is, in part, supporting the evaluation of the potential for development of streptozotocin induced diabetic retinopathy in rat and hereditary diabetes in the hamster. Hormone manipulation of the streptozotocin-induced diabetic rat is being investigated, and only preliminary studies have been conducted in the Chinese hamster. The definitive data, when available, may enhance investigations of retinal disease processes and use of animal models.

Fluorescence angiograms are performed routinely in the investigation of the vasculature in subjects with diabetic retinopathy. It is not known how accurately visualized lesions reflect the retinal vascular pathology. Retinas from diabetics have abnormal blood vessels which are easily damaged during laboratory study. Despite numerous technical difficulties, the laboratory at Montefiore Hospital and Medical Center, New York City, does find close correlations between structures seen in fluorescence angiograms and in histological preparations. The major observations are that: (1) perfused vessels have an intact endothelium, and non-perfused vessels lack endothelium; (2) perfused and non-perfused microaneurysms have a cellular wall; (3) dilated perfused vessels are hypercellular; and (4) arteriovenous communications are hypercellular channels.

An animal for study of the disease could permit experiments which are not feasible in human subjects. At the Johns Hopkins University, a diabetic dog colony has been maintained. Several dogs in the colony now have diabetes of five or more years duration. In view of the relationship of longevity of diabetes with incidence and severity of proliferative retinopathy, proliferative disease may yet develop in animals with retinopathy of longer duration. In previous examinations of dogs with spontaneous diabetes mellitus, histological examinations of retinas have revealed background diabetic retinopathy only and no proliferative retinopathy. It is possible that in the course of

preparation of the retinas for histological study, some delicate neovascular tufts might have been removed inadvertently. Different methodology must be explored in order to leave any intravitreal or any surface neovascularization, which may be present, undisturbed.

A project at the University of Wisconsin reports that a colony of healthy dogs of mixed breeds and sex, after being shown to have normal blood sugar, normal glucose tolerance and normal fundi, were made diabetic by intravenous injection of alloxan. These animals have been studied for periods of up to six years. In a group of 23 long-term diabetic dogs, 14 are controlled poorly and 9 are controlled so that hyperglycemia and glycosuria remain mild. The onset of capillary aneurysm formation is found to be accompanied by degeneration of retinal capillary cells, and a thickening of capillary basement membranes. Overt neovascularization has been identified in two of the diabetic dogs. Clinical observations of the course of retinopathy has been impeded by development of cataracts within two years. Similar phenomena may appear in rhesus monkeys with diabetes of 8 to 9 years duration. Spontaneous or idiopathic retinal lesions clearly must be considered in the interpretation of these results but appear inadequate to account for the extent of the development of retinopathy in these diabetic animals.

CLINICAL STUDIES

A group of 50 diabetic patients have been under study at the Horacio Ferrer Eye Institute, Miami. The number in this study has now increased to include 70 patients, with plans for a total of 100 diabetic patients. About half of the group are juvenile-onset diabetics and the other half are adult-onset diabetics. In each of the groups, about half of the subjects had evidence of diabetic retinopathy when the study began. Standard fluorescein angiograms are recorded by a technique in which photographs are taken at about four frames per second. Retinal circulation times are being correlated with the clinical course of diabetes. It is expected that the circulation patterns and correlations will be useful in predicting visual loss and in assessing the hemodynamics of the diabetic retina. The data indicate that the more advanced the retinopathy, the higher the value of the time from the moment of injection of fluorescein to the filling of the four main retinal arteries in the absence of dye in the veins. The average retinal artery times in healthy individuals aged 7 to 25 years and in adults aged 28 to 60 years were essentially the same at 9.6 and 10.2 seconds, respectively. The younger and older age group with retinopathy had average values of 13.5 and 14.8 seconds, respectively. These data suggest that a sluggish circulation may produce an elevated back-up pressure which could induce an aneurysm or possibly contribute to the proliferation of blood vessels.

A large group of diabetic patients with and without retinopathy are to be studied with the photographic and scanning eye oximeters which are being developed at Boston University Medical Center. The ability to assess retinal and choroidal blood flow in a quantitative fashion using arterial-venous (A-V) oxygen differences in the retinal and in the choroidal vessels should significantly enhance an assessment of the role of the vasculature in the pathogenesis of diabetic retinopathy. This study will also permit the assessment of any

form of therapy by looking at changes in flow rates rather than flow patterns as determined by fluorescein angiography or by effects on vision which are non-specific and latent.

A long-term study of the course of diabetic retinopathy has been in progress at the University of Wisconsin. This program has been involved with a detailed analysis of the natural course of pre-proliferative and proliferative diabetic retinopathy. The overall objectives of the research are the improved understanding of diabetic retinopathy through study of: (1) its natural course; (2) development and validation of an objective classification; (3) relationship between severity of diabetic retinopathy and degree of control of blood and urine sugar; (4) criteria for estimating the prognosis for vision in various stages of diabetic retinopathy; (5) pathogenesis of retinopathy; and (6) photocoagulation in various stages of retinopathy. More than 1,100 patients have been observed, of whom more than 400 have been examined at intervals of one to twelve months over a period of one or more years. Eyes from 45 patients have been obtained at autopsy, and ten eyes have been subjected to careful histological examination. One hundred and ninety-one patients with hypertension have been examined, 82 of whom had been subjected to renal transplantation. A group of 199 insulin-dependent diabetic patients have been recruited from the practices of 50 family physicians. Stereographic fundus photographs have been taken for the assessment of possible relation of retinopathy to the control of the patients' blood and urine sugar levels. All insulin dependent patients have had diabetes for at least five years. To date, the efforts have been focused chiefly on serial observations of retina and vitreous in patients with retinopathy of sufficient severity to cause visual symptoms. This study has emphasized the importance of retinal edema in diabetic retinopathy as a cause of impaired vision in the pre-proliferative stage. It has been hypothesized that retinopathy is the result of gradually increasing ischemia due to progressive narrowing and/or obliteration of capillaries and terminal arterioles.

At the Jefferson Medical College a series of 210 patients with moderate to advanced diabetic retinopathy were studied with a variety of clinical techniques which include visual fields, ophthalmodynamometry, electrophysiology and fluorescein angiography. A study correlating the appearance of apparent microaneurysms seen with fundus color photography and with fluorescein angiography demonstrated that microaneurysms can leak blood, and that some of the small red dots seen with the ophthalmoscope are actually hemorrhages rather than microaneurysms. The frequency of such findings are under further study. In addition, 82 of the patients in this series underwent hypophysectomy for treatment of their retinopathy. The long-term value of pituitary ablation in diabetic retinopathy complications are also under review.

Platelet aggregation has been suspected of playing an important role in pathogenesis of diabetic retinopathy. A project at the Retina Foundation, Boston, has presented experimental evidence which incriminates the lack of de-aggregation of platelets, once they have aggregated under the influence of adenosine diphosphate. Lack of de-aggregation of platelets in vitro appears more marked in patients with diabetic retinopathy than in other subjects. In addition, changes noted in the conjunctiva of diabetic patients have been

studied clinically and by electron microscopy. Abnormal conjunctival blood vessels were studied in one patient with severe retinopathy. By electron microscopic examination it was observed that vessels were blocked by platelet aggregates.

