ANNUAL REPORT OF PROGRAM ACTIVITIES NATIONAL EYE INSTITUTE FISCAL YEAR 1979

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

ANNUAL REPORT

OF

PROGRAM ACTIVITIES

NATIONAL EYE INSTITUTE

Fiscal Year 1979

TABLE OF CONTENTS

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1978 - September 30, 1979

STATEMENT OF THE INSTITUTE DIRECTOR

The National Eye Institute (NEI) is about to complete its tenth year. During FY 1979, the budget of the Institute grew to $105 million. In FY 1969, the year preceding the first appropriation for the National Eye Institute, the NIH budget for vision research was about $20 million, and the number of grants awarded for this purpose was about 150. During the decade that has followed, this budget has more than quadrupled, and the number of grants increased more than 1,000, despite inflation and severe restrictions and limitations in the growth of Federal health programs.

Despite prospects for a modest increase in our budget for FY 1980, it is apparent that the past trend of large yearly increases in the NEI's budget has ended. It is likely that in the future increases will be in the neighborhood of 7 percent--the expected increase for FY 1980. It should be noted that all of these funds support research directed toward reducing the physical and economic hardships caused by visual impairment and blindness. It is also important to note that more than four-fifths of the NEI's funds have been consistently reserved for the support of investigator-initiated projects, the historical means by which the most original and productive research is planned and carried out.

One important program development for the NEI over the past year was the formalizing of collaborative research on eye disease between the United States and the Union of Soviet Socialist Republics. Vision research was thus afforded the same priority in the U.S.-U.S.S.R. Program for Health Cooperation as other ongoing collaborative health research efforts.

The NEI's commitment to affirmative action programs was strengthened in FY 1979 by an intra-agency agreement for support of the Minority Biomedical Support Program administered by the NIH Division of Research Resources. This new collaboration is expected to encourage and facilitate the entry of minority scientists into vision research.

In 1979, the Laboratory of Sensorimotor Research was established in the NEI intramural program to further research on the mechanisms underlying the control of eye movements, to apply knowledge of neurochemistry and neural regeneration to the visual system, and to perform electrophysiological investigations involving the visual nervous system--research which is at the frontier of neurobiology. The Laboratory's core program will concentrate on the cellular mechanisms underlying eye movements and visual perception in monkeys.

Also during FY 1979, the Extramural and Collaborative Programs was reorganized to accommodate the seven-fold increase in NEI-supported grants since this program was originally established. As a result of this reorganization, the program is now divided into the Retinal and Choroidal Diseases

Branch, the Sensory Motor Disorders and Rehabilitation Branch, and the Anterior Segment Diseases Branch. The latter encompasses the Corneal Diseases, Cataract, and Glaucoma programs.

Last year, in FY 1978, the National Advisory Eye Council published Vision Research--A National Plan: 1978-1982, a comprehensive document prepared with the assistance of more than 150 of our nation's leading experts in the visual sciences. Copies of this five-year plan have been widely distributed among members of the scientific community, and the response to it has been most favorable. During the past year a new Program Planning Subcommittee of the Council was established under the chairmanship of Thomas D. Duane, M.D., Ophthalmologist-in-Chief, Wills Eye Hospital. This Subcommittee has already begun work on evaluating the existing plan and preparing preliminary guidelines for a new document to be published in 1982.

During the past year, three distinguished scientists were appointed to the National Advisory Eye Council. The first, Herbert E. Kaufman, M.D., Chairman of the Department of Ophthalmology at Louisiana State University Medical Center in New Orleans, is internationally recognized for his studies of diseases of the cornea, especially the immunological aspects of herpes simplex viral infections.

Alan M. Laties, M.D., the second new Council member, is both Director of Research of the Scheie Eye Institute and the Irene Heinz Given and John LaPorte Given Professor of Ophthalmology at the University of Pennsylvania School of Medicine. A highly regarded scientist, clinician, and educator, Dr. Laties has a special interest in the neurochemistry of the visual system and the relationship of this discipline to degenerative diseases of the retina.

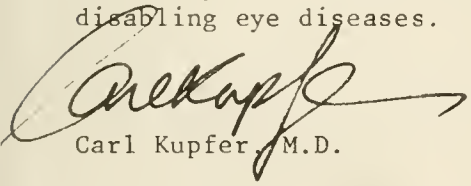
The third new Council member is Ralph W. Ryan, M.D., who is the Director of the Morgantown Eye Clinic in West Virginia and who played a key role in the creation of the National Eye Institute. His special interests include industrial ophthalmology and the social and economic consequences of visual impairment.

The role of intraocular lens implantation in the management of cataract was discussed at a National Institutes of Health Consensus Development Conference this past summer, the first such conference sponsored by the NEI. Issues that were discussed at this conference included: indications and contraindications for use of intraocular lenses; clinical experience to date with different types of intraocular lenses; long-term and short-term complication rates following intraocular lens implantation; implantation of intraocular lenses in children and in both eyes of one person; and the advantages and disadvantages of intraocular lenses in comparison with alternatives such as eyeglasses, conventional contact lenses, and long-term wear contact lenses. Program participants included 40 experts in the management of cataract patients, representatives of consumer and professional groups, and others with an interest in intraocular lens implantation. Over 300 scientists, interested laypersons, and representatives of the press attended this two-day conference.

During the past year, the NEI in cooperation with the National Center for Health Statistics and the Bureau of the Census began planning a nationwide survey of visual disorders. This survey will provide us with important data for planning research and for gauging the impact of research advances on the nation's visual health. The potential of epidemiology in the field of vision research and ophthalmology has just barely begun to be realized. We have seen in the example of the cooperative Diabetic Retinopathy Study what can be achieved by one of the most powerful tools of epidemiology, the randomized controlled clinical trial. Due to the successful outcome of the cooperative Diabetic Retinopathy Study, a number of new clinical trials are now underway employing all the essential elements of such studies, including random assignment to either experimental or control groups, use of effective masking procedures, and independent data monitoring and statistical analysis.

Approximately 13 clinical trials are currently being supported by the NEI, most of them grant-supported and conducted at single centers. The purpose of one which, however, involves several collaborating centers is to see whether photocoagulation can be effective if applied in the early proliferative and nonproliferative stages of diabetic retinopathy and also to determine whether aspirin, alone or in combination with laser treatment, can be beneficial in slowing the progression of the disease. Other trials currently underway include ones which are evaluating the role of vitamin E in preventing retrolental fibroplasia in premature infants receiving oxygen therapy for respiratory distress syndrome, looking at laser treatment of macular edema associated with early macular degeneration and histoplasmosis and laser therapy for branch vein occlusion, testing ascorbic acid for the treatment of corneal burns, and examining effects of intraocular lenses on the corneal endothelium. Altogether, nearly one-third of all the university departments of ophthalmology in the United States are participating in NEI-supported clinical trials.

Further accomplishments and examples of current research endeavors may be found in the following reports of NEI extramural and intramural programs. It is gratifying to me, as Director of the National Eye Institute since its inception, to look back upon the past decade of support of quality laboratory and clinical research and to look forward to the years to come which will most certainly lead to even more important advances against major blinding and disabling eye diseases.



Carl Kupfer, M.D.

EXTRAMURAL AND COLLABORATIVE PROGRAMS

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1978 - September 30, 1979

REPORT OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL AND COLLABORATIVE PROGRAMS

Ronald G. Geller, Ph.D.

Fiscal Year 1979 was a year of continued growth and change for the Extramural and Collaborative Programs of the National Eye Institute. In keeping with the Institute's first priority, a record number (884) of grant awards for investigator-initiated individual research projects (R01) were made covering a wealth of scientifically exciting ideas relevant to the prevention, treatment, and cure of diseases and disabilities of the visual system. The following sections highlight some of the issues and accomplishments in the NEI Extramural and Collaborative Programs during FY 1979.

For FY 1979 the National Eye Institute received an appropriation of $105,192,000--an increase of $19,792,000 over the previous year's appropriation. Of the $105,192,000, a total of $92,152,000 was allocated to Extramural and Collaborative Program activities in the following categories:

Research Grants	$81,676,000
Research Training Awards	4,643,000
Research Contracts	5,833,000
Total	$92,152,000

The bulk of the budget increase occurred in funds for research grants; an additional $16,946,000 was available in this category in FY 1979 as compared to FY 1978. These funds were distributed among the Institute's five programs as follows:

	Research Dollars (in thousands)		
	FY 78	FY 79	% Growth
Retinal and Choroidal Diseases	$24,733	$38,720	58
Corneal Diseases	8,888	14,492	64
Cataract	6,604	7,787	20
Glaucoma	8,073	10,494	30
Sensory and Motor Disorders of Vision and Rehabilitation	16,432	20,659	25
	$64,730	$92,152	42

The grant application receipt rate was slightly less than in FY 1978. The National Advisory Eye Council approval rate, however, was stable during these two fiscal years: 85 percent of grants submitted were approved for funding in both FY 1978 and 1979. The Institute was able to fund 65 percent of all approved applications, essentially the same as in FY 1978. The data are given below.

Grant Application Rate

	Received & Reviewed	Recommended for Approval	Approved & Funded	% Funded of all Approved Applications
FY 1977	512	425	225	53
FY 1978	673	552	375	68
FY 1979	577	492	323	65

The distribution of awards between competing and noncompeting research grant applications was as follows:

	FY 1977 Number of Grants	FY 1978 Number of Grants	FY 1979 Number of Grants
Prior Year Commitments	453	506	581
New Research Awards	96	272	183
Renewal Awards	129	108	120
	678	886	884

Once the prior year commitments were taken into account, there was approximately $25 million available for new and competing research grants. In FY 78, $26 million was available for competing grants and that was the largest amount of "new" money for investigator-initiated vision research ever available in one year in the history of the National Eye Institute.

The Institute's research grants are comprised of the following categories:

FY 1979 Research Grants by Mechanism
(Dollars in Thousands)

	Number	Total Awarded
Research Grants (R01, R10, R13, R23)	903	72,196
Core Center Grants (P30)	25	5,115
Specialized Clinical Research Center Grants (P50)	4	1,360
Research Career Development Awards (K04)	58	2,237
Academic Investigator Awards (K07)	24	768
Total	1,014	81,676

The codes in parenthesis in the above table are the symbols used by NIH to differentiate the various types of grant awards. A description of each of these mechanisms can be found in the Introduction to Volume Three of the publication Vision Research--A National Plan, 1978-1982 (DHEW Publication No. (NIH) 78-1260). It is noteworthy that approximately 86 percent of FY 1979 grant funds are allocated to individual investigator-initiated research project grants (R01).

The National Eye Institute complements its research grants with a program of institutional and individual fellowships. The purpose of the program is to equip young investigators with the skills, experiences and insights necessary for them to embark successfully on a career in vision science, especially its clinical aspects, and other disciplines, such as the basic medical sciences, epidemiology, engineering and biomathematics.

A total of $4,643,000 was available for support of vision research training in FY 1979, most of it for the National Research Service Awards (NRSA). The individual NRSA fellowship awards accounted for $1,529,000 or 32 percent of available training funds. The institutional NRSA training awards accounted for $3,114,000 or 68 percent of the program. A summary of the training program for FY 1979 follows.

VISION RESEARCH TRAINING FY 79
(Amounts in Thousands)

	INSTITUTIONAL (NRSA T32)				INDIVIDUAL (NRSA F32)			
	No. of Inst. Awards	Pre-Doctoral	Post-Doctoral	Amount	No. of Ind. Awards	Amount	Total (T & F)	Percent Training Budget
Retinal and Choroidal Diseases	20	16	71	$1,147	48	$ 655	$1,801	39
Corneal Diseases	11	7	27	666	5	75	741	16
Cataract	3	0	9	175	6	95	270	6
Glaucoma	4	0	18	321	7	108	430	9
Sensory & Motor Disorders of Vision	14	15	38	805	45	596	1,401	30
TOTALS	52	38	163	$3,114	111	$1,529	$4,643	100

The National Eye Institute's collaborative research activities, funded through contracts, continue to emphasize cooperative clinical trials for the treatment of diabetic retinopathy. The estimated distribution of contract awards and funds is as follows:

	Number	FY 1979 Total Awarded (in thousands)
Diabetic Retinopathy Study	17	$ 1,007
Diabetic Retinopathy Vitrectomy Study	15	1,880
Early Treatment Diabetic Retinopathy Study	23	2,486
Other	2	460
Total	57	$ 5,833

Several staff and organizational changes in the NEI Extramural and Collaborative Programs occurred during the past year:

1. The Scientific Programs Branch has been replaced by three new branches: Retinal and Choroidal Diseases Branch, Sensory and Motor Disorders and Rehabilitation Branch, and Anterior Segment Diseases Branch (includes the Cornea, Cataract, and Glaucoma Programs).

2. Dr. Thomas C. O'Brien has been designated as Deputy Associate Director, Extramural and Collaborative Programs.

3. Dr. Israel A. Goldberg has been selected as Chief, Retinal and Choroidal Diseases Branch. Dr. Thomas C. O'Brien has been selected as Chief, Anterior Segment Diseases Branch and the Sensory and Motor Disorders and Rehabilitation Branch.

4. Ms. Judith Connor has joined the Program Information Section as a Fiscal Analyst (Grants).

Immunology Workshops

In its 1977 report, the National Advisory Eye Council identified the need for expanded research on the immunological aspects of ocular disease and for application of the concepts from recent advances in the field of immunology to the study of the visual system. Such a transfer of knowledge from other fields of immunology to vision research will be effected through the National Eye Institute's sponsorship of three immunology workshops in FY 1980. The National Institute of Allergy and Infectious Diseases has assisted in the development of these workshops. The titles and dates of the three workshops are:

(1) "Immunogenetics and Transplantation Immunity," December 5-7, 1979;
(2) "Autoimmune Phenomena and Ocular Disorders," March 5-7, 1980; and

(3) "Infection, Inflammation and Allergy," June 25-27, 1980.

At these conferences ophthalmic immunologists will meet with immunologists from other fields. Their task will be to assess the state-of-the-art in each of three broad research topics indicated above and to make recommendations for encouraging scientific investigations into those areas which appear most promising for development within vision research.

Immunology Announcement

A full year has now passed since the NEI request for research applications entitled "Immunological Aspects of Ocular Disease" was published as an announcement in the NIH Guide for Grants and Contracts (Vol. 7, No. 10, August 4, 1978). This broad-based solicitation for immunological research encouraged new approaches to studies of:

- o fundamental immunologic processes of the normal eye including the roles of, and interactions among, various immunoreactive cell types, humoral factors, and immune recognition;
- o the roles of steroid hormones, autacoids, and other biochemical compounds and enzymes in inflammatory responses;
- o the etiology of ocular autoimmune diseases;
- o immunogenetic correlations with predisposition to ocular diseases, success of drug therapy, and success of corneal grafts; and
- o effects of immunosuppressive therapies on both normal ocular function and resistance to ocular infections.

The initial response to this announcement has already proved encouraging and has led to the funding of several immunologically-oriented research proposals in various program areas which were approved at the May 1979 meeting of the National Advisory Eye Council. Examples of such applications were research on the suppression of corneal allograft rejection and the alteration noted in the immune response which results from placing transplantation antigens in the anterior chamber of the eye.

Research Program Information

A very important aspect of our program involves our ability to provide detailed information on our research activities. The Program Information Section, Extramural Services Branch, is responsible for collecting, organizing and interpreting data on the size, scope, trends, patterns, and funding of the extramural activities supported by the NEI. In FY 1979, 828 research and training applications to the NEI were processed by the Section. Each grant was assigned to a program area coded for research objective, methodology, ocular area, and type of research subjects used. Information is also captured on the principal investigator including degree, sex, and the department, institution, city, and state in which the research is being conducted.

Budget information is recorded including requested dollars, study section recommended dollars, original commitment, and dollars paid and committed by the NEI. Institutional allowances and stipends for fellowship awards are also stored in the data base.

All applications to the NEI are classified according to a SCORE (Scientific Coding Of REsearch) system. This coding is done in four areas: research objective, methodology, ocular area, and research subjects. Under research objective, basic studies, developmental studies, and ocular disease states are coded. In the Retinal and Choroidal Diseases Program, disease states such as retinitis pigmentosa, macular degeneration, retrolental fibroplasia, retinal detachment, diabetic retinopathy, and aging-related disorders can be identified. Corneal dystrophies, burns, ulcers, and keratoconjunctivitis are coded in the Corneal Diseases Program. Several types of cataracts are included under the Cataract Program, such as congenital, sugar, radiation, and senile. Congenital, open angle, narrow angle, and pigmentary glaucomas are among the areas identified in the Glaucoma Program. Several different neuro-sensory and oculomotor disorders as strabismus, amblyopia, and nystagmus are coded as part of the Sensory and Motor Disorders of Vision Program. A number of infections, immune states, systemic disorders, and tumors as they relate to ocular disorders are also identified.

Methodology, the second area of SCORE, covers drug studies, neurotransmitters, hormones, laser, and several research and diagnostic methods. The third area of SCORE, ocular area, identifies general or specific individual structures delineated for each ocular area, i.e., corneal epithelium, lens cortex, and retinal pigment epithelium.

The use of human subjects, tissue, or animals in a research project is captured as the fourth area of SCORE for each study. A number of animal species is delineated, from fish to specific small animals or large mammals. All of this information is stored in the NEI data base which is constantly updated by the Section. Analyses can be done on a wide range of disease states or basic studies with a number of variations in parameters possible.

Analyses are done each year by the Section on NEI involvement in several special projects including the National Toxicology Program, the Clean Air Act, Environmental Health Related Research, clinical trials, and Basic/Applied/Development funding.

Three new computerized data bases were created by the Section this year. An intramural file was established with coding for research activities in the Clinical Branch, the Laboratory of Sensorimotor Research, and the Laboratory of Vision Research. A contract file of all NEI-supported contracts was initiated enabling the Section to provide timely and accurate status reports. An Institutional Overlap File was created to detail the equipment, personnel, supplies, and other expenses at institutions supported by the NEI. The reports from this data base will allow the Grants Management staff to monitor these expenses and reduce overlap.

Information is provided from the NEI data base for trans-NIH committees, including diabetes, nutrition, genetics, rehabilitation, behavior-social sciences, epidemiology, arthritis, and cystic fibrosis. The Section identifies NEI projects, coordinates their review, and is responsible for updating reports to the trans-NIH committees. A liaison is maintained with other NIH information offices which provide information on their institute's activities. The CRISP system of the Division of Research Grants is also used by the unit for data on NIH projects outside the NEI.

RETINAL AND CHOROIDAL DISEASES

Introduction

The Retinal and Choroidal Diseases program encompasses research on specific clinical disorders as well as basic research on development, structure, and function of the eye as related to disease. The program consists of the following subprograms:

Problem Areas and Specific Disorders

- Developmental and Hereditary Disorders
- Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities
- Myopia
- Tumors
- Macular Diseases
- Retinal Detachment
- Inflammatory Disorders

Development, Structure, and Function as Related to Disease

- Uveal Tract
- Vitreous Humor
- Visual Cells and Pigment Epithelium
- Retinal Organization and Visual Adaptation

Special Areas of Future Interest

- Toxic and Environmental Disorders
- Low Vision
- Retinal Regeneration and Transplantation
- Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models

In Vision Research--A National Plan: 1978-1982[1] the National Advisory Eye Council's Retinal and Choroidal Diseases program planning panel stated the following goals for the program:

- Enhance understanding of fundamental processes underlying good vision and of their derangement in diseases of retina and choroid.

- Improve the means to diagnose diseases of retina and choroid.

- Discover ways to prevent retinal and choroidal diseases or to treat these diseases effectively once they occur.

The panel, in its deliberations, identified a broad spectrum of areas of need, research problems to be pursued, and recommendations for resource allocation which cut across the subprograms delineated above.[2] During FY 1979, considerable progress has been made within each of the subprograms in responding to these recommendations. The staff of the Retinal and

Choroidal Diseases program have chosen to report in particular on advances in the following areas in this Annual Report: diabetic retinopathy, clinical trials of laser photocoagulation therapy, disorders of the vitreous, the roles of cyclic nucleotides in retinal metabolism, retinal neurotransmitters, and the toxic effects of light. In addition, we conclude this Annual Report with a section on the program announcements we have released during FY 1979 in support of the goals of the program and the recommendations embodied in the National Plan.

Diabetic Retinopathy

Overview. Vascular and circulatory abnormalities of the eye are the leading causes of new blindness and visual impairment in the United States today. The most prevalent of such disorders are due to diabetes. The clinical manifestations of diabetes include ocular complications such as retinopathy, cataract, neovascular glaucoma, and disorders of the vitreous. Because of the magnitude of the public health problem and the vision research community's recognition of the need for additional knowledge, National Eye Institute support of research on the causes, treatment, and prevention of diabetic retinopathy and other ocular complications of diabetes increased from approximately $5 million in FY 1975 to over $14 million in FY 1979. These funds are supporting a wide array of studies ranging from biochemical and physiological investigations designed to elucidate the detailed pathogenetic events associated with diabetic eye diseases in general to clinical trials of new treatments for diabetic retinopathy in particular. Especially noteworthy are:

(1) the continuing success of the Diabetic Retinopathy Study in establishing a scientific basis for the use of photocoagulation in the treatment of proliferative diabetic retinopathy;

(2) the completion of the planning phase for the new multicenter clinical trial, Early Treatment Diabetic Retinopathy Study, to determine the best timing during the course of the disease to administer photocoagulation or other therapies;

(3) the initiation of clinical trials of therapies for other retinal-vascular disorders: the Branch Vein Occlusion Photocoagulation Study, the Prematurity, Vitamin E, and Retrolental Fibroplasia Study, and the Macular Photocoagulation Study;

(4) fundamental and clinical research studies utilizing fluorometric techniques to examine the normal and pathological properties of the blood-retina barrier and to improve capability for early diagnosis of retinopathy;

(5) increased emphasis on transferring knowledge gained through research to practitioners who treat patients with diabetic retinopathy; and

(6) participation of the NEI staff in various interagency committees, for example, the National Diabetes Advisory Board, the Diabetes Mellitus Coordinating Committee and the NIH Diabetes Coordinating Committee.

Diabetic retinopathy is a progressive eye disease that occurs as a concomitant of systemic diabetes. In the severe or proliferative stage of the disease, new, abnormal blood vessels form along the inner surface of the retina (retinal neovascularization). The vessels may bleed into and cloud the vitreous body and the retina may become detached from underlying tissue layers as a result of shrinkage of the vitreous gel and/or of the scar tissue which often accompanies the newly-formed blood vessels. As a result, vision is reduced or lost.

During FY 1979 primary emphasis has been given to expanding the fundamental base of knowledge of retinal microangiopathy through research projects on physiological factors influencing blood flow, edema formation, and capillary closure; angiogenic substances, antagonists, and inhibitors; and transport processes betweeen the retina and its blood vessels. The large number of papers presented on these topics at the 1979 meeting of the Association for Research in Vision and Ophthalmology is evidence that research related to diabetic retinopathy has been increasing as the potential for identifying the nature of the fundamental defect in diabetic retinopathy has become more of a reality.

Epidemiology. In a recent summary article, Coughlin and Patz[3] review review the extant biostatistical data on diabetic retinopathy. They report that there are some 4 million known diabetics in the United States. Approximately 40 percent have some form of retinal microangiopathy. Ten percent of these individuals develop proliferative retinal changes. The risk of legal blindness from diabetic retinopathy is correlated best with age at onset of the diabetes. In addition, risk for retinopathy (as measured with ophthalmoscopy) increases from 30 percent at first diagnosis of diabetes to 50 percent after 10 years to 90 percent after 20 years. The average life expectancy following blindness from this disorder is less than eight years.

The relative risk for blindness is also correlated with sex and race. Almost four times as many nonwhite females with diabetic retinopathy become blind as do white males. Twenty-five percent more white females and twenty-seven percent more nonwhite males become blind than do white males.

Epidemiologic studies to determine risk factors for proliferative retinopathy and blindness in diabetics have the potential for providing important information which could be applied to the improved eye care of diabetic patients and may identify new avenues for pursuing fundamental knowledge relative to the etiology of retinopathy. The National Eye Institute has therefore been supporting a number of grants for research on the epidemiology of diabetes and has included natural history components in the contract-supported DRS, DRVS, and ETDRS.

For example, Ramsey and associates at the University of Minnesota have been studying HLA and genetic factors in diabetic retinopathy. They recently reported that one HLA factor (B7) is significantly less frequent in patients who have developed proliferative retinopathy than in matched diabetics who did not. A second HLA antigen (B15) is significantly more frequent. These differences suggest that genetic factors may play an important role in the predisposition to proliferative retinopathy.[4]

Rand at the Joslin Diabetes Foundation and Klein at the University of Wisconsin are also conducting large-scale epidemiologic studies. The Joslin study is a case-control evaluation of some 30 risk (or protective) factors in matched groups of diabetics with proliferative retinopathy (cases) and without (controls). The Wisconsin study is examining the prevalence and severity of all forms of retinopathy in a group of 2,400 diabetic patients.

Management of Diabetic Retinopathy. The primary research tool employed in NEI-supported studies of therapies for diabetic retinopathy and other vascular and circulatory abnormalities of the retina is the randomized controlled clinical trial. The current status of the Diabetic Retinopathy Study (DRS), the Early Treatment Diabetic Retinopathy Study (ETDRS), and the Diabetic Retinopathy Vitrectomy Study (DRVS), and of other related clinical trials, is discussed in the annual report of the NEI Office of Biometry and Epidemiology and in the section on Grant Supported Clinical Trials.

Etiology of Diabetic Retinopathy. The problem of the pathogenesis of diabetic retinopathy can be succinctly stated as follows: Diabetes is a disease characterized by high blood-sugar levels. There is some relationship between elevated blood sugar and microangiopathy in the eye. The nature of this relationship, how high blood sugar causes retinal vascular damage, is the major research problem being addressed by NEI-supported investigators. Some of the questions being asked are: (1) Is the defect in the blood cells, affecting the viscosity and flow of blood through the fine vessels of the eye? (2) Is the defect in metabolic, transport, or anatomical properties of the vessels themselves? (3) Is the neovascularization which is due to the hypoxia which results from nonperfusion in some retinal vessels mediated by a vasoproliferative factor?

(1) Investigators in Palo Alto, Baltimore, Philadelphia, and Boston are conducting rheological studies in diabetic animals and patients, with particular emphasis on retinal capillary blood flow. The work of Riva and associates is illustrative of these efforts. Primary attention during recent years has been devoted to the development of accurate, noninvasive techniques. One such technique is laser dopler velocimetry which can be used to measure continuously the speed with which red blood cells flow through the retinal vessels.[5] Other techniques, such as two-point fluorophotometry, allow determination of retinal mean circulation time and mean segmental-blood-flow.[6] These techniques are now being examined in a study by McMeel with over 100 patients with diabetes.[7]

(2) Major advances have recently been made in studies of the pathologic effects of diabetes on the walls of the retinal vessels. These studies have

concentrated on the role of vascular basement membrane as a barrier separating the blood from the neural retina. The characteristics of this blood-retina barrier (BRB) greatly influence the metabolic state of the retina. Disruption of the BRB, therefore, easily results in retinal pathology. Investigators are studying the biochemistry (and eventually the pharmacology) of the BRB, its morphology in health and disease, and the gross physiological effects of its disruption.

Studies of the biochemistry, anatomy, and physiology of the BRB in retinopathy require the use of appropriate animal models. While some retinal changes that resemble those of human diabetes have been achieved in dogs and monkeys with chemically induced diabetes,[8] it takes over five years for retinopathy to develop in these animals. In addition, they are hard to maintain and require considerable attention. Engerman and associates at the University of Wisconsin have been refining this model and are now conducting studies of the eyes from some of these animals.[9]

Rats and mice with diabetes induced by streptozotocin or alloxan are used for most animal studies. Although these animals do show retinal vascular changes, neovascularization is never seen. In addition, the retinal vascular changes do not always occur. Rather good success in inducing retinal vascular changes can be achieved in rats with streptozotocin and a diet rich in sucrose.[10]

This rat model is now being used by Frank at Wayne State University to study the metabolism of sugars in the cells of the retinal vessel walls. Glucose and galactose are metabolized in these tissues by a number of mechanisms. In hyperglycemia, when the usual pathways are saturated, excess sugars are converted to sugar alcohols by the enzyme aldose reductase. Aldose reductase is normally inactive and is present in a number of tissues such as lens epithelium. As reaffirmed by recent studies on aldose reductase inhibitors, the enzyme is strongly implicated in cataracts resulting from the accumulation of sugar alcohols in the lens. Aldose reductase is also found in the Schwann cells of peripheral nerves and has been implicated by Gabbay in the development of diabetic neuropathy.[12] The enzyme has also recently been found by Buzney and associates in cultures (but not in fresh tissue) of pericytes from rhesus retinal vessels.[13] Frank is using newly developed, highly sensitive biochemical techniques which can be utilized with small samples of fresh tissue to assess the role of aldose reductase in the metabolism of retinal vessel membranes. (It should also be noted that Thoft and associates at the Eye Research Institute of Retina Foundation have recently initiated studies of the corneal effects of aldose reductase in diabetes.)

The morphology of the BRB in health and disease is studied with both light and electron microscopy. Both require the use of tracer substances of high molecular weight: primarily fluorescein for the former[14] and horseradish peroxidase for the latter.[15] Laties and associates at the Scheie Eye Institute in Philadelphia demonstrated leakage of fluorescein through the retinal capillary BRB when hyperosmotic agents were used to stress the cellular transport system in otherwise normal animals.[16] They also demonstrated leakage of fluorescein through the BRB between the retinal

pigment epithelium and the choroid with hyperosmotic stress and have recently shown similar effects in streptozotocin-diabetic rats.

Electron-microscope studies by Wallow and Engerman on the retinas of dogs with alloxan diabetes show extensive leakage of horseradish peroxidase around the retinal blood vessels.[17] The tracer appears to penetrate interendothelial cell junctions, and it is hypothesized that the opening of interendothelial tight junctions accounts for the plasma leakage seen clinically in human diabetic retinopathy. Tso and associates at the Illinois Eye and Ear Infirmary have been using kittens with induced retrolental fibroplasia also to study the ultrastructure of the BRB. The role of endothelial tight junctions and of the barrier between the choroid and retinal pigment epithelium are being evaluated.[18]

Studies of the gross physiological effects of disrupting the BRB have resulted from the recent application of a technique called vitreous fluorophotometry.[19] For a number of years, fluorescein angiography in which repeated photographs are taken of the retina following intravenous injection of fluorescein, has been used as the primary clinical tool to evaluate the nature and extent of retinal vascular disturbance. Fluorophotometry is a refinement which allows the measurement of minute quantities (fractions of nanograms per millimeters) of fluorescein in various ocular tissues. Waltman and associates at Washington University in St. Louis have recently confirmed that both juvenile-onset[20] and adult-onset[21] diabetics show significantly more leakage of fluorescein into the vitreous than do nondiabetic controls. This breakdown of the BRB is observed even when the retinopathy has not yet progressed to a point where it can be seen with ophthalmoscopy or angiography.[22]

These investigators have also been employing vitreous fluorophotometry in evaluating the management of diabetes in streptozotocin-induced diabetic rats.[23] The concentrations of fluorescein in the vitreous humor were found to be higher following streptozotocin treatment than before. Moreover, these levels could be reduced following pancreatic islet transplantation.

Vygantas and associates at the Illinois Eye and Ear Infirmary have also initiated studies to evaluate the effects of photocoagulation therapy on the BRB. In one study,[24] they looked at the effects of xenon arc photocoagulation in normal rabbits. They reported that over the first few days fluorescein leakage is elevated. However, by 10 to 15 days, leakage returns to the pre-photocoagulation normal level.

(3) A number of vision scientists have been examining the hypothesis that the abnormal proliferation of retinal vessels commonly observed in retinopathy is due to the production of a vasoproliferative substance that stimulates an ingrowth of vessels. Suggestive evidence comes from a number of recent findings. For example, Patz and associates at Johns Hopkins University have demonstrated that neovascularization can be induced in kitten retinas with tumor angiogenesis factor (TAF).[25] They also found that neovascularization occurs when tumor cells are in direct contact with the retina but not when they are as little as 100 microns from the retina.

This latter finding is unique to the retina for TAF usually stimulates new vessel formation at distances of several millimeters. This suggests the possibility that there might be an inhibitor of neovascularization present in the vitreous body.

The natures of these putative substances and their roles in the normal and disease states require further investigation. The major obstacle to their evaluation has been the lack of sensitive biochemical techniques for their assay. Chen and Chen recently report that they have refined the methodology for isolating and purifying the angiogenesis factor.[26] They are now proceeding with fundamental biochemical studies to assess its role in the normal development of eye vessel and in ocular vascular diseases.

Grant Supported Clinical Trials

Branch Vein Occlusion Study. The nationwide Diabetic Retinopathy Study has demonstrated the value of photocoagulation in preventing blindness when administered at certain stages of proliferative diabetic retinopathy. Obstruction of a major branch vein may also result in retinal neovascularization and macular edema. Athough there is considerable suggestive evidence that photocoagulation therapy might also be of benefit in reducing the loss of vision due to branch vein occlusion, its efficacy is uncertain; no carefully controlled studies have been performed. Recognizing this need, the National Eye Institute awarded a grant to Finkelstein at Johns Hopkins Hospital in 1977 to develop the protocol for a clinical trial to assess the efficacy of photocoagulation in treating branch vein occlusion and to study the natural history of the disease. In June 1978, the trial's manual of procedures was completed, and awards were made to five clinical centers: Wilmer Ophthalmological Institute, Johns Hopkins Hospital; University of Illinois Eye and Ear Infirmary; Bascom Palmer Eye Institute, University of Miami; University of Southern California; and the Eye Research Institute of Retina Foundation, Boston.

The study plans to recruit a total of 310 patients with branch vein occlusion. Patients are stratified across three clinically distinct groups, each with its own recruitment requirement, under a set of strictly-followed eligibility criteria: (1) major branch vein occlusion without neovascularization, (2) major branch vein occlusion with neovascularization, (3) branch vein occlusion with macular edema and decreased vision. The patients in each group are randomized to laser photocoagulation treatment or to observation and are then followed for at least three years. At the end of 11 months of active recruitment, 132 patients have been enrolled. It is expected that the required number of study patients will be enrolled by mid-1981.

The Safety and Data Monitoring Board for the Branch Vein Occlusion Study meets twice each year. To date, the Board has found no need to take any actions based on the data accumulated.

Macular Photocoagulation Study. In 1976, Fine at Wilmer Ophthalmological Institute, Johns Hopkins Hospital, submitted research grant applications to the NEI proposing clinical studies of photocoagulation

therapy for two retinal disorders involving choroidal neovascular membranes: senile macular degeneration (SMD) and presumed ocular histoplasmosis (POH). As recommended by the National Advisory Eye Council, the NEI provided support initially for the development, refining, and testing of detailed manuals of protocols and procedures for each study in June 1977.

Two points became apparent during the development of the details of the two studies: (1) The experimental and administrative requirements for the two studies were very similar. (2) Large numbers of patients (736 with POH and 522 with SMD) would have to be entered into each study if meaningful statistical analyses were to be done. The two studies were therefore combined as the Macular Photocoagulation Study for administrative purposes and the participation of additional clinics was solicited. A committee was organized which continued the planning for the study and preparation of the manual of procedures. Meanwhile, interested investigators were invited to submit grant applications.

The National Advisory Eye Council, in September 1978, once again reviewed the study proposal and designated the Macular Photocoagulation Study as being of high programmatic relevance. In January 1979, the NEI awarded grants to the study's coordinating and fundus photograph reading centers at the Wilmer Institute and to eight clinics which are located in Baltimore, Miami, Indianapolis, St. Louis, New Orleans, Seattle, Milwaukee, and Iowa City. Training and certification of the clinic staffs for participation in this clinical trial is nearly completed, and recruitment of patients with SMD and POH has begun in the certified clinics.

Additional clinics will be needed in order to complete recruitment in a timely fashion. Therefore, the National Eye Institute issued a request for additional grant applications for potential participating clinics in February 1979.[27] It is expected that, depending on the outcome of the review of responsive proposals, two to six additional clinics will join the Macular Photocoagulation Study by late 1979.

Disciform Macular Disease Study. This study is also a clinical trial of laser photocoagulation treatment for SMD. The Disciform Macular Disease Study differs from the SMD portion of the Macular Photocoagulation Study in a number of respects, most important of which is that the Disciform Macular Disease Study is evaluating the safety and efficacy of treatment in patients with avascular lesions as well as in patients with vascular lesions.

The Disciform Macular Disease Study is a single-center clinical trial conducted by Bird and associates at the Institute of Ophthalmology, Moorfields Eye Hospital, London, England. Because of the unique pattern of patient referral to that hospital, it will be possible to enroll the close to 300 required study patients in a timely fashion at that one clinic. During the past year, the study's manual of procedures was completed and the availability of eligible patients demonstrated. Patient recruitment is well along.

In order for an eye to be considered eligible for treatment by argon laser photocoagulation in this study, the subretinal neovascular membrane must be clearly defined and at least 100 microns from the foveola (compared with 200 microns in the Macular Photocoagulation Study). The investigators at Moorfields have recently evaluated the nature of patients with SMD who might meet this eligibility criterion.[28] From a retrospective analysis of their one-year experience in which 530 eyes (398 patients) with SMD were evaluated, they found that most SMD patients who still had good central acuity would have been eligible but few with poor acuity. Many patients with a short history of SMD symptoms would have been eligible but few with a long history of symptoms. They conclude that "as many as 50% of all patients with senile disiciform macular degeneration may be amenable to treatment if seen early enough in the course of their disease." The reader will note, as do the investigators, that these data are concerned with the eligibility of patients for treatment. Whether laser photocoagulation treatment is beneficial remains to be determined from the results of the Macular Photocoagulation and the Disciform Macular Disease clinical trials.

The Vitreous Body and Vitreoretinal Disorders

In primates, the vitreous body occupies almost 90 percent of the total volume of the eye. Considerable information has recently become available regarding its physical and structural properties. It has been postulated that the vitreous matrix helps separation of the image formed by the anterior optical system and light absorption by the visual cells. Vitreous matrix is a transparent system that is highly permeable to small molecules but impermeable to cells. In addition, the vitreous provides protection for the retina which is sensitive to mechanical dislocation.

The mechanical properties of the vitreous body are determined by hyaluronic acid and collagen which form a double network; the hyaluronic acid macromolecules fill the space between the collagen fibrils. It has been suggested that interaction between the fibrils and the hyaluronic acid molecules plays an important role in the mechanical stabilization and volume control of the gel. The viscoelastic properties of the hyaluronic acid prevent the fibrils from aggregating and precipitating under the stress of vibration and shock to which the eye is exposed. Consequently, the hyaluronic acid acts as a "sponge" which can be regarded as the vibration isolator and shock absorber of the vitreous. The distance between the collagen fibrils is determined by the volume of the hyaluronic acid "sponge." The volume of the vitreous gel can therefore be regulated by the volume changes associated with the hyaluronic acid molecules.

Knowledge of embryonic development is important to comprehending the function of adult vitreous tissue. More than 90 percent of visible light is transmitted through the normal vitreous to the retina; therefore, development and maintenance of a transparent vitreous body is of obvious importance. During human embryonic development of the vitreous gel, three major stages can be distinguished: (1) the primary, or hyaloidal, vitreous body; (2) the secondary, or definitive vitreous body; (3) the tertiary vitreous body, or the zonule of Zinn. The primary vitreous body begins to form when a space

opens between the lens plate and the optic vesicle and becomes filled with a protein-rich PAS-positive material. In the fifth week, a vascularized mesoderm enters the optic fissure and the hyaloid artery develops as does the posterior pole of the lens vesicle. The secondary vitreous body which has a finer network of filaments and fewer cells than the primary vitreous body then begins to appear at the margin of the retina. By the ninth week, the hyaloid artery and the primary vitreous body are fully developed and the secondary vitreous body is also evident. The primary vitreous body atrophies during further development. By the seventh month, the hyaloid artery no longer carries blood, and around the time of birth it is reabsorbed. The factors which are responsible for the change from a vascular to an avascular vitreous tissue are not clear but continue to be studied by Jacobson,[29] Swann,[30] Hultsch[31,32] Balazs[33] and others.

Vitreous Body and Neovascularization. New blood vessels from the retina rarely enter directly into the formed vitreous gel when retinal neovascularization occurs. Neovascularization is one of the most important clinical problems of ophthalmology, since new blood vessels may contribute to pathological conditions. The main objective of research conducted by Jacobson and associates,[29] therefore, has been to determine if the conversion of the embryonic vitreous body from a vascular to an avascular tissue is due to the production of an inhibitor of neovascularization, and if so, whether hyalocytes (vitreal cells) are responsible for its production; the proliferation of hyalocytes coincides with the reduction in vascularity during embryogenesis. Preparations of vitreous tissue from calf embryos, newborn calfs, and cows appear to contain a nondialyzable factor which inhibits the proliferation of aortic endothelial cells. The stimulation of aortic endothelial cell proliferation by angiogenic factors, and prevention of proliferation by inhibitory substances is a common assay for such substances. In vitreous gel preparations, collagen is removed by high speed centrifugation, hyaluronic acid by precipitation with cetylpyridinium chloride (CPC), and other low molecular weight material by dialysis. Cartilage is similar to the vitreous gel in that it is vascular before birth and becomes avascular during development. Neonatal cartilage also synthesizes a diffusible factor that inhibits capillary proliferation induced by tumors.

Studies by Chen and associates[34] have raised the possibility that a vitreous soluble protein may be fundamental to both normal vascularization and abnormal neovascularization in developing retina. In their preliminary studies, apparent vasoproliferative activity has been demonstrated in the vitreous body homogenate using the corneal micropocket technique and vascular endothelial cell culture bioassays from puppies. The investigators demonstrated that when vitreous tissue obtained from a young puppy with retinal capillary closure is placed in a corneal micropocket in rabbit, the nearest limbal vessels are stimulated to grow into the cornea and towards the pocket. Vitreous tissue from a normal adult dog showed no such activity. However, with the aorta endothelial cell culture method, which contains no immune competent cells, almost all of the vitreous tissue (from various sources) tested showed approximately the same specific activity per mg protein. Furthermore, the activity per unit volume is significantly higher in the vitreous tissue from fetal and newborn animals and from animals with retinal capillary closure,

than in vitreous tissue from normal adults. This may be because fetal and newborn animals have much higher concentrations of vitreal protein than do adults.

It is known that there is no active protein synthesis in the vitreous body. Although current studies do not explain how and from where proteins are delivered to the vitreous body, Chen's data raise the possibility that there may be a barrier system, or a transport system, that maintains vitreal protein concentration at a constant level. The level is close to or slightly higher than that of the eye's anterior chamber, but much lower than that of serum. A pattern of declining vitreal protein concentration has been observed in newborn animals and suggests efflux of vitreal proteins. A steady increase in total protein per eye as animals grow older indicates the influx of protein in order to maintain a constant concentration as the volume of the eyeball increases with age.

Vitreous Body and Hyalocytes. The hyalocytes are the cells which are embedded within the vitreous gel. A detailed understanding of their metabolic role, and those factors which regulate it, is of considerable significance for a more complete understanding of vitreous metabolism.

Production of an abnormal vitreous gel, due to changes in the anabolism or catabolism of the extracellular matrix macromolecules, leads to degenerative changes which play a prominent role in the pathogenesis of retinal detachment. In view of the role of hyaluronic acid and collagen in the maintenance of normal vitreous gel, studies designed to clarify the process of vitreous homeostatis and studies on maintenance of normal vitreous gel structure, may aid in understanding of vitreous gel pathology. Such studies are in progress in the laboratories of Jacobson,[29] Hultsch,[31,32] Balazs[33] and others.

Vitreous Detachment. Although the vitreous body apparently performs similar functions in most species, the amounts and proportions of its extracellular macromolecular constituents vary markedly among species. It is thus difficult to relate tissue function to the concentration of the macromolecular constituents. An important common property, however, is that the vitreous in most species occurs as a gel, and liquefaction is often associated with pathological changes, such as the formation of fibrotic scar tissue. Vitreous collagen, therefore, and the other structural proteins associated with it appear to play key roles in the normal functioning of the tissue.

Posterior vitreous detachment (PVD) is a pathological condition of the vitreous body which is often related to aging and is the result of "fibrillary degeneration" of the vitreous gel. In view of the reported relationship between aging, myopic conditons, the appearance of vitreous "floaters" and PVD, the question seems to be to what extent does the normal aging of the vitreous, in terms of changes in the distribution of collagen fibrils and the concentration and molecular size of hyaluronic acid, contribute to these pathological conditions. Retinal breaks and hemorrhages occur in 10-15 percent of PVD cases in elderly persons. Thus, an important disease of

the eye, retinal detachment, may result from the aging of the vitreous.

More research is needed to obtain detailed information about the properties of the vitreous body and to determine how the vitreous maintains both its avascularity, which is of basic importance to its transparency, and its internal gel structure, which is of importance to its shock-absorbing role. Information on the type of collagen present in the vitreous body is also lacking, as is knowledge of the mechanism by which the vitreous body is displaced by invading tumors such as retinoblastoma.

Vitreous Body and Blood-Vitreous Barrier. Research which contributes to an understanding of the flow of nutrients through ocular humors is needed to understand active transport, effects of drugs and metabolic inhibitors, and the possible search for therapeutic regimes. Fowlks and associates[35,36] for example, are studying the role of barrier mechanisms. The "blood-vitreous body barrier" (BVB) refers to any barrier which separates the choroidal or retinal blood and the vitreous body. The term "overall barrier" refers to all barrier functions of the eye which include the BVB, the blood-aqueous humor barrier (B-A barrier), the aqueous humor vitreous body interface, the lens vitreous body interface, and any other barrier function of the eye which has any influence on the composition of the vitreous humor.

Although considerable research has been done on many aspects of the physiology and biochemistry of the eye, not much is known about the "overall barrier." Without the "overall barrier," which appears to be an extension of the blood-brain barrier, vision would undoubtedly be impossible. Among the functions of the "overall barrier" seems to be the protection of the very delicate nervous tissue of the retina from toxic substances and the maintenance of a milieu favorable to the proper functioning of the various structures.

Cyclic Nucleotides in the Retina

Cyclic guanosine monophosphate (c-GMP) and cyclic adenosine monophosphate (c-AMP) are synthesized and held in steady state in tissues by the actions of specific enzymes. Among the biological activities of these compounds is the regulation of intracellular metabolism and cellular function. Cyclic nucleotides interact as "messengers" with hormones, neurotransmitters, free radicals, ions, fatty acids, vitamins, and light. More is known about c-GMP metabolism than about c-AMP metabolism in rod photoreceptors. Much less is known about cyclic nucleotide metabolism in retinal pigment epithelium. The subject of cyclic nucleotides in the eye has been reviewed by Lolley and associates.[37,38,39,40,41]

The distribution of c-GMP and c-AMP in the retina has been demonstrated their localization in specific cell types. The lowest concentration both of c-AMP and c-GMP within the retina is found in the pigment epithelium. This, and the small amount of retinal pigment epithelium tissue relative to other eye tissues, makes investigation difficult and demonstrates a need for further technological development before the function of cyclic nucleotides in the retinal pigment epithelium can be determined. The highest concentration

of c-GMP within the retina is found in the photoreceptors of the rod-dominant retina which contain only moderate amounts of c-AMP. The speculation is that c-GMP may act in some aspect of scotopic vision. There is a paucity of information about the biochemistry of cones; however, recent observations by Lolley,[38] Farber,[42] Cohen,[43] and Ferendelli[44] suggest that rods and cones may use different cyclic nucleotides in the regulation of their functions.

For example, in the cone-dominant retina of the ground squirrel, the content of c-AMP surpasses that of c-GMP. Within the retinal layers that lie between the photoreceptor layer and vitreal border there is a neural organization containing levels of c-AMP that are relatively higher than those of c-GMP. The high concentration of cyclic nucleotides in the visual cell layer of the retina implies that these compounds have a role in photoreceptor cell function. This idea has been strengthened by the demonstration that light modulates cyclic nucleotide metabolism in the visual cells and that an enzymatic defect in cyclic nucleotide metabolism can initiate photoreceptor cell degeneration. Therefore, a role for cyclic nucleotides in the regulation of visual cell metabolism appears to exist; in particular, they may help coordinate biochemical events within the morphological compartments of these cells.

The possible action of cyclic nucleotides in regulating the function of retinal cells may be vitally important to the maintenance of vision.[45] The action of c-GMP may prove to have clinical relevance in those inherited disorders characterized by visual cell degeneration and blindness. Elucidating the role of c-GMP in these disorders might provide insight into how drugs could be used to retard or prevent visual cell death and blindness.

Lolley and associates[38] have shown that an abnormality in c-GMP metabolism, whether mutation-induced (rd mouse), debris-induced (RCS rat) or drug-induced (Xenopus eye rudiment cultures), is associated with photoreceptor cell degeneration in the vertebrate retina. The molecular biology which underlies this degeneration is unknown, but it may be related both to the specialized function of visual cells and to the central role of c-GMP in the modulation of biochemical events within the rod outer segment. The investigators' data confirm that c-GMP is the prevalent cyclic nucleotide in visual cells, and that c-GMP is especially concentrated in rod outer segments. Moreover, the content of c-GMP is reduced when rhodopsin is bleached by laboratory illumination. The light-induced reduction in c-GMP results from coupling the bleaching of rhodopsin either to the activation of c-GMP-phosphodiesterase, which increases the rate of c-GMP hydrolysis, or to the inhibition of c-GMP synthesis via guanylate cyclase.

The relevance of c-GMP changes to dark/light adaptation is still unclear. However, the light-induced changes are indicative of a role for c-GMP in the function of photoreceptor cells. An abnormality in c-GMP metabolism can disrupt the homeostatic balance within the photoreceptors and thus may initiate visual cell degeneration. Systematic investigation of the synthesis, degradation, and role of phosphoproteins in visual cells may help establish a biochemical basis for understanding how c-GMP modulates the function of normal

visual cells and how it may act to disrupt photoreceptor cell homeostasis and initiate the degenerative process.[45]

Farber and associates[42] are working to gain insight into the regulation of cone function and to provide information that may be useful for the treatment or prevention of human cone degeneration by investigating the metabolism of cyclic nucleotides in this cell. Assessment of whether cyclic nucleotide metabolism is linked to cone function will require study of modulation of the c-AMP or c-GMP content in cones and of regulation of the rate of phosphorylation in specific proteins. As an adjunct to the study of normal cones, it is essential to assess whether abnormalities in cyclic nucleotide metabolism are associated with the onset of cone degeneration. Preliminary results suggest that cones differ greatly from rods with regard to cyclic nucleotide metabolism. It is hoped that such studies will provide useful information about the normal biochemistry of cone visual cells and, perhaps, reveal an early defect that leads to cone degeneration. In view of the importance of cone degeneration in human blindness, these studies could also provide findings of far-reaching significance for clinical research.