THERAPEUTIC STUDIES

The study of retinal photocoagulation as a means of treating diabetic retinopathy has been in progress at the University of Chicago. One eye of the diabetic patient was treated and the natural course of the disease has been followed in the untreated eye. Data from 56 of these patients have been summarized and are based on ocular evaluations which include: (1) routine examination and (2) perimetry, fluorescein angiography, fundus photography and drawings. The indications for treatment have been: (1) bilateral early proliferative retinopathy with pre-retinal and vitreous hemorrhage; (2) massive vitreous hemorrhage; and (3) chronic macular edema with significant visual impairment and loss. The results of this trial indicate: (1) 31 patients had some benefit from photocoagulation on the basis of visual acuity and classification of changes in the treated eye; (2) of the 15 patients who did poorly, 10 of these had advanced proliferative retinopathy, and 4 were thought to have had inadequate photocoagulation; and (3) of the 10 patients who had inconclusive results, 7 did well in both treated and untreated eyes, and 3 had deterioration in both eyes. It appears that photocoagulation may have both local and widespread effects. Probably the two most important effects are the elimination of areas of surface proliferation and a general decrease in the permeability of small retinal vessels. This group of investigators has concluded that photocoagulation, if properly used, is of value in certain patients with early and moderately proliferative diabetic retinopathy; however, further study is in progress to evaluate this clinical impression.

At the Joslin Diabetes Foundation, investigators have carried out extensive histopathological and electronmicroscopic studies on both ruby and argon laser photocoagulation lesions in 36 rabbits and in 8 rhesus monkeys. This project has as its aim the investigation of the short and long term effects of laser radiation on retinal vasculature as well as the damage and repair of lesions produced by laser radiation. Observations on the effect of laser radiation of retinal vasculature suggest a slight damaging of capillaries as compared to the severity of damage of the neural elements of the retina. Even with argon laser radiation, there appears to be relatively little comparable absorption in the retinal capillaries. However, obstruction of the choriocapillaris in the center of the lesions was large one month past photocoagulation, and after 7 months, recanalization of the choriocapillaris occured. In the study of retinal damage and repair by laser radiation, there was evidence of active repair at the radiated area. It is believed that the degree of the initial damage is dependent mainly upon the effect on the pigment granules or at the pigment epithelium, and the repair is due to chorioretinal circulation.

The National Eye Institute has developed a collaborative study whose primary objective is to determine whether photocoagulation treatment will preserve vision for patients with diabetic retinopathy. Although this

therapeutic technique is in use, its effectiveness in treating diabetic retinopathy has not been adequately evaluated. Current views are based upon clinical impressions, and so a collaborative study is necessary in order to provide a sufficiently large number of patients for an objective evaluation of the efficacy of photocoagulation. This study is also designed to compare two treatment techniques--argon laser and xenon arc photocoagulation. The evaluation of photocoagulation therapy requires the simultaneous long-term observation of retinas not exposed to treatment. Sixteen ophthalmic clinics are engaged in recruitment of approximately 1800 patients. Patient recruitment started in the spring of 1972, and each clinic is expected to enter 100-150 patients. The study is being monitored by the Coordinating Center at the University of Maryland and the fundus photography Reading Center at the University of Wisconsin.

NEURO-SENSORY DISORDERS

EXTRAOCULAR MUSCLES

PROBLEM

Each eye is moved by six extraocular muscles. In order to see an object clearly, it is necessary to have the image maintained on the fovea of the retina. During this fixation period which may be a minute or more, very high velocity binocular movements (saccades), up to 400 degrees per second, or low velocity binocular movements (drifts) may occur. These movements serve to return the image of the object to the fovea and also prevent fading of vision which occurs if an image is completely motionless on the retina. Some of these motions are also due to inherent instability in the nervous system. Saccadic motion also occurs when the eyes are searching the visual field. For following a moving object, the eyes execute a slow motion termed smooth pursuit. In saccades, drift, and smooth pursuit both eyes move in the same direction. Another type of movement is vergence in which the eyes move in opposite directions; divergence refers to outward motion; convergence to inward motion.

STRABISMUS

Normal eyes are aligned so that an object in space is imaged simultaneously on the fovea of each eye. Thus, the eyes are parallel in all directions of gaze, except when they converge on a nearby object. In approximately 1 to 2% of all children, the visual axes of each eye are not so related and the condition of strabismus (squint, wall eyes, or cross-eyes) is present. Surgery for correction of strabismus is a common procedure but the amount of correction is still empirically determined, and the reoperation rate is approximately 20 to 40 percent. A quantitative knowledge of the mechanical characteristics of the human motor control system, such as the actual tension executed by the various oculorotatory muscles during fixation, vergence, pursuit and saccadic movements, is needed. In addition, a study of the innervation of the ocular muscles is necessary to arrive at a complete model of the human oculomotor system.

Investigators at the Institute of Medical Sciences, San Francisco, have been developing a model of the human oculomotor system for application to strabismus. The techniques used include: (1) placing miniature force transducers in extraocular muscles to measure in situ oculomotor forces; (2) electromyographic recording of the changes in electric potential of the muscle. It is useful for determining whether or not a muscle is contracting or for detection and location of motor unit lesions; and (3) intracellular recordings from oculomotor neurons. In this project, many of the important mechanical characteristics of the orbital and extraocular muscles have been determined. For example, the forces required to hold the eyes in any angle of fixation will vary between 5 and 40 grams. An interesting recent finding is that a force difference of only 10 to 20 grams is sufficient to produce even the largest saccadic movements. This research group has also identified the quantitative relationships between innervation and the resulting muscle forces. They have performed clinical and animal studies of ocular deviations and the

research findings have already resulted in improved clinical management. The model which is being developed is based upon the physiological findings, particularly from the implanted miniature force transducers and multiple-electrode electromyographic needle. This eye movement model satisfactorily duplicates the length-tension characteristics of the muscles, the nonlinear innervation-force transfer function, the static locus of fixational forces, the pre-emphasis peak of muscle force overshoot producing a saccade, and the unexpected initial peak force occurring before an antagonist muscle relaxes. The model also serves as a sensitive indicator of the required innervation modulation envelope responsible for various types of eye movement. Further studies along these lines should put strabismus diagnosis and management on a sounder basis.

OCULOMOTOR CONTROL SYSTEMS

In order for the eyes to function in binocular vision, it is necessary that there be an extremely accurate control system for the various types of eye movement. The control system may be considered to be divided into four independent, parallel systems: the saccadic, pursuit, vergence and the vestibular system. The latter system makes it possible for the eyes to fix on an object when the head or body is moved or rotated by providing the eyes with appropriate compensation for movement. A number of structures appear to be involved with the control of eye movements, such as superior colliculus, visual cortex cerebellum, pretectum and oculomotor nucleii. The pathways by which visual input eventually reach the oculomotor nuclei from visual cortex, frontal eye fields, pretectum and superior colliculus are not well known. Each of these areas, however, have been implicated in eye movement control.