Ferrendelli and associates[44] also believe that the anatomical location of retinal cyclic nucleotides and their biochemical metabolism and actions are important. Because the retina is a heterogeneous organ, both morphologically and functionally, it seems essential that any study of this tissue must take these differences into consideration. The above investigators' studies correlate retinal biochemistry and morphology.[46] Therefore, they can elucidate the various roles of cyclic nucleotides in the retina and explain some molecular mechanisms underlying vision. They have developed techniques and methods which allow quantitative measurement of cyclic nucleotide content and the activities of enzymes which catalyze their formation and degradation, in microscopic samples. They now propose to utilize these techniques to define the locations and characteristics of specific c-AMP and c-GMP systems in microscopic regions of retina. Once this is accomplished, definition of the roles of c-AMP and c-GMP in the retina may be advanced.

Cohen and associates[43,47] have noted that other investigators have given serious consideration to a transduction mechanism for photoreceptors which depends on a phosphodiesterase modulation of the level of cyclic nucleotides, as opposed to free calcium as a modulator of electrophysiologic activity. Their data bear importantly on these alternatives as well as on calcium plus cyclic nucleotide hypotheses. Receptor dysfunction underlies several major causes of blindness, and the data of these investigators contribute importantly to understanding the normal metabolism of these cells. Chelating external calcium was shown to increase markedly the "dark" levels of c-GMP but not of c-AMP. This dark effect was reversible by either using light or restoring external free calcium levels. Evidence was obtained to indicate that these changes were occurring in rods. However, no manipulation of external free calcium could force the c-GMP level to fall in a manner equivalent to that obtained with bright light. Cyclic AMP levels were also seen to be light-sensitive under certain circumstances. Progress has been achieved in analyzing the distribution and light behavior of the cyclic nucleotides in cone-rich retinas. Amplification of the role of cyclic nucleotides in visual cells is a relatively

recent advance, and its impact upon vision research has yet to be fully realized.

Neurotransmitters in the Retina

Although the structure, synaptic connections, and physiological responses to light stimulation of the various types of cells in the vertebrate retina are being well studied, little is known about the neurotransmitters used by these cells. Also, much is yet to be learned about the chemical events at the synapse or the development and kinetics of transmitter synthesis and storage during retinal development. During recent years research has focused on the identity, function, and synthesis of known and suspected neurotransmitters. One way to obtain this information is to examine the ability of single intact cells to synthesize and accumulate known or suspected neurotransmitters. For example, electrophysiological and pharmacological methods have been used with intact retinas and isolated retinal cells in order to determine if individual cells synthesize or store transmitter substances.

The complexity of this area of investigation is illustrated by the fact that even within one major class of retinal neurons, different transmitters may be used by cells which have different synaptic connections and functions.[48] In view of these complexities, Lam and associates are studying known and suspected transmitter candidates in vertebrate retinal cells. They are also searching for possible new transmitter substances which might be used by those cells which do not synthesize or store any known transmitters. The development and kinetics of transmitter snythesis and storage during retinal development and regeneration are also being examined and the results correlated with morphological and electrophysiological findings.[49]

Sarthy and Lam[50,51,52] are conducting research to determine the neurotransmitters used by each type of neuron in the vertebrate retina. Earlier studies have shown that of all the known neurotransmitters, the retina contains significant quantities of acetylcholine (ACh) gamma-aminobutyric acid (GABA), and dopamine. The immediate objective is to localize the neurons that use each of these substances as a neurotransmitter. The following methods will be used for the proposed research: (1) biochemical studies of the endogenous levels, synthesis, and accumulations of ACh, GABA, dopamine, and amino acids, (2) light and electron microscopic autoradiography to utilize the neurons in the retina that possess high affinity uptake systems for GABA and dopaminergic synapses with other retinal cells and (3) electrophysiological and pharmacological studies of the effects of known agonists and antagonists of neurotransmitter candidates in the retina by intracellular and extracellular recordings. Because earlier work suggested that some retinal cells may not use any of the known neurotransmitters, an additional goal is to search for possible neurotransmitters.[53]

Masland and associates[54] have pointed out that the existence of a significant number of acetylcholine-synthesizing cells in the ganglion cell layer of the rabbit retina suggests either that the layer contains a larger number of displaced amacrine cells or that a subset of ganglion cells is cholinergic. The former possibility could modify popular concepts about the structural arrangement of the inner plexiform layer, and the latter could provide a new approach for the study of central visual system structure and function.

Masland's current program is designed to contribute to understanding of the characteristics and functional role of the cholinergic synapse or synapses. The approach to this goal is developmental, and a substantial part of the work concerns the cholinergic system of immature retinas. In adult tissue the experiments are designed to characterize the pharmacologic behavior of the retina's cholinergic synapse. The experiments are primarily concerned with the nature of the acetylcholine receptor in retina and involve detailed electrophysiological studies of the effects of pharmacologic and biologic receptor antagonists on the retina's function. A complementary investigation seeks to evaluate the functional role of the retina's cholinergic system independent of acetylcholine receptors by studying the activity of ganglion cells in retinas depleted of acetylcholine or retinas in which acetylcholine release is suppressed. Another aim is to learn how the effects of acetylcholine interact with those of GABA since there is evidence that both are neurotransmitters in the inner plexiform layer, and they probably act together to shape ganglion cell receptive fields.

These studies are aimed at an in-depth understanding of the function of the retina's cholinergic system. They should provide knowledge of the internal working of the retina's circuits and the selective projection of ganglion cells to the central visual system. The choline metabolism of the photoreceptors is interesting in its own right, but more importantly because it is a possible site of the primary lesion in certain retinal degenerations.

Redburn and associates[55] have attempted to identify and localize the functional retinal neurotransmitters as a basis for further understanding of visual information processing. Eight putative neurotransmitter agents have been screened to determine their ability to meet specific criteria established for functional neurotransmitters by direct biochemical analysis of uptake, storage. and spontaneous and evoked release characteristics. Subcellular fractionation procedures for the separation of two synaptosomal fractions from rabbit retina have been established. Efforts have been directed toward the cellular and subcellular localization of specific neurotransmitter systems. Cellular and subcellular fractionations have been correlated with light and electron microscopy. Autoradiograhic analysis has been used to ascertain the specific cell populations associated with the GABA, dopamine, glutamate and acetylcholine neurotransmitter systems. Two techniques were adapted for these investigations, receptor binding assays and neurotransmitterstimulated adenylate cyclase analysis.

These studies have potential for adding important information to the knowledge of functional neurotransmitters in retina. The ability to identify

neurotransmitter compounds will be a tremendous advantage in subsequent experimental analyses. Chemical transmission is specific in the sense that uptake, release, and receptor sites have a high degree of specificity for each transmitter system and in the sense that individual transmitter activities might be easily modified by chemical or pharmacological intervention. Thus, specific chemicals might be used to manipulate discrete elements within retinal pathways so that biochemical correlates can be established for electrophysiological and visual perceptual events.

Relative to some other neural systems, the retina appears to have a morphological and functional simplicity. Its accessibility for physiological stimulation by noninvasive techniques is also an advantage for research. A good example of the suitability of the retina for neurotransmitter studies is the isolation of a synaptosomal fraction from a single identified cell type with which in vitro analysis of synaptic biochemistry is feasible. The techniques, developed in rabbit, can be applied to small amounts of human material which could be made available within several hours after surgical enucleation or autopsy. Transmitters in human retina could then be identified. Such are the necessary prerequisite for the development of a rational pharmacology for modification of human retinal function. If biochemical correlates are to be established for various retinal diseases, the use of rapid biochemical analyses of small amounts of retinal tissue is essential.

Effects of Light on Visual Processes

Toxicity to Photoreceptors. A unifying concept in this area of research is that the investigations deal with the toxicology of an organ system rather than that of single substances. Such research is relevant to determining safe limits of exposure of the eye to visible radiation. It seeks to establish the maximum safe dose-rates for long-term exposures to different wave-bands in the visible spectrum. The program, however, should also examine the questions of how much exposure, to which wavelengths, and for how long, yields retinal damage.[56]

These mechanisms are being examined with psychophysical, structural, and ultrastructural studies of receptor cells and pigment epithelium, and by application of multidisciplinary techniques to measure altered metabolic events. Sperling and associates[57] are conducting studies on goldfish, a cone vision animal, in which they can precisely identify which photopigment lies in a given cone by observing its size, shape, and position in the retina. This preparation allows extensive parametric studies of threshold energies and dose rates for producing different types of retinal damage. Beyond their immediate concern with photic radiation damage, these studies have provided important basic information about the functional and structural relationships underlying the mechanisms of color and brightness in vision.

The goldfish is an inexpensive and readily available subject. It is already known from histochemical studies to which class each morphologically different color receptor belongs. Therefore, spectral sensitivity can be

exploited in the goldfish in order to produce "color-blinding" effects in this animal similar to those found in the monkey. This approach permits an assigment of the effect either to photoreceptors or to some higher neural process. It also affords a sufficiently inexpensive and easy way to conduct parametric studies of intensity-time-wavelength interactions and a means of obtaining irreversible photic damage to the different classes of photoreceptors.

These studies provide a model of primary cone dystrophies in vertebrates. This is achieved with time-divided exposure to moderately intense spectral lights. These studies also aim to determine the maximum allowable exposure to intense spectral lights without permanent damage to the eye. In the course of these studies, it has been possible to compare the results obtained by divided doses with continuous doses of the same total energy. Results to date indicate that continuous doses produce damage resembling the pigment epithelial disorders such as retinitis pigmentosa, while divided doses simulate the cone cone dystrophies. Continuing studies will be directed towards establishing and validating light-induced animal models of retinal degenerative disorders and and seeking to understand the putative interaction between genetically determined retinal dystrophies and intense light exposures.

Toxicity to Optic Nerve. Potts and associates[58,59] have invested their efforts in establishing the cat as a test subject. Since the cat has an area centralis (without a fovea), they have attempted to investigate how far this change will carry toward the goal of separating the biology of those fibers mediating central perception compared with those mediating peripheral perception.

They have produced argon laser lesions in the area centralis of one eye. After fixation, epon-imbedding and sectioning of optic nerve, photographs have been submitted to the Argonne National Laboratory for counting, sizing and comparison of cells from injured and control eyes. Initial results indicate that the central area of the retina is the bearer of high acuity and color vision. It is affected selectively by some diseaes and spared by others at the level of the optic nerve. This research should provide information which bears on the problem of regional differences in toxic succeptibility.

Toxicity to Short-Wavelength and Near-Ultraviolet Light. Ruffolo and associates[60] are conducting studies which are aimed at contributing new basic information about retinal photopathology, cataractogenesis, and the photobiological properties of the eye in general. This research is potentially pertinent to several areas of practical application, such as ocular hazards, use of lasers in opthalmology, retinal inflammation, reactionary hyperplasia, foveomacular retinitis, solar retinitis, eclipse blindness, senile macular degeneration, aphakia, hazards of phototherapy, photobiology of melanin pigmentation, and photochemical aspects of cataractogenesis. The primary objective of these investigations is to determine the photobiological mechanisms by which short wavelength light and near ultraviolet radiation cause, or contribute to, retinopathy in primates after extended low level exposure.

Future experiments will use aphakic monkeys to determine the paramacular and macular thresholds for damage by near-ultraviolet radiation and to study the histopathology of retinal lesions. In one case a monkey has been trained

to carry out a visual task and is participating in a study aimed at correlating solar retinitis and eclipse blindness with retinal pathology due to short wavelength and photchemical lesions.[61,62]

Program Announcements

During FY 1979, the National Eye Institute released three program announcements encouraging the submission of grant applications for specific kinds of research related to retinal and choroidal diseases: (1) Vascular and circulatory abnormalities of the retina including diabetic retinopathy -- research and training grants,[63] (2) Macular diseases research grants,[64] and (3) Ocular melanoma.[65] The first two announcements were distributed to the vision research community via the NIH Guide for Grants and Contracts, the third via a letter to pertinent journals. In addition, the NEI participated with seven additional components of the NIH in an NIH Guide announcement on the epidemiology of diabetes.[66]

Vascular and Circulatory Abnormalities. This program announcement provided examples of fundamental research areas requiring additional attention as identified by the National Advisory Eye Council in Vision Research--A National Plan: 1978-1982.[67] In addition, it reaffirmed the interest of the NEI in fostering the training of laboratory scientists and clinical investigators in research on diabetic retinopathy and related disorders. Some of the highlighted areas are as follows:

- anatomy and pathology of blood vessels in different regions of the retina;
- physiological studies on factors influencing blood flow, mechanisms of edema formation, closure of capillaries and large blood vessels, and protein transport into and across the neural retina;
- angiogenic substances, antagonists, and inhibitors;
- biochemical abnormalities of diabetic retinas obtained from human and animal models;
- clotting mechanisms;
- mechanisms by which the permeability of retinal blood vessels and pigment epithelium is controlled;
- how normal blood vessel structure is maintained, how tight junctions form, and what factors are necessay for them to remain functional;
- the metabolic relationships of neurons and glial cells;

- the retina under conditions of stress; how and to what extent the retina tolerates ischemia, hypoglycemia, and other derangements.

Macular Disease. This announcement expressed the NEI's continuing interest in research on abnormalities associated with the macular region of the retina. Macular degenerations constitute an area of exceptional importance in terms of retinal disease and resultant vision loss, as well as in terms of scientific interest and research opportunity. They are among the most difficult visual disease problems to manage clinically and, until more fundamental information is obtained, little more can be done for the patient than observe the development of the macular disorder, attempt to treat the symptoms, and localize the disease process on an anatomical basis.

The need for new and more comprehensive interdisciplinary studies to increase fundamental knowledge of the structure and physiology of this region of the retina, as well as of the etiology and pathogenesis of its disorders, is cited in Vision Research--A Naitonal Plan: 1978-1982.[68] Examples of problems requiring further investigation are:

- temporal events (anatomical, physiological, biochemical) in the pathogenesis of macular diseases;

- differences between macular and peripheral retinal structures and functions so as to better understand the increased susceptibility of the macular region to disease;

- retinal edema and abnormal accumulation of fluids under the retina -- the roles of blood-retina barriers and photoreceptor metabolism;

- normal, pathogenic, and toxic effects of various chemicals and drugs on the macula.

Ocular Melanoma. At its January 1979 meeting, the National Advisory Eye Council took note of the increasing number of grant applications from investigators who wish to study the effects of nodispersing enucleation techniques and adjunctive immunotherapy in treating ocular melanoma. Agreeing that we presently do not know which treatment offers the best chance of survival for patients with this tumor, the Council proposed that the various groups interested in the therapy of ocular melanoma be invited to NIH to attend a "consensus conference" to discuss its treatment.

The Council was particularly interested in the natural history of ocular melanoma and in the effects of enucleation on metastatic dissemination. The Council also proposed that a protocol for a possible randomized controlled clincial trial of various proposed therapies be considered at the conference. The feasibility, potential scientific value, and ethical aspects of such a trial should also be considered. In addition, because the published results of previous studies are conflicting, because other studies are in progress, and

because one mode of therapy (methanol extract residue of BCG) may be toxic, the opinions of experts in tumor immunotherapy should be included.

The Council's proposal for a consensus conference on the management of ocular melanoma has been communicated to the vision research community via an announcement in ophthalmic journals and other publications. The consensus conference is being planned for late 1979.

The Epidemiology of Diabetes. In cooperation with other components of the NIH, the NEI has participated in a number of program announcements encouraging research in diabetes. In FY 1978 we joined in the announcement "Studies of diabetes mellitus and related problems."[69] This past year we participated in a request for research grant and fellowship applications to study the epidemiology of diabetes and its complications. Some of the research areas highlighted are:

- studies on etiology including nutritional, environmental, virological, immunological, and genetic components;
- epidemiologic approaches to the natural history of the disorder;
- assessment of risk factors;
- the causes of differences betweeen populations and groups in the frequency of development and extent of the complications of diabetes, including cardiovascular, renal, neurologic, ocular, dental, and pregnancy complications;
- epidemiologic assessment of the relationship with obesity;
- assessment of changes with aging;
- problems of patient compliance.

References

Retinal and Choroidal Diseases

1. Vision Research--A National Plan: 1978-1982, Vol Two, Panel Reports. US DHEW Publ No (NIH) 78-1259, 1978, p 10.

2. Vision Researh--A National Plan: 1978-1982, Vol Two, Panel Reports. US DHEW Publ No (NIH) 78-1259, 1978, p 1-142.

3. Coughlin WR, Patz A: Diabetic retinopathy: Nature and extent. J Vis Impairment and Blindness 72:343-347, 1978.

4. Barbosa J, Ramsay RC, Noreen H, Chern MM, de Leiva A, King R, Yunis EJ: Population studies of histocompatability antigens in diabetes mellitus. I. Diabetic proliferative retinopathy and the HLA. New Engl J Med (in press).

5. Feke GT, Riva EC: Laser Doppler measurements of blood velocity in human retinal vessels. J Opt Soc Am 68:526-531, 1978.

6. Eberli B, Riva CE, Feke GT: Mean circulation time of fluorescein in retinal vascular segments. Arch Ophthalmol 97:145-148, 1979.

7. McMeel, JW: Annual Progress Report, EY 02164-02, February 1979.

8. Engerman R, Bloodworth JMB, Nelson S: Relationship of microvascular disease in diabetes to metabolic control. Diabetes 26: 760-769, 1977.

9. Engerman RL: Annual Progress Report, EY 00300-14, September 1978.

10. Papachristodoulou D, Heath H, Kang SS: The development of retinopathy in sucrose-fed and streptozotocin-diabetic rats. Diabetologia 12: 367-374, 1976.

11. Varma SD, Mizuno A, Kinoshita JH: Diabetic cataracts and flavonids. Science 195:205-206, 1977.

12. Gabbay KH: Hyperglycemia, polyol metabolism, and complications of diabetes. Annu Rev Med 26:521-536, 1975.

13. Buzney SM, Frank RN, Varma SD, Tanishima T, Gabbay KH: Aldose reductase in retinal mural cells. Invest Ophthalmol Vis Sci 16: 392-396, 1977.

14. Tripathi RC, Tripathi BK: New methods for light and electron microscopic localization of fluorescein-labelled dextran in ocular tissue using epoxy-resin embedding. Exp Eye Res 25:259-262, 1977.

15. Peyman GA, Bok D: Peroxidase diffusion in the normal and laser coagulated primate retina. Invest Ophthalmol Vis Sci 11:35-39, 1972.

16. Laties AM, Rapoport S: The blood-ocular barriers under osmotic stress. Arch Ophthal Mol 94:1086-1096, 1976.

17. Wallow IHL, Engerman RL: Permeability and potency of retinal blood vessels in experimental diabetes. Invest Ophthalmol Vis Sci 16:447-461, 1977.

18. Tso MO: Annual Progress Report, RO1 EY 01903-04, January 1978.

19. Cunha-Vaz JG, Abreu JRF, Campos AJ, Figo G: Early breakdown of the blood-retinal barrier in diabetes. Br J Ophthalmol 59:649-659, 1975.

20. Krupin T, Waltman SR, Oestrich C, Santiago J, Ratzan S, Kilo C, Becker B: Vitreous fluorophotometry in juvenile-onset diabetes mellitus. Arch Ophthalmol 96: 812-814, 1978.

21. Waltman SR, Krupin T, Kilo C, Becker B: Vitreous fluorophotometry in adult-onset diabetes mellitus. Am J Ophthalmol (in press).

22. Waltman SR, Oestrich C, Krupin T, Hanish S, Ratzan S, Santiago J, Kilo C: Quantitative vitreous fluorophotometry. Diabetes 27:85-87, 1978.

23. Krupin T, Waltman SR, Scharp DW, Oestrich C, Feldman SD, Becker B, Ballinger WF, Lacy PE: Ocular fluorophotometry in experimental diabetes mellitus: The effect of pancreatic islet isografts. Invest Ophthalmol Vis Sci (in press).

24. Noth JM, Vygantas C, Cunha-Vaz JGF: Vitreous fluorophotometry evaluation of xenon photocoagulation. Invest Ophthalmol Vis Sci 17:1206-1209, 1978.

25. Finkelstein D, Brem S, Patz A, Folkman J, Miller S, Chen HC: Experimental retinal neovascularization induced by intravitreal tumors. Am J Ophthalmol 83:660, 1977.

26. Chen CH, Chen SC: Angiogenic activity in vitreous and retina. Invest Ophthalmol Vis Sci (in press).

27. Request for research grant applications: Cooperating clinics in the Macular Photocoagulation Study, NIH Guide for Grants and Contracts, Volume 8, Number 3, February 15, 1979.

28. Grey RHB, Bird AC, Chisholm, IH: Senile disciform macular degeneration: features indicating suitability for photocoagulaton. Br J Ophthalmol 63:85-89, 1979.

29. Jacobson BV: Annual Progress Report, EY 00810-06, November 1978.

30. Swann DA: Annual Progess Report, EY 01030-06, January 1979.

31. Hultsch E: Annual Progress Report, EY 02427-02, February 1979.

32. Hultsch E: Annual Progress Report, EY 02895-01, March 1978.

33. Balazs EA: Annual Progress Report, EY 01747-04, January 1978.

34. Chen CH: Annual Progress Report, EY 01399-05, March 1979.

35. Fowlks WL: Annual Progress Report, EY 01258-04, April 1979.

36. Fowlks WL, Conway JH, Wedekind TD: Solute and osmotic gradients in the rabbit's ocular vitreous body. Fed Proc 37(3): March 1978 (abstract).

37. Farber D, Brown BM, Lolley RN: Cyclic nucleotide dependent protein kinase and the phosphorylation of endogenous proteins of retinal rod outer segments. Biochemistry 18:370-378, 1979.

38. Lolley RN: Annual Progress Report, EY 00395-10, January 1979.

39. Farber D, Brown GM, Lolley RN: Cyclic GMP: Proposed role in visual cell function. Vision Res 18:497-499, 1978.

40. Lolley RN: Cyclic nucleotide metabolism in the vertebrate retina. Personal communication of manuscript.

41. Farber, DB, Lolley RN: Modulators, mediators and specifiers in brain function, in Ehrlick YH, Volavka J, Davis LG, Brunngraber EG (ed): Advances in Experimental Medicine and Biology, Vol 116. New York Plenum Press, 1979, pp 103-115.

42. Farber D: Annual Progress Report, EY 02651-01, May 1979.

43. Cohen AI: Annual Progress Report, EY 00258-16, July 1978.

44. Ferrendelli, JA: Annual Progress Report, EY 02294-01, January 1979.

45. Aguirre G, Farber DB, Lolley RN, Fletcher RT, Chader GJ: Rod-cone dysplasia in Irish Setters: A defect in cyclic GMP metabolism in visual cells. Science 201:1133-1134, 1978.

46 Troyer EW, Hall IA, Ferrendelli JA: Guanylate cyclases in CNS: enzymatic characteristics of soluble and particulate enzyme from mouse cerebellum and retina. J Neurochem 31:825-833, 1978.

47. Cohen AI, Hall IA, Ferrendelli JA: Calcium and cyclic nucleotide regulation in incubated mouse retinas. J Gen Physiol 71: 595-612, 1978.

48. Lam DMK: Annual Progress Report, EY 02423-02, June 1978.

49. Lam DMK: Annual Progress Report, EY 02608-01, June 1979.

50. Sarthy PV, Lam, DMK: Biochemical studies of isolated Muller cells from the turtle retina. J Cell Biol 78:675-684, 1978.

51. Sarthy PV, Lam DMK: The uptake and release of [^{3}H] dopamine in the goldfish retina. J Neurochem 32:1269-1277, 1979.

52. Sarthy VP: Annual Progress Report, EY 03137-01, October 1978.

53. Vision Research--A National Plan: 1978-1982, Vols One and Two. US DHEW Publ No (NIH) 78-1258 and 1259, 1978.

54. Masland RH: Annual Progress Report, EY 01075-06, January 1978.

55. Redburn DA: Annual Progress Report, EY 01655-03, April 1978.

56. Vision Research--A National Plan: 1978-1982, Vols One and Two. US DHEW Publ No (NIH) 78-1258 and 1259, 1978.

57. Sperling HG: Annual Progress Report, EY 02388-05, April 1979.

58. Potts AM: Annual Progress Report, EY 01704-04, June 1978.

59. Potts AM: Annual Progress Report, EY 01591-02, April 1978.

60. Ruffolo JJ: Annual Progress Report, EY 02324-02, February 1979.

61. Moon ME, Clarke AM, Ruffolo JJ, Mueller HA, Ham WT: Visual performance in rhesus monkey after exposure to blue light. Vision Res 18:1573-1577, 1978.

62. Ham WT, Ruffolo JJ, Mueller HA, Clarke AM, Moon ME: Histologic analysis of photochemical lesions produced in rhesus retina by short-wave length light. Invest Ophthalmol Visual Sci 17:1029-1035, 1978.

63. Vascular and circulatory abnormalities of the retina including diabetic retinopathy -- research and training grant applications, NIH Guide for Grants and Contracts, Volume 8, Number 3, February 15, 1979.

64. Macular disease research grant applications, NIH Guide for Grants and Contracts, Volume 7, Number 19, December 15, 1978.

65. Announcement on refractive keratoplasty and ocular melanoma, National Eye Institute, Spring 1979.

66. The epidemiology of diabetes, NIH Guide for Grants and Contracts, Volume 7, Number 13, September 22, 1978.

67. Vision Research--A National Plan: 1978-1982, Vol Two, Panel Reports. US DHEW Publ No (NIH) 78-1259, 1978, p 25-35.

68. Vision Research--A National Plan: 1978-1982, Vol Two, Panel Reports. US DHEW Publ No (NIH) 78-1259, 1978, p 51-57.

69. Studies of diabetes mellitus and related problems, NIH Guide for Grants and Contracts, Vol 7, Number 10, August 4, 1978.