QUANTITATIVE MEASUREMENT OF OCULAR MOVEMENTS

At the University of Maryland, research is being conducted on the quantitative aspects of ocular movements and the degree of voluntary control which a subject has when presented with a stimulus. Recent research with human subjects has demonstrated that a number of important ocular movements such as saccades and pursuit can be brought under voluntary control. Research is still in progress on separating the voluntary from the reflexive aspects of oculomotor behavior leading ultimately to descriptions of the high and low corrective mechanisms that are used when subjects fixate stationary targets or pursue moving targets. One technical accomplishment has been on-line processing of a number of statistics to describe ocular control quantitatively. These measurements include horizontal and vertical positions of each eye, velocity of movement, and other parameters.

Research completed this year may be particularly interesting clinically because it has been shown that extremely small step displacements of a target can be accurately tracked by a normal observer; also, such small movements can be made voluntarily with a stationary target. It may be possible to provide earlier diagnosis of diseases with oculomotor symptoms if disturbances in oculomotor control show up first in such very fine eye movements. This possibility requires, of course, clinical eye position monitors capable of registering movements as small as 1 minute of arc conveniently. Such instruments may not be easy to construct.

STRUCTURAL AND FUNCTIONAL ORGANIZATION OF THE OCULOMOTOR SYSTEM AND ITS CONTROLLING MECHANISM

At the University of California, Berkeley, a research project has been designed to elucidate the manner in which the decision to move the eyes is translated into the pattern of excitatory and inhibitory neural impulses channeled to the muscle fibers of the twelve extraocular muscles in the primate. Experiments are to be carried out on monkeys. Visual and vestibular stimulation is given to an alert monkey in whom electrodes have been implanted and the eye movement responses are correlated to unit firing. Such experiments, and the knowledge obtained from post-mortem histology, will enable the tracing of the supranuclear pathways which are utilized by the saccadic, pursuit, vergence and vestibular eye movement systems. At present, knowledge of the coordinating pathways for eye movements is quite fragmentary. The pathways immediately preceeding the motoneurons of the six nuclei subserving the extraocular muscles are being studied. Eye movements are recorded by electro-oculography (electrical potential between the cornea and an electrode on the head), while simultaneous unit recordings are made from strategic sites in the oculomotor pathways. Anatomical studies are used to locate the cells from which recordings are made and fiber connections between these loci and other eye movement centers are located by histological and degeneration techniques.

This kind of knowledge will be integrated in the prognostic and therapeutic approaches to oculomotor dysfunction, such as strabismus and the various ophthalmoplegias. A rather straightforward and immediate extension of the work is the study of the effect of drugs (applied systemically and locally) on eye movements and unit responses. The effect of L-dopa on Parkinson's disease is now used as a clinical tool. Significant drug influences at the mesencephalic level have recently been discovered. The close proximity of the eye muscle nuclei and center to the reticular formation and extrapyramidal pathways (red nucleus) suggests possible functional connections.

At the Good Samaritan Hospital and Medical Center, Portland, Oregon, another aspect of control mechanism of eye muscles is being conducted. Two basic hypotheses are under investigation. It is proposed that the high frequency bursts of discharge in extraocular motoneurons which initiate saccadic eye movements are caused by synaptically driving the motoneuron into a very steep "secondary range" of discharge. If this were true, then saccadic and smooth pursuit eye movements would not have separate, parallel neural substrates, but could be attributed directly to properties of the motoneuron membrane. If experiments now in progress confirm the above hypothesis, then a functional importance could be attached to the terms "primary" and "secondary" ranges of motoneuronal discharge. The second hypothesis under investigation is that the distinction between multiple innervated and singly innervated extraocular muscle fibers should be based on motor unit size and not on the frequency responses of these two kinds of fibers. If this hypothesis were confirmed, it may invalidate the suggestions that multiple innervated extraocular muscle fibers operate as exclusive elements of control for smooth pursuit, vergence, "slow" or "tonic" eye movements. If the proposed experiments for intracellular recording from extraocular motoneurons in alert monkeys succeed, then an analysis of the synaptic events producing motoneuron

discharge may be possible. Since eye movements can be measured accurately and produced reliably by either visual or vestibular stimulation, the synaptic events at extraocular motoneurons could be interpreted directly in terms of their functional consequences for the first time.

The clinical importance of these experiments is emphasized by the current lack of knowledge of the oculomotor mechanisms which might be defective in patients with strabismus. Surgical treatment of the tension of appropriate extraocular muscles is the common treatment for this disorder and this treatment has only limited success. It is hoped that the proposed experiments would provide a mechanistic understanding of the simplest elements of oculomotor system and that this understanding would suggest either improved methods of diagnosis or treatment of oculomotor disorders.

ROLE OF VARIOUS ELEMENTS IN THE OCULOMOTOR CONTROL SYSTEM

SUPERIOR COLLICULUS:

The superior colliculus is a midbrain structure which receives visual input from the retina and from several different regions of the cerebral cortex. The colliculus sends projections to the cortex via nuclei in the posterior thalamus, and descending projections to the brain stem oculomotor centers. The colliculus is laminated; neurons in its upper layers respond vigorously to visual stimulation while those in the lower layers fire in association with the eye movements.

One of the great difficulties in understanding the higher functions of the nervous system is in distinguishing between perceptual, attentional and motor mechanisms. The superior colliculus receives afferents from both lower and higher centers. It sends efferents toward association areas of the cerebral cortex and also toward regions of the brain stem related to both attentional and motor mechanisms. By continuing the examination of how visual information is sequentially processed on its way through the superior colliculus, the proposed studies should add to our basic knowledge of relation between perceptual, attentional and motor mechanisms, and thus, provide a better understanding of their disorders. In addition, study of the colliculus may contribute to the understanding of how cortical structures work and how other cortical structures may be studied.

At the University of Pennsylvania, a study is being made of the anatomy and physiology of visuo-motor systems especially with respect to the superior colliculus. As mentioned above, the colliculus has a layer structure. From electron and light microscopic studies, it was shown that dendritic connections between cells, are between cells in the same layer; connections between layers are by means of axons. By studying the distribution of synapses from electron micrographs, it was shown that the retinal input is almost entirely confined to a 50-100μ band in the uppermost superficial layer of the contralateral colliculus.

At the Massachusetts Institute of Technology, the role of the superior colliculus is being investigated by ablation and by stimulation and recording studies. Recent results suggest that after removal of the superior colliculus,

monkeys show an impairment in the acquisition of targets by saccadic eye movement. These findings lend support to the hypothesis that the colliculus contributes to mechanisms of foveal fixation. Stimulation and recording studies tend to confirm this hypothesis. Using monkeys with one eye immobilized, the receptive field location of the units in the superficial layers of the colliculus was determined and followed by electrical stimulation of the same site. The data for recording and stimulation show good correspondence. Electrical stimulation at each site produces a saccade which brings the fovea onto the receptive field of the units studies at that site prior to stimulation. Saccade parameters were independent of eye position in orbit. These investigations are directed toward the understanding of visuo-motor interaction. The colliculus is believed to play a central role in eye movement activity inasmuch as it probably contributes to the mechanism of foveal fixation whereby the eyes are directed at objects for central (foveal) viewing. Clarification of how this is carried out by the nervous system should contribute to an understanding of the underlying mechanisms and of the malfunctions in this sphere.

Another study of the superior colliculus is being conducted at the Johns Hopkins University School of Medicine. It also confirms the hypothesis that the superior colliculus produces saccades which bring the target onto the fovea. Stimulation of the superior colliculus creates saccades whose direction and amplitude form a motor map. This motor map corresponds to the sensory map created by retina tectal projections. From this, the following mechanism for moving the eyes to see a specific object (referred to as the "grasp reflex") appears to operate. If a novel visual stimulus is positioned 20° to the right and 10° up with respect to the fovea, local neuronal activity will appear at a certain place in the tectum according to the sensory map. As revealed by stimulation, this electrical activity can produce a saccade which is 20° to the right and 10° up, thus bringing the fovea to the visual stimulus.

Other evidence, concerning the role of the superior colliculus in lower animals, has been developed at the Massachusetts Institute of Technology where studies are being conducted on the effect of brain lesions on behavior of the Syrian hamster. Bilateral removal of the visual cortex results in a drastic impairment of pattern discrimination, but leaves nearly intact the ability to locate objects by visually elicited turning movements. By contrast, removal or disconnection of the superior colliculus spares pattern discrimination ability, but abolishes visually elicited turning, so that the animal behaves as if blind in simple tests of orienting ability. However, if comparable bilateral lesions are made in neonatal hamsters and the animals are tested at maturity, the behavioral defects are considerably less severe or even absent; furthermore, neuroanatomical experiments reveal certain abnormalities in the connections of the optic tracts in the brains of hamsters with early lesions. The apparent recovery of neonatal animals indicates that the animal was able to develop alternate pathways ("plasticity" of neural nets). It should be noted that the role of the superior colliculus may be quite different in man than in the lower species.

The role of the superior colliculus with respect to auditory and somatic stimuli as well as visual stimuli is being studied at the University of Oregon.

The colliculus appears to be involved in integrating information from more than one sensory modality and in controlling visual responses to auditory and somatic stimuli.

VESTIBULAR REFLEX ARC:

The vestibular reflex arc is the movement of the eyes to compensate for movement or rotation of the body in order to maintain fixation on an object. This subject is being studied at the Johns Hopkins School of Medicine as part of a program whose objective is to work out the brain stem neuronal circuits involved in oculomotor control. The principal method is that of single unit recording, starting at the motor nucleus and working centrally, step by step. Stimulation methods include stimulation and lesions in visual motor way stations in the alert monkey and the cat. From the experimental results mathematical models are derived. The approach uses modern cybernetics and system engineering methods. Recently these investigators completed a project which resulted in developing an equation which relates the firing rate of abducens neurons in the behaving monkey to eye position and velocity. This equation is obeyed by motoneurons during fixation, saccades, pursuit, vergence and vestibular-induced movements brought about by rotation of the test monkeys. The conclusion is that motoneurons cannot be divided into types to participate in some types of movements but not others. The motoneurons do not distinguish between eye movement types; their behavior is simply related to eye position and eye velocity regardless of the origin or purpose of the eye movement command. Current studies on a neural integration in the vestibulo-ocular reflex are underway; it appears to have the function of converting velocity commands into eye movements for quick phases of nystagmus, saccades, pursuit movements and maybe even vergence movements.

EXTRAOCULAR MUSCLE PATHOLOGY

Several projects are being supported by the National Eye Institute for a study of the classification of types, structure, and organization of extraocular muscles. In addition, studies are being conducted of the effects of aging and disease.

Extraocular muscles are composed of muscle fibers that show wide variations in speed of response. Muscle fibers for convergence and fusional movement are utilized in a very slow manner with the movement extending over several seconds. Following movement is a smooth change in eye position which can track an object at intermediate speeds. Saccadic movement is the fastest system that is used to shift the eye and is completed in less than 150 milliseconds and is capable of velocities of 400 arc degrees per second.

Fibers located within extraocular and middle ear muscles have a distinctive cellular organization which has not been reported in other skeletal muscles. Typical twitch fibers are also found. The combination of the two may account for the involvement in generalized or selective muscle diseases which are hereditary (progressive external ophthalmoplegia), occur spontaneously, or have autoimmune histologic characteristics (myasthenia gravis and ophthalmopathy of thyroid disease).

At the New York University Medical Center, a project has as its objective the further elucidation of: (a) fiber types and their organization in extraocular muscle, and (b) the nature and etiology of various oculomotor pathologies. From a phase and electron microscopic study of extraocular muscle of the dystrophic mouse, it was found that the changes in fiber morphology were similar to those found in dystrophic human and mouse peripheral musculature. Insofar as these changes are comparable to those reported in human extraocular muscle dystrophy, mouse extraocular muscle appears to be a good model for the study of oculomotor dystrophy.

A related program is being conducted at the University of California, San Francisco. The aim of this study is to correlate morphologic and functional studies of extraocular muscles of the cat. Techniques used include intracellular glass microelectrodes and motor endplate staining, subsequently followed by electron microscopy. This study may resolve the present uncertainty regarding the types of muscle fibers in mammalian extraocular muscle: as few as two and as many as seven have been proposed. These studies will be correlated with those of human extraocular muscle.

For the past three years, research has been conducted at Washington University, St. Louis, on the effects of aging and disease on extraocular muscle. Eye muscle appears to undergo a selective degeneration throughout life with structural alterations beginning about puberty. Initial changes consists of an increase in the amount of sarcoplasm that is more apparent near the tendon of origin. At a later age, irregularities develop in the course of the myofibrils and ringbinden was noted. The most advanced alterations consisted of loss of myofibrils so that the fiber contains mostly sarcoplasm or hyaline degeneration and fibrosis. Other ultrastructural changes have been observed in muscle disease. Round Z-line enlargements resembling those found in nemaline myopathy have been noted in biopsies of eye muscles from patients with polymyositis and ocular myasthenia. An unusual structure consisting of markedly convoluted tubules or membranes located near the sarcoplasmic reticulum were found in myasthenia gravis.

During this year, the aging study involving human and rhesus extraocular muscle has been completed. Specimens were collected from autopsies and orbital operations from individuals age three months to seventy-six years. Rhesus monkeys were evaluated from birth to seventeen years of age. In childhood, the muscle fibers show crisp details with well-defined intracellular compartments and organelles. In puberty, there is a loss of preciseness. Young adult animals have fibers which contain ribosomes and small areas of vacuolation which are adjacent to motor end plates. Lysosomes are not found in childhood muscle, but are prevalent in adult specimens and are more frequent in fibers without M-lines. In middle adult life, there is breakdown of the contractile elements with fragmentation of myofibrils; large electron lucent areas become apparent, primarily in fibers with M-lines. From middle to old age, there is a continual accumulation of disorganized fibers. These are characterized by myofibrils, sarcoplasmic reticulum, and T-tubules which may project in any direction. Other structures which seem peculiar to extraocular muscle are "light bodies" consisting of small particules about one-half the size of glycogen granules intermingled with micro-filaments.