CORNEAL DISEASES

Introduction

The 1977 report of the National Advisory Eye Council, Vision Research--A National Plan: 1978-1982, summarizes the need for continuing research on corneal diseases, and disorders and refractive errors. This report states that every year corneal problems necessitate approximately 10 million annual office visits for professional eye treatment or care, accounting for one-third of all visits to eye care specialists.. These conditions account for over 100,000 hospital days and for over $12 million in surgical costs alone. Disorders which require only optical correction account for an additional 9 million visits per year to ophthalmologists and 25 million visits annually to optometrists. It is estimated that 80 million people wear corrective lenses for which as much as $2 billion is spent on an annual basis.

The following topics highlight research areas where successes have been achieved during the past year in conformity with the priorities identified for the Corneal Diseases program in the Council's plan.

Histocompatibility Antigens and Corneal Disease

Evidence linking specific histocompatibility (HLA) antigens with particular human diseses has rapidly accumulated in recent years. All diseases associated with HLA antigens can be considered of unknown etiology, do not exhibit a clear Mendelian pattern of inheritance, and are often suspected of being immune-related.

The gene complex for histocompatibility antigens in humans is located on the short arm of the number 6 chromosome. This complex is recognized by four closely linked but distinct loci: A,B,C and D. These loci, in turn, have been determined to be highly polymorphic with many alleles occurring with appreciable frequency in the same population.

Mondino and associates[1] noted an increased prevalence of the HLA-B12 antigen in lymphocyte testing of 45 patients with ocular cicatricial pemphigoid as compared to controls. The results of this study remained statistically significant even when corrected for the number of antigens tested. In addition, about one-half of the patients with ocular pemphigoid had circulating auto-antibodies against non-ocular tissue and elevated levels of IgA.[2] These observations suggest that an immunogenetic susceptibility exists among these individuals for the development of this disease.

Zimmerman and coworkers[3] reported finding the HLA-B5 antigen significantly more common in 46 persons suffering from recurrent corneal herpes simplex infections than in an appropriate control population. Brown[4] and Meyers,[5] however, in their tissue-typing studies of patients with recurrent herpetic keratitis were unable to demonstrate a similar association with HLA-B5. This disease has been reported to recur in about one fourth the persons who have been exposed to one infection.

Associations between various histocompatibility antigens and the success of corneal transplantation have also been investigated during the past year. A retrospective analysis of corneal donors and recipients matched for HLA antigens was performed in 103 high-risk cases by Stark and coworkers.[6] No consistent correlation was observed between the number of HLA antigens shared between the donor's lymphocytes and patient's serum and the outcome of the graft. In this study the overall rate of graft failure from rejection was 15 percent. These findings indicate that negative cross-match or a lack of circulating antibodies in the recipient's serum specific for the antigens on the cell surface of the donor's lymphocytes is an important in donor selection for keratoplasty in high-risk cases.

Stark[7] has extended these observations and is presently engaged in a randomized clinical trial which assesses the long-term effects of demonstrable recipient preimmunization against HLA antigens of the donor on the outcome of corneal transplants in patients with densely vascularized corneas who are at risk.

Corneal Vascularization

The limbal episcleral vascular plexus is the only portion of the systemic blood circulation which impinges on the avascular cornea. In the normal eye these vessels are the single source of direct corneal contact with circulating antibodies, normal serum components, and circulating pharmacologic agents. Thus, the cornea, because of its clarity and proximity to the limbal vasculature, is an ideal model for studying the effects of possible angiogenic agents on the development of new capillaries through the increased proliferation of vascular endothelial cells.

In recent years, certain specific angiogenic factors have been described which may play an important role in the cornea's response to tissue injury that leads to the induction of limbal vascularization. Aviner and coworkers[8] have studied the effects of intracorneal rabbit cartilage implants on opacification and vascularization of guinea pig corneal xenografts in rabbits. The cartilage inserts delayed the advancement of limbal vessels toward the xenografts but were not capable of preventing neovascularization. These experiments confirm the close linkage of vascularization to opacification of corneal grafts and suggest that antiangiogenic factors are present in neonatal cartilage which can delay, but are not capable of preventing, graft rejection. The inhibitory effect of cartilage on angiogenesis has been attributed to several polypeptides with antiprotease activity within cartilage which are now in the process of being analyzed.[9]

Eliason[10] has examined the growth of limbal blood vessels toward experimental thermal corneal burns in both normal rabbits and rabbits which have been previously depleted of their white cells by exposure to whole body X-irradiation. The corneas of both the irradiated animals and the normal controls demonstrated the capacity to induce limbal vascularization in response to the corneal burn which suggested that the corneal epithelium might be the source of a vasostimulating substance.[10] Gospodarowicz and associates[11] have recently reported that 10μg of purified epidermal growth factor and 25μg of purified fibroblast growth factor respectively, when implanted in the

corneal stroma of rabbits, consistently induces neovascularization of the corneal pocket without inflammation. Implants of equivalent amounts of serum albumin within the stroma do not produce a similar angiogenic effect.

Additional research must be conducted into finding ways to ameliorate the intact but chronically inflamed corneal epithelium. More new angiogenesis inhibitors which potentially could be used clinically to prevent vascularization of the stroma and epithelial opacity are needed.

Thoft and coworkers[12] recently have produced a new model of corneal vascularization in the rabbit which permits reproducible superficial vessel proliferation in the anterior portion of the cornea. If penetrating wounds are made into epithelium derived from conjunctiva which has been permitted to resurface the area originally occupied by corneal epithelium, a slight but consistent peripheral vascularization of the cornea occurs in 75 percent of the cases. Normal rabbit eyes and rabbit eyes resurfaced by peripheral corneal epithelial cells were not found to vascularize after such wounds. At present these investigators have not determined the mechanism by which vascularization occurs in this model but have found that increased stromal hydration of the cornea does play a role in this process.

Study of the effect of angiogenic agents on the proliferation of limbal vessels in the cornea may provide insight into factors which control neovascular proliferation in ocular vessels which are more anatomically isolated within the eye, e.g.the iris and the retina. Further progress in such vascular research can be expected.

Lacrimal and Accessory Gland Secretions

Little is known about the nature and physical characteristics of the proteins synthesized and secreted by the lacrimal gland and their relation to tear production. Basic information obtained about any individual lacrimal gland protein may provide insight into what role such a macromolecule plays in maintaining normal tear flow and/or tear film stability. Most previous research on this tissue has been directed at localizing by specific immunohistologic methods the various immunoglobulins which exist there. Franklin and coworkers[13] have shown that the lacrimal gland contains a preponderance of plasma cells of the immunoglobulin A (IgA) type. These investigators also identified the presence of secretory component (a polypeptide which attaches to the IgA molecule before it is secreted) and lactoferrin in the glandular tissue. Allansmith and associates,[14] however, in their examination of the lacrimal gland found about an equal preponderance of IgA-, IgG-, IgD-. and IgE-plasma cells.

Preliminary isotope incorporation studies have been performed by Chao and associates[15] on in vitro explants of human and rabbit lacrimal glands. This investigation suggests that the lacrimal tissue primarily contributes immunoglobulins and other plasma type proteins to the tears but not mucous glycoproteins. Immunodiffusion experiments on these explants have successfully identified albumin, transferrin, and ceruloplasmin in addition to IgA and IgG as secretory products.

Future in depth investigations of lacrimal gland metabolism will undoubtedly profit from the recent achievement by Putney[16] who was able to prepare isolated lacrimal gland acinar cells by dispersion with enzymes. Individual lacrimocytes were prepared by incubation of pieces of the gland in trypsin, followed by trypsin inhibitor and collagenase. Cell suspensions, thus derived, were subsequently incubated for an hour in Ringer's solution which was free of bovine serum albumin. These preparations were found to have intracellular Na and K concentrations comparable to lacrimal gland slices. In addition, these cells appeared normal by phase contrast microscopy and retained the ability to exclude trypan blue.

Collagens and Glycosaminoglycans in Corneal Wound Healing

Dissolution of the corneal stroma as a result of serious injury, infection, systemic disease, or nutritional disorder represents, at best, a painful and costly experience and, at worst, results in perforation and loss of the eye. The normal vertebrate corneal stroma is a translucent avascular connective tissue containing a tight interlacing meshwork of extracellular fibers interspersed with only a few cells. In the rabbit cornea the deep layers of the stroma contain sharply defined ribbons of fibers (lamellae) which interweave and cross each other at different angles parallel to the corneal surface. These fibers are composed of numerous fibrils which travel in the same direction within a given fiber. Each fibril, in turn, is made of collagen which is intimately associated with an apparently amorphous matrix.

According to current theory, corneal transparency is dependent on restrictions in the cross-sectional diameter of stromal collagen fibrils and restrictions in the size of the interfibrillar spaces. Corneal transparency is, therefore, determined at least in part by the physical and chemical nature of the collagen in the organ. Cintron and associates[17] have followed the course of wound healing in rabbit corneas both biochemically and ultrastructurally over extended periods of time after the infliction of penetrating ocular wounds. Such wounds were found to heal initially as an avascular, opaque tissue that after about 1.5 years became transparent and appeared normal. However, electron microscopy and biochemical analyses of the collagen of these healing rabbit corneal wounds showed that the ultrastructural dimensions of the collagen fibers and fibrils and the pattern of collagen cross-linking did not return to normal. The collagen from the healing corneal wounds and the protein from fetal rabbit corneas were observed to have very similar cross-linking patterns, but these patterns were noted to be different from normal adult collagen.

In another paper,[18] these investigators extended their observations to include a survey of the glycosaminoglycans (GAGs) in the wound tissue. The healing tissues were found to be capable of synthesizing low-sulfated keratan sulfate, hyaluronic acid, and heparan sulfate. These GAGs have not been found in normal adult corneas but have been reported in fetal corneal tissue. The similarities between collagen and GAGs in healing corneal wounds and fetal tissue suggest that some ability to recapitulate ontogenetic processes exists. Thus, although the cornea can regenerate itself, as indicated by eventual recovery of transparency within the wound, the reducible intermolecular crosslinking in collagen and its lamellar organization in the tissue

continue to remain abnormal. Further experiments will be conducted by these investigators to estimate the size and conformation of the interfibrillar spaces during wound healing. Such information is needed in order to refute effectively the current concept of corneal transparency.

Gross and coworkers[19] have taken another approach to the study of corneal wound healing by investigation of cell interactions in alkali-burned, perforating rabbit corneas. Primary cell cultures derived from both the epithelium and stroma of alkali-burned corneas were compared for their ability to produce collagenase, an enzyme involved in collagen breakdown. It was observed that epithelial cells failed to produce the enzyme, whereas presumed stromal cells produced collagenase continuously at a constant rate for a period of nine days. On the other hand, epithelial-stromal cell cultures where epithelium was plated at a high density ($5x10^5$ epithelial cells per $3x10^5$ stromal cells) produced collagenase for only three days, then ceased production of the enzyme for the remainder of the incubation period. These results suggest that a high concentration of epithelial cells inhibits collagenase production by wound fibroblasts.

On the other hand, medium derived from low density epithelial cell culture was found capable of stimulating collagenase production in stromal cells derived from alkali-burned corneas which failed to produce the enzyme after exposure to multiple passages in culture. Thus, epithelial cells of alkali-burned corneas, depending on their concentration, appear to be capable of stimulating or inhibiting collagenase production by stromal cells from the same source. An added soluble factor for collagenase production is either already present in the latter cells or is not required by those cells.

More research is needed into the identification of those macromolecules which are altered in the regenerative and healing processes which produce scars following corneal injury. Increased knowledge of the cellular and biochemical mechanisms which regulate the level of collagenase in the cornea should permit the design of more effective therapy for corneal conditions characterized by deleterious collagen breakdown as well as scarring.

Nutritional Aspects of Corneal Disease

The growth, development, and maintenance of the eye, like any other organ, are intimately dependent upon the supply of nutrients provided to it from the bloodstream. The cornea, under normal conditions, has direct access to the vascular system only through the limbal vessels, which form a vascular plexus around its periphery. This anatomic arrangement of vessels make this tissue particularly vulnerable to structural damage if a period of prolonged systemic inanition occurs. Keratomalacia in infants is such a corneal condition which results usually from a severe deficiency of vitamin A in the mother during the period of rapid intrauterine development of the fetus. The worldwide prevalence of keratomalacia is 20 per 10,000 children aged 1 to 6 and represents one of the leading causes of pediatric blindness in developing nations. This deficiency state is characterized by a softening (colliquative necrosis) of the entire thickness of, at least, part of the cornea followed by a rapid melting of the structure of the cornea into a

cloudy gelatinous mass. This process ultimately leads to deformation or destruction of the eyeball.

A study, conducted in Madurai, India by Pirie and associates[20] on malnourished, vitamin A-deficient children with severe dryness of the cornea or xerophthalmia showed that there is an abnormal accumulation of retinyl esters in the serum of these children as compared to a control population. Injections of large doses of vitamin A (40,000 IU/kg. body wt.) to these malnourished children usually failed to restore the serum retinol-binding protein (vitamin A transport protein) and prealbumin to normal levels. These observations suggest that the capacity to synthesize and store the above proteins was impaired due to severe dietary protein deficiency. Hence the condition could not be corrected by administration of the vitamin alone.

Peterson et al[21] has examined the route by which vitamin A gets to the cornea from the bloodstream. Their experiments have ruled out the possibility that retinol-binding protein enters the cornea by way of either the tears or aqueous humor. The concentration of the protein in the central cornea has been found to be lower than in the peripheral portion, which suggests that the retinol-binding protein may enter by diffusion from the limbal vessels. In another study, Wolf and coworkers[22] were able to demonstrate in vitro a two-fold stimulation in uptake of labeled glucosamine into a specific, glycopeptide of corneal epithelium purified from whole vitamin A-deficient rat corneas that had been incubated with retinol. The glycopeptide was found to be a moiety of a glycoprotein isolated from the incubation medium. It was not a glycosaminoglycan for it failed to take up labeled sulfate. Its origin tentatively was considered to be the basal cells of the corneal epithelium, and its function may be related to the control of the mitotic rate of the cells of the epithelial layer.

A second vitamin has been found to be useful in the alleviation of corneal degeneration. Pfister and his collaborators[23] have studied the effects of the use of topical ascorbic acid (vitamin C) on experimental alkali burns in rabbit eyes in a double-masked study.

Individual rabbit eyes, after being subjected to 1.0 N sodium hydroxide for twenty seconds were given two drops of 10 percent ascorbate in Adsorbotear vehicle hourly for a period of fourteen hours of each day. The control group received Adsorbotear alone in the same manner. Over a six-week period, the experimental group showed a significant reduction in corneal ulcer formation and a significant increase in aqueous ascorbate levels as compared to the control group. These studies are in agreement with prior research done by these investigators which demonstrated that subcutaneous administration of ascorbate decreased corneal ulceration after alkali burns.

In order to increase in a substantive way the number of biomedical scientists who ultimately will pursue a career in nutritional research, the National Eye Institute (NEI) participated along with seven other NIH institutes in the issuance of two trans-NIH announcements in August 1979 which requested grant applications for research training in clinical nutrition. These requests were entitled, "National Research Service Awards for Institutional Grants in Nutrition," and "National Research Service Awards for

Individual Grants in Nutrition," respectively. The NEI portion of each announcement stressed the institute's interest in supporting research training in both animal and clinical nutritional studies which are related to the structure and function of the various tissues of the eye in health and disease.

Clinical Trials: Vitamin C and Acute Alkali Burns

Recognizing an opportunity to transfer knowledge gained through research on experimental corneal injuries in animals to clinical problems, the National Eye Institute provided financial support to investigators at the University of Alabama at Birmingham in September 1977 to develop the protocol for a clinical trial to assess the efficacy of the combined use of oral and topical ascorbic acid in the treatment of human alkali-burned corneas. A manual of operations for the trial was completed in December 1978[24] and an award was made to the principal investigator's institution for payment of costs incurred in recruitment of individual patients from any of six collaborating clinics. The institutions which agreed to participate in the study outlined were the University of Washington, Kresge Eye Institute, Wayne State University, University of Michigan Hospital, Wilmer Ophthalmological Institute, Johns Hopkins Hospital, University of Illinois Medical Center, and the Detroit Institute of Ophthalmology.

Refractive Keratoplasty

At its November 1978 meeting NIH Visual Sciences A Study Section wrote a letter to the National Advisory Eye Council asking for guidance in the evaluation of grant applications for research on refractive keratoplasty. After considerable discussion, at its January 1979 meeting, the Council agreed that refractive keratoplasty represented a potentially important subject for research and proposed the following guidelines for the study section's consideration.

1. Refractive keratoplasty as a clinical procedure must be considered experimental.

2. Animal experiments which employ this surgical technique should be encouraged.

3. No clinical research should be supported by the NEI until the results of animal research can be evaluated.

4. Because the cost of the necessary surgical equipment is high, sharing or non-government funding of instrumentation should be encouraged.

5. Keratometer readings and information on refraction are considered important in the postoperative evaluation of animals undergoing keratoplasty.

6. The National Eye Institute should attempt to gather follow-up data on patients who have undergone the procedure.

References

Corneal Diseases

1. Mondino BJ, Brown SI, Rabin BS: HLA antigens in ocular cicatricial pemphigoid. Arch Ophthalmol 97:479, 1979.

2. Mondino BJ, Brown SI, Rabin BS: Autoimmune phenomena of the external eye. Ophthalmology 85:801-817, 1978.

3. Zimmerman TJ, McNeill JI, Richman A, Kaufman HE, Waltman SR: HLA types and recurrent corneal herpes simplex infection. Invest Ophthalmol Vis Sci 16:756-757, 1977.

4. Brown SI: Annual Progress Report, EY 01991-02, May 1979.

5. Meyers RL: Annual Progress Report, EY 02646-01, July 1979.

6. Stark WJ, Taylor HR, Bias WB, Maumenee AE: Histocompatibility (HLA) antigens and keratoplasty. Am J Ophthalmol 86:595-604, 1978.

7. Stark WJ: Procedural manual for a controlled clinical trial investigating the importance of HLA cross-match testing in the selection of donor material for penetrating keratoplasty in high-risk cases. March 1, 1979.

8. Aviner A, Henley WL, Podos SM: The effect of intracorneal cartilage implants on the survival of corneal xenografts. Invest Ophthalmol Vis Sci 18:366-372, 1979.

9. Langer R, Brem H, Falterman R, Klein M, Folkman J: Isolation of cartilage factor that inhibits tumor neovascularization. Science 193:69-72, 1976.

10. Eliason JA: Leukocytes and experimental corneal vascularization Invest Ophthalmol Vis Sci 17:1087-1095, 1978.

11. Gospodarowicz D, Bialecki H and Thakral TK: The angiogenic activity of the fibroblast and epidermal growth factor. Exp Eye Res 28:501-514, 1979.

12. Thoft RA, Friend J, Murphy HS: Ocular surface epithelium and corneal vascularization in rabbits. 1. The role of wounding. Invest Ophthalmol Vis Sci 18:85-92, 1979.

13. Franklin RM: EY 0919-03 (preliminary findings), September 1979.

14. Allansmith MR: EY02882-01 (preliminary findings), September 1979.

15. Brown SI: Annual Progress Report, EY 01489-04, May 1979.

16. Putney JW: Annual Progress Report, EY 01978-03, February 1979.

17. Cintron C, Kublin CL: Regeneration of corneal tissue. Dev Biol 61:346-357, 1977

18. Cintron C, Hassinger LC, Kublin CL, Cannon DJ: Biochemical and ultrastructural changes in collagen during corneal wound healing. J Ultrastruct Res 65:13-22, 1978.

19. Gross J: Annual Progress Report, EY 02252-01, November 1978.

20. Venkataswamy G, Glover J, Cobby M, Pirie A: Retinol-binding protein in serum of xerophthalmic, malnourished children before and after treatment at a nutrition centre. Am J Clin Nutr 30:1968-1973, 1977.

21. Peterson PA: Annual Progress Report, EY 02417-01, February 1979.

22. Kiorpes TC, Kim Y-CL, Wolf G: Stimulation of the synthesis of specific glycoproteins in corneal epithelium by vitamin A. Exp Eye Res 28:23-35, 1979.

23. Pfister RR, Paterson CA, Hayes SA: Topical ascorbate decreases the incidence of corneal ulceration after experimental alkali burns. Invest Ophthalmol Vis Sci 17:1019-1024, 1978.

24. Pfister RR, Gartseff GV: Manual of Procedures: Ascorbic acid in the treatment of alkali burns of the eye. December 1, 1978.

Introduction

In its 1977 report, Vision Research--A National Plan: 1978-1982, the National Advisory Eye Council emphasized the great importance of cataract as a major medical disability. In the United States alone about 400,000 operations for removal of cataractous lenses are performed annually, and there are 1,670,000 people who have vision difficulties because of developing cataract. Also, cataract accounts for more than half of the hospitalizations necessitated by eye disorders. Unlike many other serious vision disorders, cataract rarely results in permanent blindness. Surgical removal of the opaque lens is a viable medical solution; surgery is costly and not without hazard, and the prosthetic devices required to restore normal vision, i.e. spectacles, contact lenses, or intraocular lenses, are not always completely effective and are sometimes difficult to use. For all of these reasons, it is clearly important to seek means for cataract prevention and cure.

Cataract results from a variety of causes. These include aging, congenital defects, viral infections, metabolic disorders, and various types of physical and chemical insult to the lens. Because of the diversity of cataractogenic agents, no single mechanism can account for all of the different forms of cataract. Thus, osmotic imbalance produced by polyol accumulation within the lens has been shown to be the causative agent in sugar-induced cataract, while light scattering by aggregated protein has been offered as a cause for senile cataract. Recently, it has been suggested that syneresis, in which there is drawing together of the dispersed phase of a gel, may also play an important role in cataract formation.[1] Syneresis produces a modification in the micro environment of macromolecular aggregates through changes in hydration level; these changes, in turn, produce fluctuations in refractive index, which when of sufficient magnitude result in opacity.

This report will deal in greater detail with three aspects of NEI's Cataract research program: lens proteins, lens electrophysiology, and animal cataract models. Lens protein studies constitute a major program area. In lens electrophysiology there have been new and promising developments, and animal cataract models have been identified which point to a wide range in cataract research opportunities.

Lens Proteins

The chemical composition of the lens is unusual in that it consists almost entirely of water and protein.[2] Not only is protein by far the major chemical component of human lens other than water, but because its concentration (33 percent) is unusually high, the possible role of protein changes in cataract development has received much attention. Early studies dealt principally with structure and composition; lens protein aggregation was proposed as a possible cause of senile cataract. Recently an increasing number of studies have been concerned with proteolysis, turnover, synthesis, and post-translational changes in lens proteins, particularly with regard to their possible role in cataract development.

The Nakano mouse strain spontaneously develops cataract. During cataract formation marked changes occur in the levels of lens protein synthesis and degradation along with a reduction in lens sodium/potassium ATPase activity. The reduction in sodium/potassium ATPase activity, which causes a marked reduction in lens potassium levels together with an increase in sodium levels, is due to the presence in this mouse strain of a naturally occurring peptide inhibitor of sodium/potassium ATPase.[3] Lens protein synthesis in cultured chick lens is sensitive to changes in the sodium/potassium ratio,[4] and treatment of the cultured chick lens with ouabain, an alkaloid ATPase inhibitor, produces cataract and a pattern of protein change and leakage similar to that found in the Nakano mouse.[5] Thus, cataract is associated with inhibition of sodium/potassium ATPase and with changes in protein levels in cultured normal lenses and in vivo in one strain of mouse. Further study is required to determine whether the patterns can be duplicated in vivo in the normal mouse and, if so, to determine the sequence of events and the ultimate cause of this cataract.

On the basis of light scattering theory, it has been proposed that a random array of lens macromolecules greater in molecular weight than 5×10^7 daltons, if present in sufficient concentration, can cause opacification because of refractive index differences from other elements in their cellular environment. Such large protein macromolecules have been detected both in animal and human lenses. The discovery that Bragg diffraction patterns can be obtained from cataractous lenses irradiated with visible laser light suggests an association between cataract and large refractive index differences between membrane and cytoplasm, possibly due to high molecular weight components in lens fiber cell membranes. Evidence for such association has recently been found.[6] From studies utilizing immunofluorescent staining procedures, it appears that soluble crystallins are coupled by disulfide linkages to a 43,000 dalton extrinsic membrane protein to yield membrane bound proteins of very high molecular weight.

Recent studies of protein turnover in the cortical region of cultured rabbit lenses for periods of as long as two days indicate that during the course of incubation the rate of protein synthesis falls while the rate of protein degradation increases. Yet, in spite of the overall loss of soluble protein, the lenses remain clear. The implication of these results to lens homeostasis and to cataract development are uncertain; but it is noteworthy that the lens appears able to accommodate itself to changes in protein concentration without the obligate development of opacity.

Evidence of association between cataract and lens protein composition, concentration and local environment remains suggestive but uncertain. Detailed studies seeking relationships between cataract and protein composition, concentration, and turnover remains an important part of the cataract research program.

Electrophysiology

Study of ion gradients, the movement of ions between lens elements and the electrical currents which result from ionic movement, can offer important basic information on the structure and function of individual lens

elements. It provides clues to our understanding of how the lens maintains homeostasis and clarity and conversely why it fails to do so and becomes opaque.