These are usually found in young animals and are adjacent to nuclei. Another bizarre structure is dense glycogen-like particles encapsulated with mitochondria.

OPTIC NERVE AND NEUROPATHIES

At Boston University, an investigation of degenerative diseases of the optic nerve is in progress based upon studying experimental lesions that result from (a) induced experimental glaucoma; (b) chronic cyanide poisoning; (c) occlusive vascular retinopathy; (d) vitamin deficiency; (e) ultrasonic and electrosystemic radiation; and (f) toxic neuropathies. These experimentally-induced neuropathies in animals make it possible to define their histologic features and to attempt to determine the mechanism by which the optic nerve is damaged. This research has demonstrated that chronic cyanide intoxication of rats does produce lesions in the retrobulbar portion of the optic nerve in twenty percent of the animals, and that the distribution of these lesions corresponds to a capillary-poor zone which is found in a minority of normal rats. The vascular distribution of the rat optic nerve was mapped out by a variety of perfusion and histochemical techniques. Baseline information on the histochemistry of the rat optic nerve has been obtained as well as some studies of the effects of lead and ethambutal.

The pathologic anatomy of optic nerve diseases in their end stages are known, but specimens of naturally occuring human diseases are rarely available during the early or active stages of disease and the changes occuring during these active phases have not been documented. It is, therefore, evident that an experimental animal model would be highly desirable for investigating optic nerve diseases. Experimental models using monkeys for (1) the effects of abnormally high intraocular pressure on the optic nerve in cases of glaucoma and (2) optic atrophy are being developed at the Bascom Palmer Eye Institute, University of Miami. On the glaucoma model, studies are in progress on the ability of the various tissues to survive artifically produced intraocular pressure. Regarding the optic atrophy model, both ascending and descending optic atrophy was produced in a series of monkeys by sectioning of the optic nerve at the orbital apex and by photocoagulation of the nerve fibers in the retina. Electron microscopy was done since then on all the specimens and showed a slow dissolution of the nerve fibers with minimal reaction of the glial cells and no macrophagic response. The end result was a histologic picture of columnar optic atrophy. This lack of response to necrosis of the nerve fibers is remarkable in that necrosis is usually accompanied by a marked inflammatory response. Even the myelin remained behind as long as six months, and only in the six month specimens was there any sign of lipid being taken up by the macrophagic cells. Despite the myelin appearing intact by electron microscopy, its staining reaction was altered when studied by methods used in routine histopathology on paraffin sections.

As an aid to basic and clinical research and to diagnosis and treatment, an atlas of neuropathology is being prepared on a grant at the Wilmer Institute, Johns Hopkins University. This atlas aims to integrate basic information regarding neuro-ophthalmological diagnosis now scattered in publications throughout the literature.

Support is being given to research on the use of the electroretinogram (ERG) and the visually evoked cortical response (VECP or VER) for studying the etiology and diagnosis of diseases of the visual system. (The ERG is the electrical potential wave present when the retina is stimulated by a light flash, usually measured on the cornea relative to the body as a whole. The VECP is the sequence of potential waves recorded on the head over the visual cortex when the retina is stimulated by a light flash.) The University of Chicago group is deeply involved in measurements of cortical electrical responses to visual and electrical stimulation of the eye. This project leans heavily on computers, programming, and automatic administration of stimuli. The major interest of this laboratory continues, however, to be the application of these techniques to visual dysfunctions, particularly retinitis pigmentosa, chloroquine and phenothiazine retinopathy, congenital night blindness, congenital achromatopsias, retinal degeneration of all age groups, and fresh cases of closure of the central retinal artery. Animal preparations which have isolated ERG components only are made, for example, by rapidly achieved hypoxia. The configuration of the visual evoked response in terms of numbers, polarity, shapes of subcomponents, and intersubject constancy is being studied.

Another project in a similar field is being conducted at Ohio State University. The overall objective is to study human visual functioning, both retinal and cortical, by means of the ERG and the VER using both an experimental and a clinical approach. Diseases such as uveitis and macular degnerations are being studied by the previously developed macular ERG and also by pattern stimulation of the electroretinogram.

The significance of the study of the pattern ERG on ophthalmological patients is the possibility of developing an objective test of retinal functioning which is more sensitive than the present macular ERG. Although the macular ERG is quite useful in clinical work in distinguishing retinal from retrobulbar ocular disease when the visual acuity has decreased to about 20/60 it would be even more desirable to have an objective clinical test which could detect and localize visual deficits smaller than this. The pattern ERG needs to be tested on a patient population to determine if it is such a more sensitive test. The findings on normal subjects will also be of basic scientific interest for correlation with psychophysical findings in the same area (modulation transfer functions).

Another project is to determine the sites of origin of the visual evoked response resulting from stimulation of local retinal areas. Based on potential contour mapping and theoretical considerations of spread of current in volume conductors, research is in progress to determine whether the early components of the visual evoked response originate in secondary or primary visual cortex. Also from results on normal subjects it is expected that better clinical procedures can be derived that will be tested on patients with hemianopia and optic nerve disease.

The significance of determining the sites of origin of the visual evoked response will be to increase our basic understanding of the cortical visual process. The VER provides the only means available at present of studying

cortical visual physiology on intact awake human subjects. Despite our anatomical knowledge of the visual pathways in man, past research on the alpha wave of the EEG and the VER has not added greatly to our understanding of human visual brain physiology. It is only recently that attempts are being made, of which this project is one, to understand the details of this physiology. These experiments should help to localize the origin of these evoked potential components in the primary or secondary visual cortical area, and further should suggest the possible circuitry for the generation on these potentials.

The significance of this project would be to increase greatly the clinical utility of the visual evoked response. The first study on hemianopia offers the promise of greatly reducing the variability found in clinical studies of the VER in this condition. While group differences are present in studies of hemianopes, it is sometimes difficult to make a clinical decision on the VER of an individual case. The use of electrode positions rationally selected on the basis of hemispherical differences in normal subjects, and the use of hemi-retinal stimulation, should make VER abnormalities have greater salience than they presently possess. This technique also offers promise of elucidating the mechanisms in altitudinal as well as in homonoymous and bitemporal hemianopia.

Another study on retrobulbar diseases affecting visual acuity now in progress should also increase the clinical utility of the VER. At present, the VER of a patient in response to blank flash stimulation offers very little help in evaluating how well the patient can see. A patient with very poor acuity can have a large amplitude VER with component latencies in the normal range and no hint of anything wrong can be seen from looking at his VER. It is likely, however, that by recording the VER to a pattern stimulus, it will be possible to have a much more sensitive indicator of a patient's visual status.