In early work, the interpretation of electrophysiological data obtained from study of the lens was attempted using a giant spherical cell as a model. In such a model there would be either little barrier to ionic movement or, alternatively, if resistance existed, the resistance would be of a uniform nature. All attempts to develop mathematical models based upon such assumptions were unsuccessful. The data obtained could not be related to the resultant mathematical parameters.[7] A newer model based upon the existence of the lens as a syncytium with electrically coupled elements, i.e. low resistance pathways between individual cells has met with success.[8] The high degree of electrical coupling is consistent with the very high density of gap junctions found in the lens. Further, when the dye Procion Yellow is iontophoretically injected into a single lens fiber, it moves readily from one fiber cell to its neighbor without passage into the intercellular space.[9]

Application of electrophysiological data to the syncytium model provides important new information about lens elements which need early confirmation and extension by other research procedures. Thus, calculations indicate that the membranes of fiber cells have very high electrical resistance, perhaps three orders of magnitude greater than that of most cells. Further, the lens extracellular space exhibits a high resistance to current flow. Correlation of these electrical properties with membrane composition, intercellular ion movements, and other cell functions will be an important next step. Further, these studies emphasize the need to obtain information comparing the properties, structure, and anatomical position within the lens of individual lens elements.

Animal Models

Although study of the human lens has received recent emphasis, animal models continue to be widely used to determine the precise and detailed cause of the various forms of cataract. Studies are performed for a variety of purposes in normal animals using agents that produce cataract. One purpose is to learn the mechanism by which drugs and other agents cause cataract. Such studies provide insight into the biological and biochemical functions required for maintenance of lens clarity, and also they offer information which can be used to predict which particular drug or properties will cause opacity. This may make it possible to avoid cataract formation when such drugs may be required for treatment of major disorders or diseases.

Of prime importance is the search for clues to senile cataract, the most important form of cataract in humans. At present its cause remains unknown. With an increasing understanding of the variety of biological mechanisms which can cause cataract, clues to the cause of senile cataract should appear. Recent reports implicating drugs in cataractogenesis include echothiophate-inhibition of lens acetylcholinesterase,[10] inhibition of lens sodium/potassium ATPase by a cholesterol-synthesis inhibitor, and free radical induced lipid peroxidation resulting from inhibition of lens catalase by 3-aminotriazole. Acetaminophen, an important analgesic and antipyretic, has been found to cause cataracts in genetically susceptible groups of mice. The mice are induced to produce high

levels of P_1-450-mediated monoxygenase activity which leads to tissue reactive intermediates of acetaminophen resulting in cataract.[11]

Cataract can be produced in experimental animals by various types of radiation including ultraviolet, x-ray, infrared and microwave. Yet, there is no evidence that environmental irradiation sources actually contribute to human cataract. Although some investigators have speculated that long-term low level ultraviolet irradiation may contribute to senile cataract development, this hypothesis remains unproven. While cataracts are readily produced in experimental animals by high levels of ultraviolet irradiation,[12] and epidemiological studies suggest an association in humans between cataract incidence and exposure to sunlight, further research is necessary to determine whether a causual relationship exists.

Several animal species and strains are available for study as models of naturally occurring human cataract or as models of biological deficiencies which lead to cataract. These include the Fraser mouse, the Nakano mouse, the Philly mouse and the athymic mouse of Hazlett and Bradley, all of which develop cataract at an early age. There are strains which develop cataract later in life which, it has been suggested, are analogous to human senile cataract. These include the deer mouse (Peromyscus) of Burns and Feeney and a mouse strain developed by Kuch. Gorthy, using a Wistar rat strain that spontaneously develops cortical cataracts, has associated the appearance of lysosomal hydrolases in the lenticular intercellular space with the appearance of cataracts.[13]

The dog also has been suggested as a model because the canine lens more closely resembles the human lens. Inherited cataracts have been observed in many strains, including the German shepherd, pointer, beagle, miniature schnauzer, toy poodle, cocker spaniel, Siberian husky, Old English sheepdog, wire-haired fox terrier, Boston bull terrier, Sealyham terrier, golden retriever, and Staffordshire bull terrier. Hereditary cataracts also exist in fish. Crosses between the blind Mexican cave fish and its presumed normal-eyed surface ancestor lead to cataractous progeny. At least eight different types of cataract have been observed in these offspring, some of which appear to have a common genetic basis.

Because the ultimate objectives of the Cataract program are the discovery of means for preventing cataract and, where cataract has already developed, for the discovery of means for its reversal, a detailed understanding of the events which precede cataract formation and of those involved in its reversal are clearly important Several animal model systems are being studied in which reversal from cataract occurs. Needle puncture of the rabbit lens causes cataract which ultimately reverses. Accompanying this cataract is cellular debris which is shed through the puncture wound.[14] An experimental drug-induced cataract has been used to examine the lens's ability to clear itself of debris during recovery in the absence of a wound fissure. The cataract is produced in rabbits by intracameral administration of methylene blue solution. The lens capsule remains intact while opacity develops in the entire retropupillary region including the epithelium and superficial fibers. During cataract development, there is complete disruption of the epithelial layer with evidence of cellular debris. Fiber cells are irregular in size and

in interfiber relationships and have a heterogenous cytoplasm. Vesicles appear which seem morphologically similar to lysosomes, and epithelial cell migration into the lesion begins by 48 hours. Clarification of the lens occurs rapidly, and by three weeks it is almost complete. It has been speculated that the cellular debris may be removed by lysosomal enzymes that may be present in the vesicles.[15]

Galactosemic cataract in the rat, too, is in part reversible if the animal is returned to a normal diet in the early phases of cataract development. Cortical regions of the lens clarify although the nuclear region remains opaque. Ultrastructural study indicates that the epithelial and capsular layers remain normal and that all damage occurs among the fiber cells. This damage includes membrane bound vesicles, cellular swelling, membrane disruption, and coarse fibroplasm. In some cells, the fibroplasm contains small electron dense aggregates. Enlargements occur in intercellular space. During recovery, the debris from damaged lens fibers disappears. Here, too, it has been speculated that removal of the debris is performed by lysosomal enzymes which may be present in the vesicles.[16] The extent to which the reversal of lens opacity is due to the repair of existing fibers or the formation of new ones remains to be ascertained. Biochemical studies of the lens during the development of and recovery from galactose cataract indicate progressive and marked hydration along with reduced levels of glutathione (without concomitant reduction in glutathione synthatase activity), free amino acids, taurine, dulcitol and sodium ion. At the same time, potassium levels fall. During the recovery phase, glutathione and free amino acids return to normal levels. However, water and sodium levels remain high and potassium levels become somewhat lower than normal.[17] Galactosemic cataract can also be produced in fetal rats by feeding galactose to females during pregnancy. Unlike the galactose cataracts induced in the mothers and in young animals which only partly reverse when the animals are returned to a normal diet, newborn rats fully recover when put on a normal diet.

Further studies, from molecular to cellular, of these and other animal models for recovery from cataract would seem both important and desirable.

The use of animal models will continue to be important in cataract research. Animals provide opportunities to study processes of cataract development, and to clarify issues of basic lens biology and biochemistry. They may ultimately offer clues to the cause and development of human senile cataract.

Conferences

The Second International Symposium on Red Blood Cell and Lens Metabolism, scheduled for the fall, will be partially supported by the NEI. Also, an NIH/NEI Consensus Conference on Intraocular Lens Implantation was held in September. At this conference, leading ophthalmologists and researchers discussed the major issues associated with intraocular lens implantation as a treatment for cataract.

Program Announcement

Compared to program needs and scientific opportunities indicated in Vision Research--A National Plan: 1978-1982, grant applications received by the NEI both for research and training programs have been relatively low in number. In an attempt to alter this trend, an announcement inviting research grant applications in this field was published in the NIH Guide for Grants and Contracts, Vol. 8, No. 7, May 11, 1979. The announcement indicated research needs and opportunities under four general headings: Normal Lens Development and Maintenance of Transparency; Causes and Pathogenesis of Senile Cataract; Cataract and Association with Congenital, Metabolic and Genetic Factors; Drug-Induced and Secondary Cataracts.

References

Cataract

1. Bettleheim FA: Syneresis and its possible role in cataractogenesis. Exp Eye Res 28:189-197, 1979.

2 Bloemendal H: The vertebrate eye lens. Science 197:127-138, 1977.

3. Fukui HN, Merola LO, Kinoshita JH: A possible cataractogenic factor in the Nakano mouse lens. Exp Eye Res 26:477-485, 1978.

4. Piatagorsky J, Fukui HN, Kinoshita JH: Differential metabolism and leakage of protein in an inherited cataract and a normal lens cultured with ouabain. Nature 274:558-562, 1978.

5. Shinohara T, Piatagorsky J: Regulation of protein synthesis, intracellular electrolytes and cataract formation in vitro. Nature 270:406-411, 1977.

6. Spector A, Garner MH, Garner WH, Roy D, Farnsworth P, Shyne S: An extrinsic membrane polypeptide associated with high molecular weight protein aggregates in human cataract. Science 204:1323-1326, 1979.

7. Eisenberg RS, Rae JL: Current-voltage relationships in the crystalline lens. J Physiol 262:285-300, 1976.

8. Eisenberg RS, Barcilon V, Mathias RT: Electrical properties of spherical syncytia. Biophys J 25:151-180, 1979.

9. Rae JL: The movement of procion dye in the crystalline lens. Invest Ophthalmol Vis Sci 13:147-150, 1974.

10. Philipson B, Kaufman PL, Fagerholm P, Axelsson U, Barany EH: Echothiophate cataracts in monkeys. Arch Ophthalmal 97:340-346, 1979.

11. Shichi H, Gaasterland DE, Jensen NM, Nebert DW: Ah locus: Genetic differences in susceptibility to cataracts induced by acetaminophen. Science 200:539-541, 1978.

12. Zigman S: Near UV light and cataracts. Photochem Photobiol 26:437-441, 1977.

13. Gorthy WC: Cataracts in the aging rat lens. Morphology and acid phosphatase histochemistry of incipient forms. Exp Eye Res 27:301-322, 1978.

14. Weinsieder A, Rothstein H, Gierthy J: A comparison of wound repair in cultured lenses with that occurring in the lenses of living animals. Ophthalmic Res 1:279-291, 1970.

15. Unakar NJ, Weinsieder A, Reddan JR: Ultrastructural changes associated with the induction and reversal of a chemically induced cataract. Ophthalmic Res 9:296-307, 1977.

16. Unakar NJ, Genyea C, Reddan JR, Reddy VN: Ultrastructural changes during the development and reversal of galactose cataracts. Exp Eye Res 26:123-133, 1978.

17. Reddy VN, Schwass D, Chakrapani B, Lim CP: Biochemical changes associate with the development and reversal of galactose cataracts. Exp Eye Res 23:483-493, 1976.

Introduction

Glaucoma continues to be a major cause of impaired vision and blindness. Primary open-angle glaucoma (POAG) and glaucoma secondary to other ocular diseases are nearly always accompanied by an increase in intraocular pressure (IOP), changes in the appearance of the optic nervehead, and visual field losses. Many cases of POAG and some kinds of secondary glaucoma may be controlled for long periods of time with appropriate drug therapy or by surgery; others, fortunately much smaller in numbers, are more difficult to control.

In Vision Research--A National Plan: 1978-1982, a panel of experts convened by the National Advisory Eye Council described the major outstanding problems in our understanding of the basis of glaucoma and its clinical management and suggested specific areas of research to address them.

While encouraging studies aimed at achieving a better understanding of glaucoma are in progress, work will be required to define more fully the homeostatic mechanisms which determine the level of intraocular pressure and to delineate precisely how increased intraocular pressure leads to damage of the optic nervehead. Still lacking are truly adequate tests which can predict development of POAG, secondary glaucoma, and acute angle-closure glaucoma. Much knowledge remains to be gathered regarding the etiology and natural histories of some of the low incidence secondary glaucoma types and optimal methods for their clinical management.

During fiscal year 1979, the Glaucoma program of the National Eye Institute (NEI) supported nearly 100 individual research projects and center-based projects studying many aspects of the disease. Two clinical trials were initiated: a long-term evaluation of a new drug for the treatment of POAG, timolol, and a second trial involving drugs used in the treatment of certain forms of secondary glaucoma. Four other glaucoma-related clinical trial protocols are being prepared and may be activated in the coming year. Despite a need for more highly qualified researchers in this field, the Glaucoma program traditionally has not attracted many fellowship applicants; however, in 1979 six individuals were awarded individual National Research Service Awards, and the program now supports recipients of four Academic Investigator Awards and six Research Career Development Awards.

Integration of fundamental and clinical research approaches to the problems of glaucoma is difficult because there is at present no valid natural animal model for the various forms of this disease. As a consequence, clinical research is often devoted to technical improvements in diagnosis, drug treatment, or surgery or to searches for correlates with readily observed phenomena. Conversely, experimental studies either use normal animals or those in which approximations to the human disease have been artificially created. Thus, lacking knowledge of the cellular and molecular processes underlying the disease, most clinical studies of glaucoma are limited to a "macro" approach, manipulation of known or intuitable functions. Ideally, rational disease therapies should be developed upon a substrate of fundamental

knowledge of "micro" processes at the cellular and molecular levels. A review of the research projects presently supported by the NEI's Glaucoma program indicates a strong dichotomy, with projects being either clinical or basic in nature. To advance research on the prevention and treatment of glaucoma, it will be necessary to develop more bridges---interdisciplinary studies linking cell biology (fine structure, molecular biology, physiology, biochemistry, and pharmacology) of normal and diseased tissues to macro disease processes.

The discussion which follows covers some new findings in basic research and developments in clinical research. Major items of basic research noted include studies of the ultrastructure, biochemistry, and pharmacology of iris and ciliary body. Properties of drugs used in glaucoma therapy, comparison of two surgical treatments for POAG, and the possible value of panretinal photocoagulation for treatment of neovascular glaucoma are among clinical topics reviewed.

Ultrastructure Studies

Understanding the nature of the ciliary epithelium is particularly important because the epithelial cells produce aqueous humor, and knowledge of the morphology of these cells will serve as a basis for future studies aimed at correlating their physiologic and biochemical processes with drug effects. Communications between cells, the integrity of tissues, membrane permeability or barriers, and, possibly, nutritional effects depend upon various types of cellular functions.

The fine structure of intercellular junctions in the ciliary epithelium is described by Raviola and Raviola based upon studies using transmission electron microscopy and freeze fracturing techniques.[1] In the monkey, this tissue has a rich variety of specialized junction types including zonule occludens, zonula adhaerens, gap junctions, and desmosomes. The zonule occludens, existing only between nonpigmented cells, is a permeability barrier considered to be the structural counterpart of the blood brain barrier because it limits diffusion between these epithelial cells to ions and small molecules. Gap junctions are the points of contact between pigmented and nonpigmented epithelial cells and are the most distinctive morphologic feature of the ciliary epithelium. Gap junctions are known to mediate electrical and metabolic coupling between cells, and the investigators state that in the ciliary epithelium they ensure "that all epithelial cells are joined in a metabolic syncytium---and are probably instrumental in coordinating the activity of a multitude of individual units inserted in parallel in each epithelial layer." They are concentrated between the two epithelial layers and may in some way mediate the process of aqueous formation. Desmosomes are generally thought to maintain cellular adhesion, possibly by compensating for the shearing forces generated during accommodation, thus ensuring mechanical stability of the epithelium. Puncta adhaerentia are numerous between pigmented cells and at interfaces between the two epithelial layers; they may subserve intracellular cohesiveness, or since they are associated with microfilaments, they may anchor cytoplasmic contractile elements to the plasmalemma.

A beginning attempt to correlate drug actions with iris function and morphology is reported by Szalay.[2] Topical application of beta-adrenergic drugs to rat eyes, accompanied by intravenous injection of fine carbon particles, was used to measure changes in iridial blood vessel permeability as seen by light microscopy. The beta-adrenergic agonist, isoproternol (both B-1 and B-2 activity), increased vessel permeability, with a greater effect noted in young than in old animals. Pretreatment with timolol, a nonselective beta receptor antagonist, inhibited leakage of particles, while an alpha-adrenergic blocking reagent, tolazoline, had no effect on vessel permeability to the fine particles. Electron microscopy showed that isoproterenol induced the formation of small intercellular gaps between adjacent endothelial cells of venules. Also, in old animals, platelet clumping and carbon particles were observed near these gaps. Carbon particles passed through the lumen via patent interendothelial clefts. Pretreatment with timolol prevented these changes and resulted in the formation of large spaces between the two layers of epithelial cells lining the posterior surface of the iris.

It has been believed that injured ciliary epithelium could regenerate following cyclocryosurgery, and now physical evidence of this has been provided by Yamashita and Sears.[3] The ciliary epithelium of rhesus monkeys was subjected to mild cryogenic injury. IOP fell, and sequential light and electron microscopic studies showed that pigmented cells were more severely damaged than nonpigmented cells, that ciliary tips and lateral portions reappeared by four weeks after injury, and that return to normal IOP lagged behind regeneration of nonpigmented epithelium. Following severe injury, only nonpigmented cells regenerated. Other evidence showed that nonpigmented tissue regenerated more rapidly than pigmented tissue. These experimental findings help to explain why cryosurgery only temporarily relieves the symptoms of glaucoma.

Drugs Used in Glaucoma Therapy: Basic Studies

Epinephrine has long been used to treat glaucoma, but some of its effects are still only poorly explained; for example, IOP remains reduced well beyond the life of the drug in the eye, and refractoriness to the drug wears off with time. Flach and Wood initiated their study[4] after noting that clinically applied 6-hydroxydopamine (6-OHDA) induces "chemical sympathectomy" (selective degeneration of adrenergic nerve terminals), thereby increasing the effects of epinephrine by preventing its re-uptake and inactivation. This effect, potentiation of the action of a transmitter at its receptors, is known as receptor supersensitivity. The investigators reasoned that epinephrine might exert its clinical effects by mechanisms similar to those known for 6-OHDA since the two drugs possess similar chemical structures. Cats protected by a blocking reagent against excess stimulation of alpha-adrenergic receptors by epinephrine were administered otherwise supralethal doses of epinephrine (controls were either not protected or treated only with phenoxybenzamine). Electron microscopy revealed that epinephrine-treated animals had acute sympathetic nerve terminal degeneration in the iris similar to that produced by 6-OHDA. At lower dosage levels lesser effects were noted. The authors suggest that clinical persistance of epinephrine's effects may be due to a denervation supersensitivity following an epinephrine-

induced "chemical sympathectomy."

Drug-induced changes in receptor properties are also suggested by a study of Neufeld and associates.[5] The density of beta-adrenergic receptors in purified membranes of rabbit iris-ciliary tissues was measured by use of a specific radioactive ligand. Treatment with 6-OHDA caused a small but statistically significant increase in number of receptors; topical epinephrine alone had no notable effect on receptor density of normal eyes, but occasioned a decrease in receptor density in eyes denervated surgically or by treatment with 6-OHDA. Timolol treatment resulted in an increase in receptor density. The density of beta-adrenergic receptors, therefore, seems to be related inversely to the level of adrenergic stimulation, while the affinity of receptors for the reagents remains unchanged. In line with the observations by Flach and Wood[4] above, continued use of epinephrine over five days resulted in a loss of reduction of IOP, possibly due to a drug-induced loss of receptors.

Chemical sympathectomy caused by 6-OHDA treatment of rabbit eyes was monitored by following changes in pupil diameter in a study by Colasanti, Kosa, and Trotter.[6] Following development of adrenergic supersensitivity, a marked decrease in response to cholinergic agonists was observed. The iris sphincter has both cholinergic and adrenergic innervations, and the investigators suggest that loss of inhibitory sympathetic input which leaves cholinergic input unopposed may lead to cholinergic subsensitivity.

Potter and Rowland[7] have analyzed the effects of a number of adrenergic drugs on IOP in rabbits. Drugs with strong or solely alpha-adrenergic effects caused an initial increase in IOP, followed by a more prolonged hypotensive effect. Drugs having predominantly beta-adrenergic activity immediately lowered IOP and produced neither an increase in IOP nor mydriasis, and beta-2 agonists had the most powerful hypotensive effects. Therefore, drugs having both alpha and beta stimulating properties have initially opposing effects on IOP, and selective beta-2 adrenergic agonists should be superior to drugs with mixed adrenergic effects in securing reduction of IOP.

Studies with several vasoactive drugs showed that they increased vascular permeability and had direct and independent effects on rabbit ciliary epithelium. Green, Griffen, and Hensley[8] found that acetylcholine increased both fluid secretion and passive permeability of the tissues while atropine caused a decrease. It may be significant to understanding the effects of bradykinin and its role in inflammation that, when administered at "physiologic" concentrations, the drug increased fluid secretion.

Bito and Merritt[9] have suggested, based upon studies using pilocarpine and a cholinesterase inhibitor in owl monkey eyes, that there may be two populations of cholinergic receptors responding to pilocarpine. Continuous treatment with echothiophate, which raises acetylcholine (ACh) concentrations by inhibiting cholinesterase, produced a subsensitivity to the miotic effect but not to the hypotensive effect of pilocarpine. Paradoxically, after 16 days, pilocarpine increased IOP. The cholinesterase inhibitor could have

induced receptor subsensitivity to acetylcholine in one class of receptors on the iris sphincter by ensuring local ACh excess. If another population of receptors, probably on the ciliary muscle, were to have a high affinity for pilocarpine, but respond to it poorly (low intrinsic activity), pilocarpine could act as a muscarinic antagonist rather than an agonist (i.e. by successfully competing for sites with the ACh it would raise IOP). Based upon biochemical and pharmacologic analysis of muscarinic receptors, Mittag also has provided evidence for two classes of muscarinic ACh binding sites in iris-ciliary tissues.[10]

In a similar vein, Kaufman[11] has shown that echothiopate caused subsensitivity to the accommodative response to pilocarpine in cynomologus monkey eyes. Pilocarpine is thought to increase outflow facility by causing contraction of the ciliary muscle and increasing accommodation.

An effect of norepinephrine (NE) on phospholipid metabolism of iris muscle has been established by Abdel-Latif and associates[12] which may help to elucidate its mechanism of action at the biochemical level. After pairs of rabbit iris muscles were prelabelled in vivo or in vitro with radioactive phosphate, one muscle of each pair was treated with NE and the changes in distribution of the label among phospholipids were assessed. Initially, most of the label was in triphosphoinositide (TPI), but on further incubation the radioactive label shifted to other compounds. In tissues from NE-treated eyes 30 percent more label than in control eyes shifted from TPI to phosphatidic acid and phosphatidylinositol, mediated by an alpha-adrenergic mechanism. Surgical denervation caused small increases in label uptake and an increased sensitivity to the label shift caused by NE. Also, NE increased the amount of label transferred from TPI to phosphatidic acid. The investigators speculate that since calcium ion is essential to this TPI effect, increased release of calcium stimulated by NE may be the basis of the redistribution of phosphate among the lipids of the iris.

The studies cited in this section are of considerable interest because they examine the mechanisms by which drugs act upon ocular tissue receptors for neurotransmitters. They are early steps in establishing linkages between drug and transmitter reactions with cells and the consequent intracellular biochemical events and physiologic responses. It is hoped that extensions of research in this direction will ultimately lead to an understanding of the molecular basis of the action of endogenous neuroactive substances and of the drugs which interact with them at specific cellular sites.

Cell Biology

Normal human trabecular meshwork cells have been established in culture and maintained through several passages by Polansky and associates.[13] Their growth characteristics and ultrastructural features have been described, and evidence has been presented that these cells can be distinguished by light and electron microscopy from human corneal keratocytes and corneal endothelial cells. When similar stocks of cells from eyes with glaucoma become established, research into the fundamental biochemical and physiologic cellular events in glaucoma should be greatly facilitated.

Treatment of Glaucoma: Clinical Studies

Timolol, a nonselective beta-adrenergic antagonist, has been approved by the Food and Drug Administration for use in treatment of glaucoma. Studies comparing its short-term efficacy with other antiglaucoma drugs indicate the following: timolol causes a greater fall in IOP than pilocarpine, while pilocarpine produces a greater mean increase in outflow facility; timolol appears to act predominantly by decreasing aqueous production; timolol causes a greater drop in IOP than epinephrine, with apparently none of the toxic effects seen in some patients treated with epinephrine; timolol causes a significant decrease in mean pulse rate without changing blood pressure (pilocarpine has no such effects); timolol does not affect pupil diameter; and some patients using timolol suffer annoying side effects. It remains to be seen whether serious contraindications will become evident as the drug enters wider clinical usage and what, if any, complications attend its long-term use.

Labetalol is a potential new drug for the treatment of glaucoma. This drug, which is also being evaluated for use in the treatment of hypertension, is a strong beta-adrenergic blocker and has a weaker affect on alpha-adrenoceptors. Murray and associates[14] report that labetalol lowers IOP in a dose-dependent manner without affecting pupil size and does not affect outflow facility. No significant irritation or toxicity has been reported. It is hoped that further studies will determine whether the drug will be suitable for ophthalmic use.

Caution is indicated in combined use of a carbonic anhydrase inhibitor (CAI) and aspirin. Systemic acidosis is a concomitant of CAI usage and may also accompany use of salicylates, especially at high dosage levels. For example, both groups of drugs may be used in elderly patients who suffer both from glaucoma and rheumatic diseases. Two cases of severe acidosis have been reported by Anderson and associates[15] following such combined drug therapy.

To improve the capability for differential diagnosis of POAG with a narrow-angle and true acute-angle closure glaucoma, Wand and Grant have proposed a provocative test using thymoxamine.[16] Thymoxamine is an alpha-adrenergic blocking agent, a miotic which widens the angle and can relieve mild angle closure by contracting the ciliary muscle while not affecting IOP or outflow facility. Patients were evaluated by conventional criteria plus the thymoxamine test. In eight, the angle opened but pressure did not fall, indicating POAG. Of eleven patients treated with iridectomies for angle closure, nine were relieved of symptoms. This test, or refinements of it, may become a useful addition to standard glaucoma diagnostic procedures.

A comparison of the benefits of two types of surgery for relief of otherwise uncontrollable glaucoma was made by Watkins and Brubaker.[17] Presumably, partial thickness (trabeculectomy) and full thickness (corneo-scleral trephination) filtration procedures reduce IOP through similar mechanisms. Patients were carefully matched for age, sex, race, and diagnosis. Postoperative IOP was significantly higher in the eyes in the partial thickness group than in those in the full thickness group, while in the former cases a lower incidence of flat anterior chambers and filtering blebs was

observed. Potts comments that trabeculectomy is best employed when modest pressure drops are required and that it is less successful than trephination in young and black patients.[18]

Rubeosis iridis is often an accompaniment of diabetic retinopathy, central retinal vein occlusion, uveitis, or sickle cell disease, and often precedes development of neovascular glaucoma. Neovascularization is thought to be consequent to ocular ischemia. Goldberg and Tso[19] reported a light and electron microscopic study of an eye from a patient with sickle cell retinopathy and absolute glaucoma in which there was rubeosis iridis, degeneration of the trabecular meshwork, complete angle closure, and peripheral synechiae which completely covered the angle. The cytoplasm of the iris capillary epithelium was attenuated, and open junctions explained the leakage of fluorescein previously seen by angiography. The investigators conclude that the ultrastructural changes described were nonspecific concomitants of neovascularization rather than diagnostic for this particular disease.

There is evidence that panretinal photocoagulation for treatment of diabetic retinopathy might cause regression of rubeosis iridis and prevent neovascularization of the angle. Wand and associates[20] followed 93 patients, each having had one eye treated and the other eye left as a control, for about seven years. A statistically significantly larger group of untreated than treated eyes developed rubeosis iridis; also at a significant level, fewer treated than untreated eyes developed neovascular glaucoma. These promising results are being followed up, and the possibility that photocoagulation of the rubeotic angle may prevent development of glaucoma is being investigated.

Another form of secondary glaucoma has been described as due to essential iris atrophy. Now, a study by Campbell, Shields, and Smith[21] hypothesizes that the condition may be a consequence of a particular pattern of corneal epithelial degeneration. Previously, iris nodules and an ectopic membrane had been associated with this syndrome which included peripheral anterior synechiae, corectopia, ectropion uveae, iris atrophy, and glaucoma. Reviews of case histories and light and scanning electron microscopic examination of pathologic specimens suggested that an ectopic endothelial membrane proliferates from the corneal epithelium, overgrows the angle, and as it continues to overgrow the iris, contracts and exerts traction on the iris causing most of the above noted symptoms. The iris nodules appear to be caused by the membrane and to be a clinical marker for it. The investigators suggest that this syndrome be called "Primary Proliferative Endothelial Degeneration." The description of the cellular events in this syndrome should stimulate development of experimental models for testing both the above hypothesis and potential means of therapy.

Animal Studies

Gwin, Gelatt and Chiou[22] have continued to define the hereditary glaucoma occurring in beagles. Histochemical methods have now demonstrated adrenergic fibers in the corneal stroma and fine adrenergic fibers in the trabecular meshwork and anterior ciliosclerał sinus. Dense adrenergic innervation in the subendothelial portion of the ciliary body was seen

extending into the stroma of the ciliary processes. Also, prominent fibers were observed in scleral outflow channels and iris dilator muscle, and less prominent fibers were seen in the iris sphincter muscle and ciliary body musculature. These observations may lead to an explanation of how adrenergic drugs increase outflow facility by acting upon receptors at specific tissue sites. Enzymes associated with cholinergic nerves were identified in corneal epithelium, and slight amounts were measured in the trabecular meshwork and outflow channels. The ciliary body and ciliary processes had high levels of choline acetyltransferase, and cholinesterase was present in the ciliary musculature and epithelial cells; both enzymes were found in iris tissues. In dogs with advanced glaucoma, less evidence of adrenergic fibers was seen, and there was less choline acetyltransferase activity in the tissues.

Other studies by Gelatt and associates in the normal dog have established the existence of a uveoscleral outflow pathway.[23] Fluorescein labeled dextran injected into the posterior chamber rapidly traversed the trabecular meshwork and the ciliary body musculature and passed into the suprachoroidal space and the sclera. Large increases in IOP did not cause any significant changes in this flow pattern, nor did administration of phenylephrine or atropine. Pilocarpine, however, both increased aqueous outflow and impeded the uveoscleral flow. A species difference in outflow mechanism was shown in a study by Sherman and associates using macaque monkeys, in which no uveoscleral flow could be detected; perfusion of fluorescein into the anterior chamber showed drainage only via the vortex veins.[24]

Using the technique of luminal casting, coupled with light and scanning electron microscopy, Van Buskirk has further described the aqueous drainage system in dogs.[25] Canine eyes have no canal of Schlemm and differ from primate eyes in having intrascleral mergence of aqueous and uveal venous blood flows. Drainage paths for aqueous extend radially from the filtering meshwork, through the deep intrascleral venus plexus to the mid-scleral Circle of Hovius, which also serves choroidal venous drainage. The mixed fluids leave the globe via the anterior ciliary vein or posterior vortex veins. The vessels arising from the filtering meshwork do not act as circumferential channels analogous to a primitive canal of Schlemm. Previously, the author had shown that canine outflow facility was insensitive to IOP. The joining of aqueous and uveal flows may provide an anatomic basis for a direct relationship between IOP and uveal blood pressure which may determine ciliary vascular autoregulation and homeostatic maintenance of optic disc perfusion. The investigator suggests that lacking such a regulatory system, primate homeostatic mechanisms may be more vulnerable to breakdown.

The buphthalmic rabbit, while not presently considered to be a good model for studies of POAG, continues to be of interest in glaucoma research. Lee, Fox, and coworkers[26] have shown that the ascorbic acid concentration of aqueous humor is greatly reduced in these rabbits compared to normal animals, and that there are statistically significant inverse relationships between ascorbate level and IOP and ascorbate concentration and outflow facility in buphthalmic rabbits. The low amount of ascorbate in the aqueous is ascribed to a reduced level of secretion by ciliary processes.

Genetic Correlates of Glaucoma

Although many clinicians believe that there may be a familial distribution pattern of liability to glaucoma, hard data to support this contention is still lacking. Recent reports that patterns of occurrence of cellular HLA antigens were correlated with incidence of POAG have not been confirmed; indeed, several investigations in the past year have provided evidence that there are no significant correlations between the frequency of HLA antigens at either A or B loci and the occurrence of glaucoma. It is hoped that the search will continue for such correlations using other types of genetic markers.

Some Directions for Glaucoma Research in 1980

Gaining a sound basis for new directions in the treatment of glaucoma will require an increase in interdisciplinary research. Defined cultured cells from the anterior segment should make possible fruitful developments ranging through molecular biology, biochemistry, pharmacology, and physiology to clinical studies. Controlled clinical trials should aid in determining optimal use of existing and newly developed therapies. Additional researchers should be recruited into these efforts, particularly clinically trained individuals who can translate the results of basic studies into clinical research hypotheses. To provide this necessary bridge, enthusiastic clinicians should be encouraged to enter into fellowship training programs to develop expertise in the relevant basic sciences. Well-trained persons in clinical and basic disciplines should be encouraged to establish interdisciplinary research projects.

Research into some of the areas touched upon in this review needs to be amplified. These include development of valid animal models for the various types of glaucoma, a search for predictive factors (genetic, provocative, biochemical), and the search for a better understanding of the secondary forms of glaucoma. Additionally, topics which should be definitively approached include: a thorough state-of-the-art evaluation of the use of laser treatments for glaucoma, a review of the effectiveness of the various methods for recording and evaluating changes in optic nervehead morphology, and an evaluation of the effectiveness of the more elaborate diagnostic and recording instrumentations vis-a-vis simple observations. Also, an up-to-date overview of the innervation and pharmacology of the anterior segment is needed, particularly with reference to possible roles for the many substances now defined as neurotransmitters and neuromodulators or as having vasoactive effects. A critical review of the role of inflammatory agents in the genesis of glaucoma should also be encouraged.

References

Glaucoma

1. Raviola G, Raviola E: Intercellular junctions in the ciliary epithelium. Invest Ophthalmol Vis Sci 17:958-1981, 1978.

2. Szalay J: Annual Progress Report, EY 01631, January 1979.

3. Yamashita H, Sears M: Proof that the ciliary epithelium can regenerate. Exp Eye Res 27:199-213, 1978.

4. Flach A, Wood I: An electron microscopic study of degeneration of nerve terminals after administration of epinephrine. Exp Eye Res 27:377-386, 1978.

5. Neufeld A, Zawistowski KA, Page ED, Bromberg B: Influences on the density of B-adrenergic receptors in the cornea and iris-ciliary body of the rabbit. Invest Ophthalmol Vis Sci 17:1069-1075, 1978.

6. Colasanti BK, Kosa JE, Trotter RR: Responsiveness of the rabbit eye to adrenergic and cholinergic agonists after treatment with 6-hydroxydopamine or alpha-methyl-paratyrosine. 1. Pupillary changes. Ann Ophthalmol 10:1067-1074, 1978.

7. Potter DE, Rowland JM: Adrenergic drugs and intraocular pressure: Effects of selective B-adrenergic agents. Exp Eye Res 27:615-625, 1978.

8. Green K, Griffen C, Hensley A: Effect of parasympathetic and vasoactive drugs on ciliary epithelium permeability. Exp Eye Res 27:533-538, 1978.

9. Bito LZ, Merritt SQ: Paradoxical ocular hypertensive effect of pilocarpine on echothiopate iodide treated primate eyes. Invest Ophthalmol Vis Sci (in press).

10. Mittag TW: Annual Progress Report, EY 02619, June 1979.

11. Kaufman PL: Anticholinesterase induced cholinergic subsensitivity in primate accommodation mechanism. Am J Ophthalmol 85:622-630, 1978.

12. Abdel-Latif AA, Green K, Smith JP, McPherson JC, Matheny JL: Norepinephrine-stimulated breakdown of triphosphoinositide in rabbit iris smooth muscle: Effects of surgical sympathetic denervation and in vivo electrical stimulation of the sympathetic nerve of the eye. J Neurochem 30:517-525, 1978.

13. Polansky JR, Weinreb RN, Baxter JD, Alvarado J: Human trabecular cells. 1. Establishment in tissue culture and growth characteristics. Invest Ophthalmol Vis Sci (in press).

14. Murray DL, Podos SM, Wei C-P, Leopold IH: Ocular effects in normal rabbits of topically applied labetalol. Arch Ophthalmol 97:723-726, 1979.

15. Anderson CJ, Kaufman PL, Sturm RJ. Toxicity of combined therapy with carbonic anhydrase inhibitors and aspirin. Am J Ophthalmol 86:516-519, 1978.

16. Wand M, Grant MW: Thymoxamine test. Differentiating angle-closure glaucoma from open-angle glaucoma with narrow angles. Arch Ophthalmol 96:1009-1011, 1978.

17. Watkins PH, Brubaker RF: Comparison of partial-thickness and full thickness filtration procedures for open angle glaucoma. Am J Ophthalmol 86:756-761, 1978.

18. Potts AM: Some rationalizations on chronic open-angle glaucoma. Am J Ophthalmol 86:743-755, 1978.

19. Goldberg MF, Tso MDM: Rubeosis iridis and glaucoma associated with sickle-cell retinopathy: A light and electron microscopic study. Ophthalmology 85:1028-1041, 1978.

20. Wand M, Dueker DK, Aiello LM, Grant MW: Effects of panretinal photocoagulation on rubeosis iridis, angle neovascularization and neovascular glaucoma. Am J Ophthalmol 86:332-339, 1978.

21. Campbell DG, Shields HB, Smith TR: The corneal epithelium and the spectrum of essential iris atrophy. Am J Ophthalmol 86:317-324, 1978.

22. Gwin RW, Gelatt KN, Chiou CY: Adrenergic and cholinergic innervation of the anterior segment of the normal and glaucomatous beagle. Invest Ophthalmol Visual Sci (in press).

23. Gelatt KN, Gum GG, Williams LW, Barrie KP: Uveoscleral flow of aqueous humor in the normal dog. Invest Ophthalmol Vis Sci (in press).

24. Sherman SH, Green K, Laties AM: The fate of anterior chamber fluorescein in the monkey eye. 1. The anterior chamber outflow pathways. Exp Eye Res 27:159-173, 1978.

25. Van Buskirk EM: The canine eye: The vessels of aqueous drainage. Invest Ophthalmol Vis Sci 18:223-230, 1979.

26. Lee P-F, Fox R, Henrick I, Lam WKW: Correlation of aqueous humor ascorbate with intraocular pressure and outflow facility in hereditary buphthalmic rabbits. Invest Ophthalmol Vis Sci 17:799-802, 1978.

SENSORY AND MOTOR DISORDERS OF VISION AND REHABILITATION

Introduction

Some of the more intractable disorders of vision, comprising over 25 percent of the visual problems in the United States, are the concern of the Sensory and Motor Disorders of Vision and Rehabilitation program. Some of these are strabismus, amblyopia, nystagmus, degenerations of the optic nerve, abnormalitites of gaze, third cranial nerve paralysis, oculomotor dyslexia, and refractive errors. The research supported in this program deals with the visual neural pathways, the processing of visual information, visual perception, optical properties of the eye, functioning of the pupil, and control of the ocular muscles.[1]

This research requires the contributions of a heterogeneous group of scientists: neuroanatomists, neurophysiologists, bioengineers, biomathematicians, psychophysicists, geneticists, and behavioral scientists, as well as ophthalmologists and optometrists. Furthermore, these scientists work with a large number of anatomical structures in a great variety of animals: the eyeball as a whole, and the parts of the nervous system and musculature involved in vision in invertebrates, lower vertebrates, lower mammals, and primates, including humans.

The panel of the National Advisory Eye Council's Program Planning Subcommittee responsible for sensory and motor disorders of vision in its report in Vision Research--A National Plan: 1978-1982[2] divided the program into the following six subprograms: (1) Congenital, Developmental, and Degenerative Abnormalities: (2) Strabismus and Other Oculomotor Disorders; (3) Optical and Pupilary Disorders; (4) Visual Sensory and Perceptual Disorders; (5) Sensory and Motor Disorders Related to Specific Disease Processes; and (6) Rehabilitation. In total, the Sensory and Motor Disorders of Vision and Rehabilitation Program spends almost $20 million in support of over 300 research projects.

Only a few of the research areas of greatest activity in this program during the past year will be discussed in this report. They are: color vision processing, binocularity, neural regeneration, assessment of vision in infants, and low vision.

Color Vision Processing

Disorders of color vision, although not rare, are not usually serious problems. However, many tests of color vision could yield a better diagnosis of disorders of the entire visual system from the retina to the cortex. In order to understand the visual system in humans, perception of color must also be explained. Over 30 grants deal with color vision, some including other matters as well. Generally speaking, these grants can be divided into (a) psychophysics, which is concerned with descriptions of stimuli and responses concerning color, and (b) neurophysiology, which is concerned with processing of visual color signals from the retina to the cortex.

Psychophysics. Barris and Frumkes[3] used a 10 msec. test flash to stimulate the parafoveal retina where it could be detected by rods alone. Shortly before, during, or after this test flash, a 10 msec. variable wavelength annular flash was also presented. When the annular flash preceded the test flash by more than 100 msec. or followed it by more than 150 msec., the wavelength as well as luminance influenced the threshold measured. Therefore, the rods and cones must interact in this part of the retina. Frumkes[4] also found that there are spatial constraints upon rod-cone interaction which applied during steady adaptation and during the earliest stages of light and dark adaptation. Furthermore, at scotopic levels of luminance, two different channels mediate flicker: one sensitive to very low frequencies and one to relatively higher frequencies. It seems that the low frequency channel has relatively little cone involvement, and the higher frequency has both a rod and cone input.

Boynton[5] has also found evidence of physiological interactions between red- and green-sensitive cones that is causing a reassessment of Stiles's pi-5 (red) color mechanism. Other parts of this project have established that dichromats exhibit, when large fields are used, a residual trichromacy not mediated by rods. Piantanida,[6] too, finds that dichromats are actually trichromatic and show opponent processing that appears to be the same as that seen in normal trichromats. Transient and sustained aspects of an incremental flash (normally used to measure increment threshold spectral sensitivity) were dissected out and used to generate new types of incremental stimuli. Piantanida found that the short wavelength color mechanism of normal trichromatic humans is more sensitive to offsets. Ingling and associates, comparing hue matching methods with hue cancellation procedures, found that cancellation overestimated short wavelength sensitivity. They feel this means that different mechanisms[7] control the perception of short wavelength and long wavelength redness.

In a more direct attack upon the physiological basis of color processing, Riggs is comparing electroretinograms and visually evoked cortical potentials taken from subjects who must determine minimal detectable color contrasts between all pairs of wavelengths in the visible spectrum.[8] So far the VECP part of the study has produced interesting data. Response averaging indicates that amplitude is proportional to the logarithm of chromatic contrast. Sensitivity decreases for smaller wavelength differences. Multidimensional scaling will be used to generate a "color space" with these data. Similar techniques have been used with the same stimuli in a completely behavioral experiment. Here color space seems to be a uniform, reversed U-shaped array of the sequence of wavelengths in the visible spectrum.

Neurophysiology. Granda[9] has found that turtles can discriminate red, green, and blue in such a way that responses are correlated with the time-to-peak data collected from cones of different classes. The critical duration of the stimulus is greater than 100 msec. and increases with the size of the target and with dark adaptation. Receptive fields of ganglion cells in the retina are generally most sensitive to red. The fields of green and blue ganglion cells were larger under dark adaptation than were the red cells'. Horizontal cells in the retina have the same temporal relationships and fall into three classes: red, red-green, and

blue. Measuring the quanta summated in horizontal cells, Granda found that blue needed more than green which needed more than red in order to produce a critical duration.

The retina of the ground squirrel contains two types of cones and a very small number of rods. Jacobs[10] took electroretinograms of squirrels exposed to monochromatic light, at the same time recording responses in optic nerve fibers. One cone has a maximum response at 525 nm. This mechanism dominates the ERG under conditions of light adaptation, and it provides some input to all of the fibers in the optic nerve. The other cone's photopigment has a maximum response at 440 nm. Its contribution to the ERG is minimal, but its presence is found in about one third of the optic nerve fibers tested. A third spectral peak is recorded at 500 nm which contributes to the ERG under conditions of dark adaptation and to many optic nerve fibers. Jacobs feels that the 500 nm mechanism arises from the small number of rods contained in this retina.

After the retina, the first relay station in the central visual system is the lateral geniculate nucleus (LGN). Sperling[11] and his colleagues have developed a technique for controlling fixation in the central macular area, measuring behavioral thresholds, and determining thresholds for single neural units at the same time. Comparison of data from neural units and behavioral thresholds seems to reject the hypothesis that the color-opponent LGN units can serve threshold detection. They found another type of broad-band LGN unit which could serve that function. Another relay station is the pulvinar complex of the thalamus which becomes highly developed in primates. When the same kind of experiment was performed in the pulvinar by Crawford and Espinoza,[12] they found a uniform response to flashing test lights throughout the visible spectrum from single units in the inferior pulvinar. If the unit fails to respond, the monkey himself fails to report seeing the test flash. After adaptation with monocromatic light, the threshold of the neuron shifts as does the sensitivity of the monkey. The pulvinar neurons have a spectral sensitivity comparable to other broad-band units of the geniculo-striate system. The same group of investigators[13] studied the cortices of young monkeys stimulated monocularly by monochromatic light after being injected with 2-deoxyglucose. Autoradiographs show narrow bands of increased metabolic activity extending from the cortical surface to the white matter in which layers I and II contain the highest density of label. The labeled column is significantly narrower than adjacent nonlabeled space, suggesting that only a subpopulation of neurons of the eye-dominance hypercolumn was activated. At the striate cortex, color information is processed in a mosaic of columns of neurons and relayed to more specialized prestriate areas. In the prestriate cortex a complex pattern is found throughout the inferior occipital sulcus. Here the mosaic becomes less precise. Strangly, no patterns of note were seen in the lateral geniculate.

In a series of studies further tracing neural pathways dealing with color stimuli, Marrocco[14] has found both opponent and non-opponent cells in the parvocellular layers of the LGN. Generally, the excitatory responses of these cells (e.g. to long wavelengths) are generated by the RF center and the inhibitory responses (short wavelenghts) are generated by the RF surround.

Receptive field properties of cells in both the LGN and the superior colliculus (SC) were determined, and then the cells stimulated. In the LGN, non-opponent cells had the longest discharge latencies whether they were classified as transient (brief discharges only) or sustained (long duration discharges). Among the non-opponent cells, transient units had significantly shorter latencies than sustained units. In all cases, visual latencies were highly correlated with shock latencies. In the superior colliculus, cells are almost all transient responders to stationary stimuli and sustained responders to moving stimuli. As in the LGN, shock latencies are trimodally distributed, but the best predicter of a cell's latency is the depth in SC rather than features of its receptive field. There is less overlap of latencies in the three kinds of cells in the geniculostriate system than in the retinogeniculate system.

Michael[15] reported that striate cortical cells which are color coded fall into four categories: concentric, simple, complex, hypercomplex. These cells arrange themselves in vertical columns from the surface of the cortex to the white matter. All cells in a column are color sensitive, but they have varying RF, axis orientation, and eye dominance. They are often related chromatically as if this were part of a serial processing within a single column. The complex and hypercomplex cells are found in layers II, III, V, VI. The concentric and simple cells are all in layer IV. All of the concentric cells and most of the simple cells are monocular, but the majority of the others are binocular.

The next central relay station in the visual path is the prestriate area, 18 or V2. Baizer and associates[16] found the lunate sulcus to be the richest field of visually sensitive cells. The tiny foveal receptive field of the striate (four minutes of arc) expands on the anterior banks of the lunate sulcus to 120 x 300 minutes of arc. Obviously, considerable convergence takes place between the striate cortex and area 18. In this area the same group of investigators[17] noted color selective cells both with and without orientation selectivity and cells with specific requirements for certain kinds of textures or gratings used in stimuli. Color cells are usually of the opponent variety. About half have suppressive surround (i.e. "double opponent") and the other half are single opponent color cells without surrounds. These have identifiable counterparts in area 17, but there seems to be an absence of precise orientation-specific color cells in the V2 area.

De Valois and his colleagues investigated the spatial tuning of lateral geniculate neurons in monkeys by stimulating with luminance-based and color-based lines.[18] The cells responded most to intermediate widths of luminance-based lines and to the widest pure-color lines. The same group of investigators examined the spatial tuning of cells in area 17, using both luminance and pure-color modulated sine wave gratings. They found that almost all cells respond to both kinds of stimuli, and a majority of these cells respond equally well to either color or luminance gratings.[19] Most cells show bandpass tuning characteristics for both gratings. Sometimes the tuning functions to color and luminance are identical in a single cell, but on the average, the peak spatial frequency tuning to color is lower, and the average bandpass broader. The cortical cell responses were precisely predictable

from the Fourier spectra of the patterns, and not at all from the edge orientations and bar widths.

Finally, in a psychophysical experiment, it was found that adaptation to high-contrast sinusiodal luminance gratings produces a temporary, band-limited loss in sensitivity centered around the adaptation frequency. The effect falls to zero one octave away. Enhancement of contrast sensitivity occurs for frequencies further removed, peaking at about 2 3/4-3 octaves. The investigators feel these data suggest mutually inhibitory interactions among spatial frequency-selective units of varying filter characteristics.[20]

We have come full circle from stimulus to response. We have traced the pathways the "stimulus" follows, and we have learned much about the interactions between the "stimulus" and the relay stations. Indeed, there are many gaps in the information, and some disagreements among investigators about details within the information. But a feeling of clarity, precision and agreement pervades. Yet we are still not certain how we perceive color. As Boynton says, "In vision, we are not concerned with perceiving light rays as such, but with perceiving the external objects mediated by these radiations; the eye must inform us, not about the momentary intensity or quality of the light reflected from external objects, but about these objects themselves."[21]

Central Neural Regeneration in Visual Paths

One can consider neural regeneration a special case of neural plasticity or of neural development. Both plasticity and some aspects of development were discussed in last year's annual report. This year we would like to consider this special case. Neural regeneration is an extremely active research field at this time. Although peripheral nerve regeneration has been studied intensively for many years and is still a matter of great concern, until recently regeneration in the central nervous system has received less attention. Peripheral nerve regeneration occurs in almost all species whereas central nerve regeneration, possible in some animals such as most amphibians and fish, seems impossible in mammals. In other areas of the health sciences, such as those dealing with diseases of the central nervous system or injuries thereto, scientists have been conducting a great deal of research in mammalian central nerve regeneration.

Work in the visual nervous system has lagged behind. Currently, the National Eye Institute is supporting only nine grants dealing with regeneration in the central visual pathways. Six of these, in fact, have only recently been funded.

Sharma[22] removed one eye of goldfish and noncorresponding halves of each tectum. The remaining eye projected in a compressed form to the contralateral half-tectum. Gradually, the projection there grew, and a year later the half tectum had regenerated with an appropriate topographic map. In a tectum devoid of myelin debris, the full retinal complement reinervated at the same time. In a half-tectum containing debris, only appropriate fibers reestablished contact. Behaviorally, there was no effect of partial tectal ablation, but the total loss of tectum produced greater spatial summation.