VISUAL PATHWAYS AND PERCEPTION

Impulses from the retina pass along the optic nerve to the optic chiasm, at which point, fibers from the nasal half of each retina cross to the opposite optic tract. Thus, the fibers from the nasal half of the left retina combine with the fibers from the temporal half of the right retina in the right optic tract, and, conversely, the fibers from the other halves of the retina form the left optic tract.

The fibers of the optic tract synapse in the lateral geniculate body in a somatotopic arrangement with the geniculo-calcarine fibers. The impulses from the lateral geniculate pass through the so-called geniculo-calcarine tract which spreads through the parietal and occipital lobes in the brain finally ending in an area of the visual cortex referred to as Brodmann's area 17. Here is where the initial sense of vision takes place. Two other areas in the visual cortex referred to as "areas 18 and 19" are also involved in vision and the more sophisticated aspects of perception.

SUPERIOR COLLICULUS

Another element in the visual system is the superior colliculus, from which the impulses for eye movements originate. Connections to the superior colliculus are from the visual cortex and also some from the optic tract to subserve such involuntary movements as light reflexes.

At Yale University, a detailed study of the organization of the superior colliculus was conducted on the ground squirrel. The visual system of the ground squirrel differs from that of primates and other species in the following ways: (1) the ground squirrel has an all-cone retina (other species have both rods and cones); and (2) in the case of the ground squirrel some neurons from the retina which sense moving stimuli go directly to the superior colliculus; those that respond to stationary stimuli go to the lateral geniculate. In other species all the neurons go to the lateral geniculate. To investigate the structure of the superior colliculus, a recording electrode was placed into the superior colliculus. The animal viewed test patterns consisting of moving rectangles of light of various dimensions, or dark rectangles against a light background located in different areas of the visual field. Whenever the electrode touched a cell or a nerve fiber, electrical pulses were received, if the visual stimulus excited that particular cell. In the superior colliculus two distinct classes of visually activated cells were found: (1) directionally selective (direction of movement is the only criterion), and (2) hypercomplex, (requires the stimulus to move in a specific direction and be of specific length). It was shown that the directionally selective cells are activated by the retina whereas the hypercomplex are activated by the cortex.

Because there is a topographic projection of the retina onto the superior colliculus, an electrode penetrating perpendicular to the layers encountered cells that had receptive fields in a particular part of the visual field, and the fields of cells overlap. In addition to having the same receptive-field position, all of the cells in a perpendicular penetration have the same preferred direction of motion, and their receptive fields had the same axis orientation, regardless of whether they are directionally selective or hypercomplex neurons. Thus, the superior colliculus is divided into discrete vertical columns containing directionally selective and hypercomplex cells which share the same receptive-field position, same receptive-field axis orientation and same preferred direction of motion. Each column extends perpendicularly through all of the layers of the colliculus, from the surface to the deep white matter. There appear to be as many different types of columns as there are distinguishable axis orientations and directions of motions. There is no evidence for one type being any more common than the other. The outermost layers contained only directionally selective cells while the intermost layer had only hypercomplex cells. The intermediate layers have both types of cells. The columnar organization of the superior colliculus is thus closely analogous to that of the visual cortex which will be subsequently described. These investigations on the structure of the superior colliculus and those on the structure of the visual cortex at Harvard University have greatly increased our understanding of how the ground squirrel sees, detects motion and controls eye movements which may give clues as to how these functions operate in man.

VISUAL CORTEX

At Harvard University research has been in progress on the structure of the visual cortex using the cat and the spider monkey. From intracellular recording from the visual cortex when the animal is presented various visual stimuli consisting of bars of light at different length in various and at various orientations at rest and moving at various velocities, the positions of the cells in the visual cortex which are activated by each type of stimulus was determined. From these experiments it was shown that the cortex is organized vertically and horizontally in entirely different ways. The cortex has six layers. The columns are perpendicular to these layers, cells lying along a column have common features such as response to a stimulus in the same retinal position, line orientation, and directionality of movement. The horizontal system segregates cells in layers by so-called "hierarchical orders." Examples of "hierarchical orders" are: "simple cells" which respond to stimuli of a given orientation in specific location in the visual field; "complex cells" which respond to stimuli of a given orientation in any part of the retinal field and "hypercomplex cells" which respond only to stimuli of a given length and orientation anywhere in the field.

At Emory University, the connections between the visual cortex, areas 17, 18, and 19, are being investigated. Reciprocal point-to-point connections between area 17 and areas 18 and 19 have been found in the squirrel monkey and the results demonstrate that there is a precisely organized reciprocal point-to-point connection between area 17 and areas 18 and 19. These connections could be of significance in clinical evaluation of the site of visual defects. For example, bilateral removal of areas 18 and 19 is followed by disturbances in spatial judgment and confusion of moving objects, while visual recognition and prehensile and playing reactions remain intact in the monkey.

NEURONAL CONNECTIONS

Several laboratory methods for tracing connections within the visual system are being employed by producing a lesion which causes axonal degenerations which may be visualized by suitable staining methods, by producing a lesion and observing the behavioral results in trained animals, or by injecting into the system at one level a radioactively labelled compound which is metabolized by the nerve, and transported to the neuronal synapse ("axoplasmic transport"). The location of the radioisotope is visualized by radioautography.

At the Oregon Regional Primate Research Center a study is being conducted in order to obtain a detailed morphological description of visual neural and supporting cells and their mutual disposition in the visual system of the horseshoe crab, Limulus polyphemus. Serial sectioning of the nerves is the method employed. A related program on the structural organization of the Limulus eye is also being carried at Macalester College.

Research on tracing visual pathways in the rat and rabbit is in progress at the University of Washington, Seattle. Considerable progress has been made in refining the radioautographic technique for tracing neural connections. The localization is at the light and electron microscopic levels. This has involved the comparison of different isotopes used to label the exoplasmic

transport system, a quantitative study of the distribution of labelled protein with time, and a technical study of CNS staining methods which are compatible with radioautography. The radioautographic-neuroanatomical tracing technique has been critically examined in the central nervous system. It has been found to have several advantages over lesion techniques in that it uses a physiological neural process, is less time-dependent, is unidirectional in that neither axons passing through, nor terminals ending in the injected region take up the label and transport it in a retrograde fashion, and the Nissl-stained radioautographs allow precise cryto-architectonic studies. As verified by electron microscopic quantitative date, short survivals (24 hours) demonstrate mainly labelled synaptic terminals, while axons become heavily labelled much later. The radioautographic technique has also been intensively developed at the Washington University, St. Louis, and has been applied to a study of the projection of the retina upon the hypothalamus in a large series of laboratory animals (rats, rabbits, cats, guinea pigs and rhesus monkeys).