Goldberg[23] deflected optic fibers in the ganglion cell fiber layer of embryonic chick retinas. Most fibers continued to grow in their abnormal directions. Only those in the vitread portion of the ganglion cell fiber layer showed unanimity, growing toward the optic nerve. It seems likely that this portion of the retina contains some guiding substance which allows for proper alignment during development.

Four days after crushing the optic nerve of a toad, Willard[24] identified a rapidly transported protein. This regeneration-associated protein ceased to be transported at about the time that the regeneration optic nerve reached the tectum. The protein cannot be labeled by amino acids applied to the crushed nerve, nor is it synthesized by glial cells.

Goldberg[25] summarizes the reasons for failure of mammalian nerve regeneration: (1) the fault is intracellular or in the cell's environment, (2) The deficit is in cell maintenance or in axonal growth, guidance or synapse formation, (3) these can involve chemotactic, mechanical, or electrophysiological mechanisms.

The needs for future research in this field are clear. Further studies are needed in animals that normally regenerate nerves, especially in the CNS, in order to identify the regeneration-enhancing peoperties of their nervous systems. Also needed is careful work on mammals, not aimed at the sudden dramatic breakthrough, but concentrating on the details of nerve cell death and axon growth. It is strange that so much of this work has gone on in other parts of the nervous system when the visual nervous system could be a model for such studies. Among the new grants in this program one finds examples of this:

There is work on normal nerve growth in tissue culture; on the pharmacology of neural tissue in culture; on "normal" neural regeneration in goldfish, using psychophysics to study normal visual processing; on neural transport during regeneration in fish; and on the sequence of protein synthesis in mammals. It is hoped that this recent increase in regeneration work is an indication of an active visual research area in the near future.

Binocularity: Neural Development and Organization

Visual disorders such as strabismus and amblyopia seriously affect depth perception. If we learn more about normal neural development, neural plasticity, and neural organization, prevention or correction of these problems may be possible.

Some aspects of neural development were discussed in last year's annual report in the context of neural plasticity. One of the research fields briefly mentioned was binocular vision. In this section we hope to bring the subject matter of plasticity in binocular vision up to date, speak more about normal development in regard to binocularity, and deal with the organization of the nervous system in regard to binocularity.

Stereopsis. The possession of two eyes is a precondition for seeing the world in depth. However, it does not assure stereovision. In strabismus the

foveas of the retinas do not correspond and frequently change their degree of correspondence. This is one of the conditions which sometimes causes amblyopia, a condition (usually in one eye) in which reduced visual acuity is not correctable by refractive means and is not attributable to obvious anomalies. This is a definition of what Higgins[26] would call functional amblyopia, contrasting it with organic amblyopia in which the loss of acuity can be attributed to some condition affecting the projections from the retina. Higgins feels that, for the normal viewer, the central visual field becomes increasingly scotomatous with decreasing illumination, but he believes that the reverse is true for the functional amblyope. In some testing conditions the amblyope shows increased spatial summation, i.e. finds it difficult to distinguish objects close together. Such summation may result from larger receptive fields or excessive neural excitation. However, the elevated contrast thresholds shown by amblyopes are more consistent with an increased neural inhibition which also explains better the scotomata at higher light levels. As Higgins points out, it is difficult to localize the anatomical site of the physiological problem using psychophysical methods.

Sometimes the site can be inferred. Blake and Cormack[27] flashed grating patterns into one eye of stereoblind humans whose task was to report which eye received the flash, a process called utrocular discrimination. Performance falls to chance levels in normals at higher spatial frequencies of grating. Stereoblind observers, on the other hand, are able to distingush which eye has been stimulated regardless of spatial frequency. The investigators feel that these results reflect the loss of binocular cells and the increase in monocular cells in the visual cortex of stereoblind observers. This explanation is an extrapolation from anatomical data from animal experiments in visual deprivation.

Plasticity. Animal experiments provide more direct evidence of the changes in neural connections resulting from visual experience. Eggers and Blakemore[28] raised kittens so that their only visual experience (43-80 hours) took place with a high-power negative spherical lens in front of one eye and glass in front of the other. Later, cells in the primary visual cortex were examined in these and normally raised animals. In the anisometropic animals, the proportion of binocularly driven cells was reduced compared to the normals, and in a majority of the neurons tested, monocular cells were driven by the eye that had had normal visual experience. The shift in ocular dominance, though consistent, was not as complete as in previous experiments with monocular occlusion. Cells monocularly driven by the defocused eye had lower cutoff spatial frequencies and lower contrast sensitivity.

The small number of binocular units tested had low preferred spatial frequencies, and the cutoff in the defocused eye tended to be lower than that in the normal eye.

Similar results were achieved with slightly different methods by Smith and his colleagues.[29] In order to avoid the confounding influences of surgically altered ocular muscles, these investigators raised kittens so that their only visual experience (a minimum of 208 hours) took place through goggles containing ophthalmic prisms before one or both eyes. Then

recordings of cells in the visual cortex were made. While the majority of neurons encountered in control animals were excited by stimuli presented to either eye, the majority of neurons in prism-reared animals were classified as monocular. The percentage of monocular cells encountered depended upon the direction and magnitude of the prism worn. For all prism-reared animals ocular dominance changed dramatically compared to normal. Cells were driven by one eye or the other, the influence being greater for 30 than for 15 diopter prisms and greater when prisms covered both eyes compared to one. The investigators concluded that the development and maintenance of normal binocular vision is a complicated process requiring a delicate balance in both motor and sensory processes.

Although monocular deprivation in young cats produces a deficiency of binocularly driven cells in the striate cortex, Blasdel and Pettigrew[30] showed that this effect can be reversed in cats as old as eight weeks if they have been allowed normal visual experience before eye closure. Although sometimes reversible, the loss of binocular cells is almost certain to take place. The severe deficit of binocular driven neurons as a result of monocular occlusion can occur even if the animal has had normal binocular vision up to the time of deprivation and continues to experience binocular vision from time to time during the course of deprivation.[31] In the owl, a period of normal binocular experience before deprivation and short periods during deprivation produced a normal ocular dominance distribution. Perhaps there is some difference in brain chemistry between cats and owls. Injections of the catecholamine, depleting agent, 6-OHDA, into the visual cortex of kittens can prevent the shift in ocular dominance normally resulting from monocular lid-suture in kittens. Perfusion of norepinephrine reverses the effect and restores susceptibility in adults. The proportion of normal binocular cells decreases, but the complete shift is not seen. This plasticity is found only in the local area perfused.

Development. Much of the work on visual deprivation has taken place in rats, usually albino. In addition to the usual changes in the cortex after monocular lid-closure, the albino rat shows changes in the retina. In order to avoid the possibility that the excess light striking the retina of the nonsutured eye causes some of these intraocular changes, Fifkova[32] has used hooded rats where no changes in visual receptors of the functional eye have been observed. The pigmented iris and choroidea apparently protected the retina from injurious levels of light. The changes in the visual cortex, however, were even greater than those in the albino. Some central process induced by enhanced stimulation results in a large increase in synaptic density in the functional cortex. In the nonfunctional cortex, monocular lid-suture caused a larger decrease in synaptic density in the binocular than in the monocular segment.

In order to find out if some degenerative process in the deprived visual pathway was responsible, one eye was removed. Since the "enucleated" visual cortex showed an increase in synaptic density, a degenerative process does not seem to underlie the changes resulting from deprivation. In the hooded rat considerable synaptogenesis and dendritic growth occur before eyes are open. The intermediate layer of the cortex matures faster than the more superficial layers, a difference which disappears ten days after birth. At

birth, deep layers (V and VI) contain more synapses than superficial ones (II-IV). The rate of synaptogenesis remains high in superficial layers but decelerates in deep layers by day 10.

Normal development is sometimes more graphically illustrated by study of abnormal development. In common cats, fibers from the nasal half of each retina cross in the chiasm and synapse in the most dorsal geniculate lamina, lamina A. Fibers from the temporal half of each retina innervate the ipsilateral LGN in lamina A1. From the LGN, fibers proceed to the primary visual cortex, area 17, where they remain in register and where the more midline parts of the visual field abut area 18. In the Siamese cat, Shatz and Le Vay[33] found that fibers from a region of retina on the temporal side of the midline also cross in the chiasm and terminate in the medial portion of lamina A1 on the wrong side of the brain. The cells on which they synapse send axons to area 17 in such a fashion that the temporal representation from that side of the brain no longer abuts area 18. There is another rearrangement of cortico-geniculate

fibers such that they synapse in the LGN, with retinotopically corresponding points being connected. Within the cortex, the 17-18 border region is projected massively to zones deeper in the cortex. These cortico-cortical projections link regions representing different, though mirror-symmetric, visual field positions. In the formation of associational connections, positional information from the retina is still used, but sign (left or right of vertical midline) is ignored.

In common cats, Daniels[34] is testing a model of binocular vision based on hierarchical processing (combinatorial logic) in the dendritic trees. He finds that, in both the LGN and cortex, spatial frequency patterns are no better at evoking binocular facilitation that moving edges. He also finds that neural responses in the 17-18 border area are easier to evoke than responses deeper in 18. The sum of monocular responses is greater than the best binocular presentation of the optimal pattern.

Organization. The explanation of how the brain combines with the eyes to produce binocular vision is still incomplete. More research is needed. In fact, the anatomical organization is not completely clear though work proceeds rapidly, and some of it has been discussed above. The function of cortico-geniculate fibers is unclear, but we know that most of these neurons are in the "complex" category with strong binocularity and possibly a preference for slower velocity of visual motion. Within the LGN, some cells have been identified by Pettigrew and his colleagues[31] as being strongly influenced by feedback from the visual cortex. When a stimulus in the form of an oriented contour moving at a specific velocity is presented to the normally nondominant eye for a given LGN cell, there can be a 100 percent facilitation of the usual response. This is especially true when the motion starts or stops in a location in the nondominant eye equivalent to that of the receptive field in the dominant eye.

In predatory birds, Fox[35] has found both ipsilateral and contralateral projections to the visual Wulst, evidence for binocular innervation that is congruent to prior behavioral demonstrations of stereopsis. On

the other hand, stereopsis is not automatic. Attempts to induce visually evoked potentials in anesthetized animals by moving stereoscopic contours formed from random element stereograms have been unsuccessful. Apparently, elicitation of potentials requires a conscious, attentive animal.

It is probable that all vision, certainly vision in depth, requires feedback from muscles and some kind of explicit or implicit response to the sensory portions of the experience. However, when the system is working perfectly it is truly astonishing. Pettigrew reported that, within limitations imposed by oculomotor control and by changes in eye size or position during development, the cortex is capable of detecting, in one retinal image relative to the other, a displacement which is a small fraction of the angle subtended by one photoreceptor.[36]

Assessment of Visual Function in Infants

In recent years, data from numerous studies on monkeys,[37] kittens,[38] and rodents[39] have demonstrated the role of early visual experience in the development of the visual system and visual function.[40] Taken together, these data show that the visual system is maleable (plastic) very early in life, that after some point early in life (critical period) this plasticity is greatly reduced or even lost, and that visual capability following the critical period is determined by visual experience during early development. Early experience is important in human visual development too. This can be demonstrated, for example, by the reduced visual acuity (amblyopia) in one eye evidenced in people who have suffered strabismus during infancy. Other examples abound.

It is important, therefore, to detect visual impairments in human infants at the earliest possible time if remedial therapies are to be applied so as to prevent or reverse visual loss. There are problems, however: (1) The parameters of normal human visual plasticity, and of critical periods for visual development, are not well defined. (2) Methods of early therapy for many developmental disorders of vision are yet to be formulated. (3) Techniques for the evaluation and characterization of visual capability must be developed and modified for use with infants. The third is the more serious immediate problem.

Improved detection of disorders of visual function requires knowledge of the parameters of normal functional capability. Similarly, early intervention requires early detection, and our ability to devise early interventions will be determined to some extent by our knowledge of normative matters. Increased knowledge and increased capability for intervention clearly await the development of special techniques for testing infant vision.

In the past year, NEI grantees have continued to make great progress in attacking the problems attendant to the assessment of visual function in infants. Most of this research has relied on the use of behavioral and electrophysiological techniques.

In 1958 Fantz[41] reported a new method for evaluating the nature and extent of visual form perception in infants. This method--the preferential looking technique--is predicated on the preference of infants for looking at patterned stimuli more than homogeneous stimuli. Two visual stimuli are presented to the infant, and an adult observer records the proportion of time the infant looks at each. More recently, this technique has been improved and refined by Teller and her associates at the University of Washington,[42,43] and they are now applying it to the evaluation of normal infants and infants with disorders. The primary focus of these studies to date has been on establishing the parameters of visual acuity in normal infants from birth through the first year. The data they have obtained to date in screening studies[44] indicate that this technique has great potential for identifying infants with subnormal visual acuity. In studies performed on premature infants it was found that acuity should be evaluated on the basis of due date and not actual age from birth.[45] These investigators have also initiated studies of the changes in visual sensitivity which occur under light-adaptation and dark-adaptation conditions.[46]

Held and his associates at the Massachusetts Institute of Technology have also been using the preferential looking technique in studying clinical populations of infants after refining further the procedures for obtaining visual acuity measurements.[47,48,49] In one study,[48] they have followed the development of acuity in both eyes of infants being treated for congenital deviation of one eye. They find: (a) the onset of amblyopia in the deviating eye begins in the fourth to fifth months, (b) patching the nondeviating eye results in increased acuity in the deviating eye and decreased acuity in the patched eye, and (c) following termination of the patching therapy, the now unpatched eye develops an acuity which is better than expected by the norm for that age.

In another study,[49] Held and his colleagues have been evaluating the changes in visual acuity associated with refractive error early in life. The basis for this study comes from the recent finding by Mohindra[50] and Howland[51] that astigmatism at four months of age is roughly ten times that found in adults, and declines during the next few months of life. At the New England Medical Center Hospital, Sokol has been developing and refining electrophysiologic techniques for assessing visual capability in infants. In these studies, visually evoked potentials (VEP) are used as the measurement technique. Visually evoked potentials are the summated electrical potentials evoked in the brain (as measured with scalp electrodes) by visual stimulation. In one study,[52] they find that the development of pattern vision proceeds at a rapid rate, as does visual acuity, and that the shape of the VEP function resembles the adult VEP function by six months of age. In a second study,[53] they demonstrate, using the VEP, that by six months of age infant visual acuity is comparable to 20/20 in Snellen equivalents. These investigators are currently continuing their research with the VEP technique and conducting studies on preterm infants and on infants who have been visually deprived of visual experience by early eye disorders.

Biochemistry of the Nervous System. In Vision Research--A National Plan: 1978-1982,[54] one of the research priorities recommended for the Sensory and Motor Disorders of Vision and Rehabilitation program was investigation of the possiblility of treating disorders of the visual nervous system pharmacologically. It was foreseen that basic work in neurochemistry was necessary to develop drugs that could regulate neural plasticity and treat other sensory or motor disorders. In 1978 representatives of NEI met with neuroscientists at a special meeting organized by the Society for Neuroscience and discussed these possibilities. Stemming from that general meeting, a small group of neuroscientists and vision scientists met later in order to see if there were specific recommendations that could be made. Study of the biochemistry of parts of the central nervous system other than the visual system has rapidly advanced. However, progress has lagged in studying the pharmacology of the visual system. Various means of encouraging vision scientists to use the precise techniques of neuropharmacology on the one hand, and on the other, encouraging neuropharmacologists to take advantage of a neural system whose electrophysiology is so well described were discussed. It seemed that one way of increasing this kind of research would be to combine research training in both of these areas for younger, developing scientists.

Low Vision. In its national research plan, the National Advisory Eye Council also identified the need for additional research in visual rehabilitation.[55] As a first step in this direction, a workshop on "Research Opportunities Relevant to the Management of Severe Visual Impairments" was conducted by the National Eye Institute in June 1977. The purpose of this workshop was to identify recent advances and current needs in research related to rehabilitation of the low-vision individual.

As a result of the Council recommendations and the activities of the workshop, a program announcement was developed. The announcement, released in October 1978, requested application for grants in the following (and related) research areas: (1) better diagnosis of visual system impairments and improved characteristics of residual vision, (2) development and evaluation of special devices and techniques to improve visual performance of patients with specific optical or retinal irregularities, (3) development and clinical trials of special devices and techniques to improve mobility and the performance of jobs and skills, and (4) assessment of visual functions in the infant and young child.[56]

The vision research community has not been slow to respond to the identified need for high quality research relative to low vision. During FY 1979, the NEI made 15 grant awards to initiate new projects with the potential for direct impact on clinical management of low vision problems. These include the following: Analytic Studies of Perturbed Vision (University of Pennsylvania), Psychophysical Studies of Photopic Abnormalities (University of Illinois, Chicago), Stimuli for Clinical Testing of Visually-Evoked Potentials (University of Texas, Dallas), Assessment of Visual Pathology with Psychophysics and Evoked Potentials (Dalhousie University, Halifax), Refractive Studies of Eyes (Cornell University), Mobility-Enhancement for the Blind

(M.I.T.), Psychophysical Analysis of Visual-Sensory Deficit (University of California, Berkeley), Matrix Displays in Low-Vision Reading Aids (University of Minnesota), Studies of Visual Functions in the Peripheral Retina (Johns Hopkins University), Control and Application of Laser Speckle to Macular Evaluation (University of Florida, and Clinical Application of Hyperacuity and Related tests (University of Florida). Influence of Peripheral Vision on Visual Performance (California Institute of Technology), Electroretinographic Studies in Diabetic Retinopathy (University of Wisconsin), Retinal Assessment by Laser of Visual Acuity (University of Michigan), and Development of Optimal Glaucoma Visual Field Protocols (Mt. Sinai Medical School, New York).

Orthokeratology: Clinical Trial. Orthokeratology is a procedure aimed at correcting refractive errors, primarily nearsightedness, by reshaping the curvature of the cornea. This treatment consists of fitting a series of specially designed, slightly flattened, contact lenses which are intended to remold the cornea gradually. Some vision specialists believe that the changes produced by orthokeratology will not be permanent and that the potential for damage to the cornea and other risks involved are negligible, and that any adverse effects that may occur could be detected and remedied promptly. Thus, orthokeratology remains a subject of controversy within the vision care community.

For the past two years, the NEI has been supporting a controlled clinical study of orthokeratology by Polse and his associates at the University of California School of Optometry in Berkeley. Patients who meet the elegibility criteria of the study are assigned at random to treatment with either conventional contact lenses or orthokeratology. Patients are followed for a two-year period, after fitting is completed, to provide information on the long-term effects of both methods. Changes in visual acuity, refractive error, corneal thickness, corneal endothelial cell density, corneal curvature, and general eye health are monitored.[57,58,59] The study is thus designed to provide objective information on whether orthokeratology is safe and effective and offers any advantages over conventional contact lenses.

The recruitment phase of this study is now complete. More than 250 potential participants have been screened, and 80 patients have been admitted to the study. All of the study patients have either completed or are in the process of completing their adaptation to the wear of contact lenses. Well over half of the study patients have begun the treatment phase.

The safety and data monitoring committee for this study has been meeting every six months in order to monitor the safety of study participants and to assure timely review and interpretation of the data obtained. It is estimated that initial study results will be available late in 1981.

References

Sensory and Motor Disorders of Vision

1. Vision Research--A National Plan: 1978-1982, Volume One, Summary. US DHEW Publ No (NIH) 78-1258, 1978, p 21.

2. Vision Research--A National Plan: 1978-1982, Volume Two, Panel Reports. US DHEW Publ No (NIH) 78-1259, 1978, p 397-425.

3. Barris MC, Frumkes TE: Rod-cone interaction in human scotopic vision-IV. Cones stimulated by contrast flashes influence rod threshold. Vision Res 18:801-808, 1978.

4. Frumkes TE: Annual Progress Report, EY 01802-03, April 1979.

5. Boynton RM: Annual Progress Report, EY 01541-06, May 1979.

6. Piantanida TP: Comprehensive Progress Report, EY 02357-03, May 1978.

7. Ingling CR, Russell PW, Rea MS, Tsou B: Red-green opponent spectral sensitivity: Disparity between cancellation and direct matching methods. Science 201:1221-1223, 1978.

8. Riggs LA: Annual Progress Report, EY 00744-08, October 1978.

9. Granda AM: Comprehensive Progress Report, EY 01540-10, March 1978.

10. Jacobs GH: Spectral mechanisms in the ground squirrel retina. Fed Proc 38:399, 1979.

11. Sperling HG, Crawford MLV, Espinoza S: Threshold spectral sensitivity of single neurons in the lateral geniculate nucleus and of performing monkeys. Modern Problems in Ophthalmology 19:2-18, 1978.

12. Crawford MLV, Espinoza S: Photic sensitivity of macaque monkey and pulvinar neurons, in Cool SV, Smith EL (eds): Frontiers in Visual Science. Springer Series in Optical Sciences. New York, Springer-Verlag; 1978, pp 503-517.

13. Crawford ML: Annual Progress Report, EY 00381-10, May 1979.

14. Morrocco RT: Comprehensive Progress Report, EY 01286-04, January 1978.

15. Michael CR: Color vision in monkey striate cortex. J Neurophysiol 41:572-588, 1978.

16. Baizer JS, Robinson DL, Dow BM: Visual responses of area 18 neurons in awake, behaving monkey. J Neurophysiol 40:1024-1037, 1977.

17. Dow BM, Baizer JS, Robinson DL: Functional orientation of area 18 in the rhesus monkey. Exp Brain Res 1:412-414, 1976.

18. De Valois RL, Snodderly DM, Vund EW, Helper NK: Responses of macaque lateral geniculate cells to luminance and color figures. Sens Processes 1:244-259, 1977.

19. De Valois RL: Annual Progress Report, EY 00014-11, July 1978.

20. De Valois KK: Spatial frequency adaptation can enhance contrast sensitivity. Vision Res 17:1057-1065, 1977.

21. Boynton RM: Color in contour and object perception. Handbook of Perception Vol. VIII, New York, Academic Press, 1978, p 196.

22. Sharma SC: Annual Progress Report, EY 01426-05, March 1979.

23. Goldberg S: Unidirectional, bidirectional and random growth of embryonic optic axons. Exp Eye Res 25:399-404, 1977.

24. Willard MB: Annual Progress Report, EY 02682-05, March 1979.

25. Goldberg S: Central nervous system regeneration and ophthalmology. Surv Ophthalmol 20:261-272, 1976.

26. Higgins KE: Monocular performance of functional amblyopes: Another look at the scotoma hypothesis. Am J Optom Physiol Opt 55:172-182, 1978.

27. Blake R, Cormack RH: Psychophysical evidence for a monocular visual cortex in stereoblind humans. Science 203:274-275, 1979.

28. Eggers HM, Blakemore C: Physiological basis of anisometropic amblyopia. Science 201:264-267, 1978.

29. Smith EL, Bennett MJ, Harwerth RS, Crawford MLV: Binocularity in kittens reared with optically induced squint. Science 204:875-877, 1977.

30. Blasdel GG, Pettigrew JD: Effect of prior visual experience on cortical recovery from the effects of unilateral eyelid suture in kittens. J Physiol 274:601-619, 1978.

31. Pettigrew JD: Annual Progress Report, EY 01909-03, November 1978.

32. Fifkova E: Comprehensive Progress Report, EY 01500-05, January 1978.

33. Shatz CV, Le Vay S: Siamese cat: Altered connections of visual cortex. Science 204:328-330, 1979.

34. Daniels JD: Annual Progress Report, EY 02244-02, October 1978.

35. Fox R: Annual Progress Report, EY 00931-06, August 1978.

36. Pettigrew JD: The paradox of the critical period for striate cortex, in Cotman CW (ed): Neural Plasticity. New York, Raven Press, 1978, pp 311-330.

37. Hubel DH, Wiesel TN: Functional architecture of area 17, in normal and monocularly deprived macaque monkeys. Cold Springs Harbor Symp Quant Biol 40:581-589, 1976.

38. Olson CR, Freeman RD: Monocular deprivation and recovery during the sensitive period in kittens. J Neurophysiol (in press).

39. Drager UC: Observations on monocular deprivation in mice. J Neurophysiol 41:28-42, 1978.

40. Daw N: How far does monocular deprivation affect directional sensitivity, and direction deprivation affect ocular dominance? Paper delivered at Symposium on Visual Deprivation Effects in Animals and their Analogs in Human Pathology, Center for Visual Science, U Rochester, NY, June 8-10, 1978.

41. Fantz RL: Pattern vision in young infants. Psychol Res 8:43-47, 1958.

42. Teller DY, Morse R, Borton R, Regal D: Visual acuity for vertical and diagonal gratings in human infants. Vision Res 14:1433-1439, 1974.

43. Dobson MV, Teller DY, Lee CP, Wade B: A behavioral method for efficient screening of visual acuity in young infants. I. Preliminary laboratory development. Invest Ophthalmol Vis Sci 17:1142-1150, 1978.

44. Fulton AB, Manning KA, Dobson MV: A behavioral method for efficient screening of visual acuity in young infants. II. Clinical application. Invest Ophthalmol Vis Sci 17:1151-1157, 1978.

45. Dobson MV: Annual Progress Report, EY 02581-02, May 1979.

46. Peoples D, Teller DY: White adapted photopic spectral sensitivity in human infants. Vision Res 18:49-53, 1978.

47. Held R, Gwiazda J, Brill S, Mohindra I, Wolfe J: Infant visual acuity is underestimated because near threshold gratings are not preferentially fixated. Vision Res (in press).

48. Thomas J, Mohindra S, Held R: Strabismic amblyopia in infants. Am J Optom Physiol Opt (in press).

49. Gwiazda J, Brill S, Mohindra I, Held R: Infant visual acuity and its meridional variation Vision Res 18:1557-1564, 1978.