Although it has been known for many years that the visual system exerts a dominant influence upon several hypothalmic functions (e.g., the regulation of the estrous cycle), the neural pathways by which these influences are mediated have been extremely difficult to elucidate by conventional neuroanatomical methods. By taking advantage of the autoradiographic procedure in tracing connections, it has been shown that in each of the animals examined there is a substantial retinal projection to the superachiasmat nuclei in the rostral part of the hypothalamus. The projection is always a bilateral one (even in the albino guinea pig in which all other components of the retinal projection are completely crossed) and in nearly every case the input to the contralateral nucleus is approximately twice as heavy as that to the nucleus on the ipsilateral side. Most of the fibers terminate in a restricted portion of the nucleus (in its ventral half) and electron micrographs have shown that after eye removal an appreciable number of degenerating axon terminals can be identified in this zone. The clarity with which this important pathway could be demonstrated with the autoradiographic method is a striking tribute to the efficacy of this new experimental technique. This retinal projection to the hypothalamus has been reported by the research group at the University of Washington, Seattle. At Washington University, St. Louis, this tracing technique is being applied to other neuronal pathways. Recent results indicate that there is a remarkable feedback from the lateral geniculate body to almost every level within the visual pathway central to the retina.

Another significant research finding by the group at Washington University, St. Louis, is that, in the chicken embryo, the retinal ganglion cells are specified to form connections with very particular regions of the tectum as early as the third day of incubation. In this study, a large part of the optic cup was removed on the third day, embryos were allowed to survive until the 19th day when a small volume of tritiated proline was injected into the embryonic eye. Six hours later when the embryos were sacrificed, the transported proteins from the surviving retinal ganglion cells could be shown by autoradiography to have been distributed only to that region of the optic tectum with which that portion of the retina would normally have established connections. Apparently, the optic nerve fibers will grow over an uninnervated portion of the tectum to reach their definitive region of termination.

The visual system, as in all other neural systems, has feedback from higher levels to lower levels, and there is an influence of higher levels on the output from the retina.

PERCEPTION

At Loyola University, Chicago, a study on the basic aspects of perception is in progress. The principal investigator, along with other perceptual psychologists, views perception as a series of transformations and operations on stimulus input. In broad outline, making sense out of the visual input can be divided into the sequence: registration, description and interpretation. All three categories are thought to involve various kinds of transformations on the initial stimulus; in particular, registration and description are thought to involve the abstraction of properties or features of the stimulus. The interpretive operations are thought to occur within a framework of internal models of how the world is suppoed to look.

The term "visual masking" refers to events which occur when two or more stimuli are presented close to each other in time and space, and when at least one of the stimuli is presented for a relatively short duration. The threshold of one of the stimuli (the target) is raised, or if the target is supra-threshold, its appearance changed, by the presence of another stimulus (the mask). These techniques can provide insights into spatial and temporal organization of the visual system.

The overall research strategy of this approach is to utilize visual masking techniques as a means of investigating, for that part of the pattern recognition sequence which involves the visual system, how this sequence occurs, and what, specifically, is involved in registration, description and interpretation of a stimulus. Two projects using psychophysical methods are based upon the fundamental concept that perception in the human is subserved by various analysers specialized (or "tuned") with respect to the size, the orientation and the shape of the light stimulus. Accordingly the studies are designed to identify and characterize these independent tuners. One of these projects is at Northwestern University, where some of the features of the stimulus being studied with human subjects are: orientation, length, direction of movement, velocity, temporal frequency and spatial frequency. The other project is at the University of California, Los Angeles, where the research is particularly directed toward the nature and role of analysers "tuned" to estimation and perception of size. For example, a typical experiment would involve presenting to the human subjects a group of stimuli which differ in some respect such as size, color and shape. The stimuli are presented in random order and each subject attempts to name them or rate them with respect to some dimension such as size.

Another study based upon the concept of "tuned" receptor cells but employing electrophysiological methods, is being pursued at the Massachusetts General Hospital, Boston. These studies use the cat and are designed to investigate the spatial tuning properties of single neurons in the nonstriate cortex. The response to slits of various widths, to nine wave gratings of

various spatial frequencies and to double slits with various spacings between the slits is being determined. Electrophysiological studies on animals have shown the existence of cells which respond to stimuli having a specific orientation and in some cases also length. These psychophysical studies provide an application of the work to broader problems in perception in humans and should help to clarify the relationships of many electrophysiological findings to human perception.

CONTRACT NARRATIVE

WASHINGTON UNIVERSITY (NIH-NEI-71-2289)

Title: Development and Application of a Biomedical Image Processor

Current Fund Allocation: $41,050

Objective: This project is designed to assist in the advancement of neuro-ophthalmological research and has supported the development of a completely computer controlled microscope to track and display the dendritic organization of neurons. Through the development of computer hardware and software, biological data can be collected and analyzed. The operational system permits the rapid computation of cells in nuclear areas and of axon diameters.

Progress to Date: (1) By directly interfacing a computer with an electron microscope, the contractor has effectively by-passed all photographic procedures involved in the quantitative analysis of electron microscopic material. This is a significant improvement over current techniques and is an important advance in this field. (2) An automated system has been developed which measures neurons. It estimates, from electronmicrographs, diameters of optic nerves to 1.5 microns. This automated system increases the rate at which this type of data can be collected 5 fold. (3) A semi-automated system has been designed for the collection, analysis, and display of structural data from single Golgi-impregnated neurons. This system will also allow recording data from several cells in order that new insights into neuronal interconnections may be gained. (4) A technique has been developed for the automatic counting and three dimensional location of silver grains in autoradiographs used for tracing axonal connections. (5) A semi-automatic system has been developed to measure nerve cell size.

Significance to NEI Programs and Biomedical Research: The development and application of the automated biomedical image processing system represents an important advance for neuroanatomical studies because it allows collection of data of appreciably greater statistical significance, and enables neuroanatomists to embark on research projects which, for technical reasons, are virtually impossible by other methods. With the development of a high quality automated microscope coupled to a suitable image processor. it is now possible to automatically scan and analyze such clinically relevant materials as blood films and cervical smears.

Although the main objective in developing this system was not to create a diagnostic tool, it will enable researchers to achieve

a better understanding of the mechanisms underlying the pathogenesis of various diseases. Concerning visual disorders, these systems will in the future facilitate laboratory research efforts in the development and/or study of congenital strabismus (squint or cross eyes) in animal models. By being able to analyze and evaluate the pathogenesis and time sequence of the changes in visual cell sizes occurring with this disease, one would be able to more accurately determine that critical period where correction or treatment has to be initiated prior to the condition progressing to strabismic amblyopia and possibly permanent blindness. In addition, children born with marked astigmatism, if not corrected at an early stage, have severe visual defects which are at right angles to the axis of best vision. This disorder appears to result from some defect in the visual cortex portion of the brain. These newly developed automated systems will allow researchers to perform careful anatomical studies of cells in the visual cortex or laboratory animals to observe the pathogenesis of this disorder and to determine if cells in the cortex are properly oriented with respect to each other.

Proposed Course of Project: This contract is in the final phase of support; the work requirements of this contract will be completed by September, 1973.

CONTRACT NARRATIVE

BOSTON UNIVERSITY (NIH-NEI-71-2513)

Title: Development of Clinically Useful Methods of Estimating Retinal and Choroidal Blood Flow

Current Fund Allocation: $247,430

Objectives: The objective of this contract is the development of clinically useful methods of estimating the rate of effective or nutrient blood flow to the human retina and choroid from the retinal and choroidal circulatory system.

Progress to Date: Two types of instruments are being developed for measuring arteriole-venous oxygen differences in the retinal and choroidal blood vessels. The fundus is photographed through green and red filters, and microdensitometry is performed on the photographic films. This procedure yields an estimate of the oxygen saturation of hemoglobin in the particular blood vessel analyzed. The optical system will be further refined to compensate for variations in the intensity of the illuminating flash.

A choroidal oximeter which uses photoelectric measurement of light reflected from the choroid is being refined. It will enable the operator to scan and to measure reflected light from small regions of retinal arteries and veins. The output of this measurement will be processed and the arteriole-venous oxygen differences calculated.

Research to date has indicated that the approach being utilized may achieve in the near future the desired accuracy which would permit clinical usefullness of the instruments developed.

Significance to NEI Programs and Biomedical Research: The objectives of this contract supported research are of major importance to the Institute's retinal disease program. If successful, such an instrument would provide a much needed research and diagnostic tool applicable to the study of blinding eye diseases.

Proposed Course of Project: This project is currently in its final phase of support. Further extension for 6 months of research performed under the contract is anticipated. The contractor is currently concentrating its efforts on data collection and analysis and, as progress merits, initial human volunteer testing.

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: $166,613

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: During the past year, investigations of the ultrastructural effects of various concentrations of hyaluronidase delivered into the anterior chamber of the owl monkey have been undertaken. Instrumentation and techniques have been tested, and glaucoma research patients have been identified and preoperatively studied.

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 clinical importance in overcoming disadvantages in current treatment modalities.

Proposed Course of Project: It is anticipated that this research will be completed by June 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: $51,965

Objective: This clinical trial has as its purpose the determination of the effect of DPH on 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: Thirty-three patients are currently included in the study. Evaluation of data to date will commence with the addition of a biostatistician to the research team. However, results of the study will be masked from the investigators until all 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: This project is part of a major special emphasis program of the Institute - the improvement of the prevention, diagnosis and treatment of glaucoma. The successful development of more effective means of 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.

Proposed Course of Project: It is anticipated that an additional two years will be required to complete this study.

CONTRACT NARRATIVE

STANFORD UNIVERSITY (NIH-NEI-72-2049)

Title: Design and Construction of a Prototype Fluorometer

Current Fund Allocation: (none)

Objective: The objective of this contract is the design and construction of a prototype fluorometer for the objective measurement of fluorescein in the transparent media of the eye. This instrument would permit a non-invasive, direct estimation of corneal and endothelial function and of aqueous humor turnover, as indicated by fluorescein measurement.

Progress to Date: The objective has been met; the instrument is currently being evaluated in a clinical setting.

Significance to NEI Programs and Biomedical Research: The current state of research technology related to studies of corneal disease and glaucoma has been advanced through the development of an improved instrument for the objective measurement of fluorescein in the transparent media of the eye.

Proposed Course of Project: The requirements of the contract have been successfully completed. No further extension is anticipated.

CONTRACT NARRATIVE

NEW YORK MEDICAL COLLEGE (NIH-NEI-72-2115)

Title: Relationship of Posterior Pole Microcirculation to the Pathogenesis of the Bjerrum Scotoma.

Current Fund Allocation: $118,206

Objective: The objective of this contract is to determine, through clinical studies, the relation of deficits in the capillary vascular bed of the optic nervehead and adjacent retina and choroid to glaucomatous field defects.

Progress to Date: Adequate photographic ability to record optic disc and choroidal circulation has been established; glaucoma patients already studied demonstrated marked variations from the normal pattern of posterior pole microcirculation. In addition, the densitometric technique for analysis of an angiographic series has resulted in useful quantitative data concerning the rate of perfusion in the retina, choroid and optic nerve.

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. It is essential for the understanding of visual field loss in glaucoma to determine whether field defects and capillary filling defects are related.

Proposed Course of Project: It is anticipated that this research will be completed by June, 1975.

CONTRACT NARRATIVE

YALE UNIVERSITY (NIH-NEI-71-2512)

Title: Development of Drugs Useful in the Treatment of Glaucoma and Their Evaluation Both in Animals and Man

Current Fund Allocation: $144,870

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 reuptake and binding of norepinephrine and epinephrine and those which interfere with enzymatic degradation of norepinephrine may enhance and prolong the action of these drugs. The problem is approached through the testing and screening of drugs in animal models and through iris perfusion techniques.

Progress to Date: A number of leads have been opened up by the contractor. It would appear that chemical blockade of norepinephrine uptake will, as expected, enhance the effects of catecholamines on intraocular pressure. A clear cut effect of adrenergic drugs on cyclic AMP in the aqueous humor has been established and some preliminary observations on the possible clinical utility of tricyclic antidepressants have been made.

The development of specialized techniques for pharmacologic investigations have been particularly useful. These techniques include the perfusion chamber, the adaptation of the spectrophotometer for constant monitoring of dilation, and the development of ultra micro-high pressure chromatographic systems for the analysis of catecholamine metabolites and nucleotides in aqueous humor.

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. The successful development of more effective means of drug therapy for glaucoma patients would represent a major breakthrough in the treatment of this serious visual disorder which is a major cause of blindness.

Proposed Course of Project: This contract will be extended for an additional year only to continue on-going studies to: (1) to evaluate penetration of topically administered adrenergic drugs; (2) to study the site of action of adrenergic drugs; (3) to determine the role of COMT in the inactivation of catecholamines; (4) to assess the efficacy of amitriptylene; and (5) to evaluate analogues of cyclic AMP. This research will involve *in vitro*, *in vivo* (animal experimentation), and human studies.

CONTRACT NARRATIVE

NEW YORK MEDICAL COLLEGE (NIH-NEI-72-2113)

Title: Steroid-Induced Glaucoma

Current Fund Allocation: $116,195

Objective: The objective of this contract is to obtain a systematic evaluation of the ocular effects of topical corticosteroids.

Progress to Date: Twenty-nine sets of twins are either engaged in or have completed the investigation; an additional eleven sets of twins are being rescheduled for the study. Concerning the test-retest consistency of response to corticosteroids, of the 203 individuals contacted, 108 have appeared for initial re-evaluation. Eighty-three patients have completed the test-retest consistency including seven high corticosteroid responders. Dose-response studies with the high responders have been initiated.

Significance to NEI Programs and to 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. The observation of increased intraocular pressure caused by topical corticosteroids in susceptible individuals is of considerable importance because: (1) steroid glaucoma may represent an experimental model of open-angle glaucoma and (2) pressure response to topical corticosteroids appears to be genetically determined and it is important to clarify the relationship, if any, between steroid responsiveness and risk of developing open-angle glaucoma.

Proposed Course of Project: It is anticipated that this project will be completed by June, 1974.