50. Mohindra I, Hild R, Gwizda J, Brill S: Astigmatism in infants. Science 202: 329-331, 1978.

51. Howland HC, Atkinoor J, Braddick O, French J: Infant astigmatism measured by photorefraction. Science 202:331-333, 1978.

52. Sokol S, Dobson MV: Pattern reversal visually evoked potentials in infants. Invest Ophthalmol Vis Sci 15:58-62, 1976.

53. Sokol S: Measurement of infant visual acuity from pattern reversal visual evoked potentials. Vision Res 18:33-40, 1978.

54. Vision Research--A National Plan: 1978-1982, Volume Two, Panel Reports. US DHEW Publ No (NIH) 78-1259, 1978, p 415.

55. Vision Research--A National Plan: 1978-1982, Volume Two, Panel Reports. US DHEW Publ No (NIH) 78-1258, 1978, p 107.

56. Research grant applications relative to the management of severe visual impairment, NIH Guide for Grants and Contracts, Volume 7, No 15, October 16, 1978.

57. Decker M, Polse KA, Fatt I: Oxygen flux into a human cornea covered by a soft contact lens. Am J Optom Physiol Opt 55:289-293, 1978.

58. Polse KA, Decker M: Oxygen tension under a contact lens. Invest Ophthalmol Vis Sci (in press).

59. Polse KA: Etiology of corneal sensitivity changes accompanying contact lens wear. Invest Ophthalmol Vis Sci (in press).

OFFICE OF BIOMETRY AND EPIDEMIOLOGY

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1978 - September 30, 1979

REPORT OF THE CHIEF, OFFICE OF BIOMETRY AND EPIDEMIOLOGY

Fred Ederer

The Office of Biometry and Epidemiology has three main functions: research, education, and consultation.

Research is the dominant function. It is the Office's mission to plan, develop, and carry out studies on human populations concerned with the causation, prevention, and treatment of eye disease and vision disorders, with emphasis on the major causes of blindness. This includes studies of incidence and prevalence in defined populations, prospective and retrospective studies of risk factors, natural history studies, clinical trials, genetic studies, and studies to evaluate diagnostic procedures.

Education: The Office carries out a program of education in biometric and epidemiologic principles and methods for the vision research community. This consists of courses, workshops, a pre- and post-residency fellowship program for ophthalmologists, publications, and consultation and collaboration on research.

Consultation: The Office provides biometric and epidemiologic assistance to National Eye Institute intramural and extramural staff and to vision research workers elsewhere. The assistance ranges from referral to appropriate consultants to collaboration as coinvestigator.

Research

Clinical Trials. Three clinical trials on the treatment of diabetic retinopathy are in progress. These are the Diabetic Retinopathy Study (DRS), the Early Treatment Diabetic Retinopathy Study (ETDRS), and the Diabetic Retinopathy Vitrectomy Study (DRVS).

The oldest of these is the DRS, which demonstrated the effectiveness of photocoagulation in reducing the incidence of diabetic blindness. More recently the DRS research group reported on four retinopathy factors that increase the two-year risk of developing severe visual loss: (1) the presence of vitreous or preretinal hemorrhage, (2) the presence of new vessels, (3) the location of new vessels on or near the optic disc, and (4) the severity of new vessels. The two-year risk of developing severe visual loss was shown to increase as the number of these risk factors increases. Eyes with three or more risk factors (eyes with "high-risk characteristics") are at a much higher risk than eyes with two or fewer factors.

The ETDRS has been designed to provide a better understanding of the optimum time to use photocoagulation in the course of diabetic retinopathy. Considerable staff time has been given to designing this trial, for which patient recruitment is scheduled to start in the fall of 1979. Patients with

macular edema, and preproliferative and proliferative retinopathy will be included. Three forms of photocoagulation treatment will be assessed, ranging from a restricted focal treatment to a full scatter. In addition, half the patients will be randomized to a daily administration of aspirin to test the effect of this drug on the incidence of microvascular complications. Of additional interest is whether aspirin reduces macrovascular complications in the diabetic patient. The study will also provide the opportunity to investigate the factors that are associated with the progression of disease.

DRVS has succeeded in recruiting a sizable number of patients whose vision has been severely reduced by hemorrhage into the vitreous. Half of these patients have been randomized to early vitrectomy, and half to vitrectomy one year later, if still indicated. Review of records of patients in the natural history portion of the study permitted identification of a group with especially poor prognosis for retaining their useful vision beyond a year. A national survey of retinal surgeons suggested, moreover, that vitrectomy was being used more commonly in patients who still had useful vision but were at serious risk of complications which might lead to retinal detachment. The DRVS protocol was broadened this year to include such patients who are being randomized to either vitrectomy or conventional treatment (which may include subsequent vitrectomy, if indicated).

Epidemiology. The first year of a contract to evaluate the feasibility of using existing clinical records to estimate the long-term complications following intraocular lens insertion in cataract surgery has been completed. Results from that study pointed to serious shortcomings in the use of clinical records for epidemiologic research. Patients were commonly not followed beyond the first year, records of preoperative visual acuity were occasionally not available, "aborted" cases (where lenses were not inserted) were not identifiable. With the additional disadvantages inherent in such retrospective studies, staff in OBE felt that proceeding to a full analysis of these clinical records would not offer any more insight into the efficacy of intraocular lenses than is already available in the published literature. Our staff is pursuing other ways in which information possibly can be obtained on complication rates with intraocular lens insertions. We have developed a protocol for a randomized trial and are exploring its feasibility with consultants. In addition, we are developing studies that depend on other data systems, such as prepaid insurance plans.

Dr. Seigel and Dr. Ferris collaborated in investigating the pattern of mortality following treatment of uveal melanoma. They published a paper demonstrating that the peak in mortality shortly after diagnosis is also seen in patients with cancer at other sites. This research was motivated by a publication in the British Journal of Ophthalmology suggesting that enucleation was responsible for dissemination of cancer cells and premature death of the patient. In addition to showing that the mortality pattern was not unusual, the OBE Staff members presented additional arguments challenging the claim that surgery is deleterious in cancer of the eye. With growing interest in this subject, it is likely that this publication will generate considerable discussion.

Little is known about the frequency of eye disease and visual impairment in the United States, how this frequency varies according to various demographic and social factors or how it varies over time. Yet such information is fundamental to the formulation and testing of hypotheses in the epidemiologic research of vision disorders. Previous efforts to collect such information, including NEI's Framingham Eye Study, the National Center for Health Statistics (NCHS) Health and Nutrition Examination Survey, and NIH's Model Reporting Area for Blindness Statistics, have been limited in scope or assurance of quality. During the past year, a project team, consisting of OBE Staff and several consultants, has been preparing a plan for a pilot study for a continuing 2-stage national survey of visual impairment in the United States. In the first stage visual acuity examinations would be carried out in a sample of households as part of the NCHS Health Interview Survey, a continuing survey of a probability sample of 42,000 households (120,000 individuals) per year. In the second stage all those found to be visually impaired in the first stage plus a sample of those not found to be visually impaired would be referred for a detailed ophthalmological examination. The primary objective of the study is to determine the nationwide prevalence of visual impairment, by cause. A secondary objective is to conduct case-control studies to investigate etiologic hypotheses, using cases of visual impairment from a specific cause and, as controls, a random sample of survey participants not visually impaired from that cause.

The Framingham Eye Study, a prevalence survey of four major eye diseases among 2,477 individuals aged 52-85, was completed in December 1978, and the large study file of data and documentation is now available for use by researchers. An extensive statistical monograph containing detailed clinical and epidemiologic findings and details of study methods has been completed and a suitable publisher is being sought. It is hoped that the monograph will serve as a research resource and teaching tool for ophthalmic-epidemiologic researchers.

Several OBE staff members collaborated with staff of the Department of Ophthalmology, University of Wisconsin, in an investigation into the survivorship of people with diabetic retinopathy. The study confirmed a previously found association between severity of retinopathy and survival and was able to quantify the association more precisely than was possible previously.

Dr. Cristina Leske, State University of New York at Stony Brook, collaborating with OBE staff, has completed and submitted for publication a report estimating glaucoma incidence from age-specific prevalence. The same investigative team is now starting a study of the usefulness of Framingham Eye Study data in planning glaucoma screening surveys.

Dr. Roy Milton is collaborating with Mr. Harold Kahn, a consultant in epidemiology, in two studies using Framingham Eye Study data: revised estimates of the prevalence of glaucoma and diabetic retinopathy and alternate definitions of open-angle glaucoma and resultant revised prevalence estimates and associations with Framingham Heart Study variables. Dr. Milton is working with a team of consultants to develop a plan for a nationwide prevalence survey of nutritional eye disease in pre school children in El Salvador. This survey is also being designed to correlate nutritional and biochemical data with disease state in these children. Together with Dr. Arin Chatterjee, Christian Medical College,

Ludhiana, India, Dr. Milton is completing a cataract etiology study of three areas in the Punjab.

Little is known about the etiology of senile macular degeneration, a major cause of blindness in the United States, and in an attempt to test various etiologic hypotheses about the disease, a case-control study has been started by OBE staff collaborating with epidemiology and ophthalmology staff at Johns Hopkins University.

Mr. Marvin Podgor has been working with Dr. Robert Frank, Kresge Eye Institute, Wayne State University, in a study of retinal vascular changes in juvenile onset diabetes of short duration. Five retinal sub-specialists, who were masked as to the presence or absence of diabetes in the people studied, independently evaluated fundus photographs and fluorescein angiograms. Retinopathy was judged absent in all of the nondiabetics but present in 20% of the diabetics. This latter figure is lower than has been reported recently in unmasked studies. Additionally, as has been reported in other studies, the prevalence of retinopathy was found to increase with increasing duration of diabetes. A report on the results was presented before 1979 Annual meeting of the American Diabetes Association.

Education. Seven presentations on the Diabetic Retinopathy Study (DRS) were made by DRS investigators, including one each by Dr. Ferris and Mr. Ederer, at the 1979 annual meeting of the Association for Research in Vision and Ophthalmology. Ms. Hyman made a presentation on the senile macular degeneration case-control study at that meeting and at the annual meeting of the Society for Epidemiologic Research.

At the 1978 annual meeting of the American Academy of Ophthalmology, Dr. Kupfer, Mr. Ederer, Dr. Ferris, and Dr. Matthew D. Davis, Department of Ophthalmology, University of Wisconsin, presented a course on methods of clinical research, and five DRS investigators, including Dr. Ferris, presented a course illustrating the application of findings from the Diabetic Retinopathy Study to clinical practice.

Dr. Lutza Yanko, Jerusalem Institute for the Prevention of Blindness, completed a 13-month period with the office as Visiting Scientist. Dr. Robert Sperduto began a 2-year post ophthalmology fellowship in biometry and epidemiology, and Dr. Gary Cassel a pre-residency fellowship, with the office.

Articles were published on epidemiologic methods for ophthalmologists in Metabolic Ophthalmology and the American Journal of Ophthalmology. Additional methodological papers were published in Clinical Pharmacology and Therapeutics and The American Statistician.

Dr. Seigel collaborated with staff in the NIH Division of Computer Research and Technology in preparing a workshop on computer technology in clinical trials. He was also one of four panel members in an NIH Science Writers' Seminar on the subject of clinical trials, where he spoke on biostatistical issues. He presented a talk on clinical trials before the faculty and students of the Department of Biostatistics and Epidemiology at the University of Massachusetts at Amherst and he made a presentation on the functions of data and safety monitoring

committees in clinical trials before a clinical trials study group at the University of Pennsylvania.

Dr. Ferris gave talks on diabetic retinopathy at Howard University, the FAES, and the Southwest Michigan Area Health Education Center.

Consultation. Mr. Podgor provided statistical consultation to members of the Clinical Branch and the Laboratory of Vision Research, NEI.

The National Eye Institute is represented through Dr. Milton on the NIH Advisory Committee for Computer Usage and through Dr. Seigel on the NIH Clinical Trials Committee.

Dr. Seigel is a consultant to the Boston University Collaborative Drug Study and served on the HEW Arthritis Interagency Coordinating Committee.

Both Dr. Seigel and Dr. Sperduto served on planning committees for NEI consensus conferences on Intraocular Lenses and Uveal Melanoma.

Publications

Office of Biometry and Epidemiology

1. Ederer F: Epidemiological methods. What opportunities do they afford public health workers and clinical investigators? Metabolic Ophthalmology 2:91-93, 1978.

2. Ederer F: The Framingham Eye Study. Blindness, 1977-1978, Vol 13, Washington, American Association of Workers for the Blind, 1979, pp 77-85.

3. Ederer F, Ferris FL: Studying the role of an environmental factor in disease etiology. Letter to the Editor. Am J Ophthalmol 87:434-435, 1979.

4. The Diabetic Retinopathy Research Group: Four risk factors for severe visual loss in diabetic retinopathy. The third report from the Diabetic Retinopathy Study. Arch Ophthalmol 97:654-655, 1979.

5. Ravnihar B, Seigel DG, Lindtner J: An epidemiologic study of breast cancer and benign breast neoplasias in relation to the oral contraceptive and estrogen use. Eur J Cancer 15:395-405, 1979.

6. Ferris FL, Ederer F: External monitoring in multiclinic trials: Applications from ophthalmologic studies. Clin Pharmacol Ther 25:720-723, 1979.

7. Seigel D, Myers M, Ferris F, Steinhorn SC: Survival rates after enucleation of eyes with malignant melanoma. Am J Ophthalmol 87:761-765, 1979.

8. Milton RC: Statistical computing in the United States. Environ Health Perspect (in press).

9. Davis MD, Hiller R, Magli YL, Podgor MJ, Ederer F, Harris WA, Long JW, Haug GA: Prognosis for life in patients with diabetes: Relation to severity of retinopathy. Trans Am Ophthalmol Soc (in press).

10. Seigel DG, Stanley F: Statistics on perinatal morbidity and mortality, in Quilligan EJ (ed): Basic Concepts in Perinatal Medicine (in press).

11. Ederer F: The statistician's role in developing a protocol for a clinical trial. The American Statistician (in press).

CONTRACT NARRATIVE

Fifteen Clinical Centers plus a Coordinating Center at the University of Maryland School of Medicine, Baltimore, Maryland, and a Fundus Photograph Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin

Title: Diabetic Retinopathy Study (DRS)

Principal Investigator: Matthew D. Davis, M.D., (Study Chairman)

Current Fund Allocation: $902,726 for the period June 30, 1979, through June 29, 1980.

Objectives: The Diabetic Retinopathy Study (DRS) is a multicenter clinical trial to evaluate the efficacy of photocoagulation, (argon laser and xenon arc) in the treatment of proliferative diabetic retinopathy. This randomized, controlled study involves over 1,700 patients enrolled at 15 medical centers.

Major Findings: Photocoagulation with either argon laser or xenon arc, as used in the study, is effective in reducing the risk of severe visual loss and in inhibiting the progression of retinopathy. These effects were apparent in all stages of diabetic retinopathy studied: proliferative, severe nonproliferative, and background. Also found were some deleterious effects of treatment, namely, small losses of visual acuity and constriction of the peripheral visual field.

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy, uncommon only a few decades ago, is now a leading cause of blindness and visual disability in the United States. There is a critical need to find and evaluate scientifically treatments which will reduce the risk of blindness or visual impairment from the ocular complications of diabetes. Although photocoagulation is widely used as a treatment, adequate evidence of its efficacy is not based on carefully documented research findings.

Proposed Course: Follow-up of all surviving DRS patients terminated in May 31, 1979. The remaining two years will be devoted to data editing, processing, analysis, and report writing. Data analyses for a series of papers on the following topics are in preparation: detailed description of study methods and baseline characteristics of patients and eyes, detailed description of treatment effects, macular edema, and assessment of risk factors of severe visual loss and death.

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

Publications:

The Diabetic Retinopathy Research Group: Four risk factors for severe visual loss in diabetic retinopathy. The third report from the Diabetic Retinopathy Study. Arch Ophthalmol 97:654-655, 1979.

CONTRACT NARRATIVE

Thirteen Clinical Centers, plus a Coordinating Center at the University of Maryland School of Medicine, Baltimore, Maryland, a Fundus Photograph Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin

Title: Diabetic Retinopathy Vitrectomy Study (DRVS)

Principal Investigator: Matthew D. Davis, M.D. (Study Chairman)

Current Fund Allocation: $1,313,615 for the period June 26, 1979, through June 25, 1980.

Objectives: The DRVS is a multicenter clinical trial to:

a. Evaluate vitrectomy performed in the first six months after severe vitreous hemorrhage secondary to diabetic retinopathy as compared to the more usual practice of waiting twelve months after vitreous hemorrhage to remove the vitreous.

b. Evaluate vitrectomy in eyes with good vision but with severe proliferative retinopathy and poor prognosis before vision is lost through hemorrhage or retinal detachment.

c. Study the natural history of progression of retinopathy.

Major Findings: As of June 1979, a total of 294 eyes with severe hemorrhage had been randomized to early or deferred vitrectomy. A total of 742 eyes had been enrolled in the natural history study.

Recruiting for the natural history study has been terminated, but follow-up is continuing. Following review of the natural history data at hand, and of best estimates of complication rates accompanying vitrectomy, and in light of the expanding practice of vitrectomy among retired surgeons, eligibility criteria were enlarged to include patients with good vision but with severe proliferative disease, before vision is lost through hemorrhage or retinal detachment. Recruiting of such patients began in the summer of 1979.

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 (macular degeneration, glaucoma, cataract) in that it affects a younger population. Vitrectomy has the theoretical potential of removing the "scaffolding" on which abnormal new vessels can develop, fibrous tissue can form, and retinal detachment can occur. It is important to determine when such intervention is most likely to deter this process, and reduce the incidence of loss of vision. This presents an ideal opportunity for the National Eye Institute to mobilize scientific talents to answer a significant medical question.

Proposed Course: All 13 clinics are actively recruiting and enrolling patients in the study. The Coordinating Center is processing all the data

forms, providing interim results for review by the Data Monitoring Committee, and monitoring the recruitment efforts by each of the clinics. The Reading Center continues to grade the baseline fundus photographs as well as those following treatment.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities, Vitreous Humor.

Publications: None

CONTRACT NARRATIVE

Nineteen Clinical Centers, plus a Coordinating Center at the University of Maryland School of Medicine, Baltimore, Maryland; a Fundus Photograph Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin; and a Central Laboratory at the Center for Disease Control, Atlanta, Georgia

Title: Early Treatment Diabetic Retinopathy Study (ETDRS)

Principal Investigator: Dr. Lloyd Aiello (Chairman)

Current Fund Allocation: $2,188,960 for the period September 30, 1979, through September 29, 1980.

Objectives: The Early Treatment Diabetic Retinopathy Study (ETDRS) is a multicenter, randomized clinical trial, the main goals of which are:

a. To determine whether treatment of early stages of proliferative and nonproliferative diabetic retinopathy with or without macular edema by aspirin and/or prompt photocoagulation is effective in decreasing the rate of development of known retinopathy risk factors and/or the development of severe visual loss when compared to placebo or deferred photocoagulation.

b. To determine the optimum time to initiate photocoagulation treatment in diabetic retinopathy.

c. To monitor closely the effects of diabetes mellitus and/or of photocoagulation on visual function.

d. To develop natural history data that can be used to develop or confirm etiologic hypotheses or identify risk factors in diabetic retinopathy.

Major Findings: The Study's Manual of Operations was completed during the summer of 1979. Training sessions were held for coordinators and technicians to insure that laboratory procedures, visual function tests, and other basic elements of the design were fully understood. Solution of these management problems permitted recruiting of patients to be planned for November 1979. Whether this target date is met will depend on how promptly contracts can be negotiated with participating study units.

Significance to Biomedical Research and the Program of the Institute: The Institute regards fostering careful evaluation of new and widely used ophthalmic treatments as an essential element in its mission. This study represents an extension of the Institute's interest in developing the best possible program to care for the patient with diabetes.

Proposed Course: Follow-up of all ETDRS patients is planned for five to eight years, and monitoring of accumulating data will be performed at three-month intervals.

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

Publications: None

CONTRACT NARRATIVE

J. Robb Associates, Houston, Texas (NIH 1 EY 8-2100)

Title: Safety and Efficacy of Artificial Lens Implants

Principal Investigator: Jay Glasser, Ph.D.

Current Fund Allocation: None

Objectives: Intraocular lens implants are being inserted by ophthalmologists into eyes following cataract removal with increasing frequency. There is a need to evaluate the long-term safety of these lenses. As a first step towards such an evaluation, the contractor examined the cataract patient records of ten ophthalmologists who have implanted a large number of intraocular lenses. He tried to determine whether such records contain sufficient research quality information on relevant variables prior to, during, and after surgery, and whether long-term follow-up information on nearly all patients is available. This phase of the study was intended to determine the feasibility of research on intraocular lenses using physicians' records that were originally designed for patient care. If the feasibility of such research is demonstrated, then it was planned for the contract to enter into an analysis phase, when data concerning the efficacy and complications of intraocular lens implants would be accumulated, analyzed, and published.

Major Findings: The first year of the contract the feasibility study was completed. The Principal Investigator submitted a summary report of his results. This report was reviewed by a panel from outside the Institute. The dominant view was that there were very serious limitations (losses to follow-up, missing visual acuity notes, etc.) to the clinical data in patient care files which made them unsuitable for research on the long term consequences of intraocular lens implantations, so that little could be learned from them not already available in the published literature. In light of this review the project officer recommended that the second year of the study not be carried out. This recommendation was supported by the National Advisory Eye Council.

Significance to Biomedical Research and the Program of the Institute: This contract has been important in helping us to learn more about the feasibility of research using physician records designed primarily for patient care.

Proposed Course: The contract is completed.

NEI Research Program: Cataract--Senile or Degenerative Cataract

Publications: None

OFFICE OF PROGRAM PLANNING AND SCIENTIFIC REPORTING

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1978 - September 30, 1979

REPORT OF THE CHIEF, OFFICE OF PROGRAM PLANNING AND SCIENTIFIC REPORTING

Julian M. Morris

Program Planning

The primary activity of this Office in program planning during fiscal year 1979 was responding to numerous planning and evaluation requests from the central NIH administration, from other levels within the Department of Health, Education, and Welfare, and from the Congress of the United States. Besides coordinating and writing the NEI contributions to the NIH Research and Legislative Plan and the NIH Evaluation Plan, the Office assisted in the development of the National Conference on Health Research Principles held at NIH in October. The Office also drafted the NEI Director's Opening Statement before the House of Representatives and Senate Appropriations Committees and prepared testimony for the NEI Director before the Subcommittee on Health and Scientific Research of the Senate Committee on Labor and Human Resources. The Office wrote the NEI Director's testimony for Senator Kennedy's Health Resources Bill and met for preliminary briefings with members of the staff of the Subcommittee on Health and Scientific Research, of which Senator Kennedy is the chairman. The Office also prepared the NEI contribution to the Special Reports to Congress on Genetic Diseases and Diabetes and wrote the NEI contribution to a report related to the Clean Air Act.

During FY 1979, the Office developed initial plans for two important projects related to evaluation of the National Advisory Eye Council publication, Vision Research--A National Plan: 1978-1982. The first of these is a proposed evaluation of the scientific community's subjective response to the plan. The information gained from the study will help the NEI gauge the likelihood that the plan's goals and objectives will be carried out and help improve the Institute's evaluation and planning processes in general. The outcome of this study will be weighed carefully and given serious consideration in modifying current NEI plans and policies and in preparing future evaluation and planning documents.

The second evaluation project developed over the past year comprises initial aspects of a system for tracking the responses of vision researchers to the priority recommendations in the national plan. Fundamental to any assessment of the effectiveness of the vision research plan in directing NEI resources toward high priority areas is the development of a system which will compare incoming grant applications with the plan's recommended levels of support in each priority area. The purpose of this project is to build such a capability into the present NEI information system and to generate reports periodically on our progress in achieving our planning goals. The system will show not only what correspondence there is between grant applications and identified program priorities but how well such applications fare in the review and funding process and what discrepancies there may be between receipt of applications and funding in priority areas.

Plans for these evaluations of the national plan were included in the National Eye Institute's FY 1980 Evaluation Plan which included descriptions of these and six other evaluation projects proposed by this Office and the Extramural and Collaborative Programs, plus a policy overview and projections of evaluation staff resources and budgets for the coming year. The Evaluation Plan, which was coordinated by this Office, was presented to and approved by the Deputy Assistant Secretary for Evaluation and Technical Analysis, DHEW.

The Chief and staff of the Office also:

- edited the proceedings of the NEI-sponsored Workshop on Psychophysics and Physiological Optics;
- extensively discussed the NEI's evaluation and planning procedures with two teams of accountants from the General Accounting Office;
- participated in two contract Request for Proposal review boards, one for the National Cancer Institute and the other for the National Institute on Aging;
- made a presentation to a staff planning seminar conducted by the National Cancer Institute;
- supervised on a temporary basis a Grants Associate during his assignment at the NEI to assist in developing the Evaluation Plan;
- participated in and made presentations before the Grants Associate Seminar on Program Planning, the STEP Seminar, and the Program Planning Subcommittee of the National Advisory Eye Council.

In reference to requests from other National Institutes of Health offices, this Office wrote, compiled, or contributed to:

- a compendium of ongoing NIH research programs in primary prevention;
- a calendar for prevention activities in 1979;
- NEI comments on the Surgeon General's Report on Prevention;
- the FY 1980 Consultant Services Plan;
- Trans-HEW Research Initiatives;
- the NEI's contribution to the new booklet NIH's Extramural Programs;
- the 1981 PHS Program Plan;
- a summary of NEI Science Base Activities;
- the NIH Almanac; and
- PHS Strategy Highlights, FY 1980-82, in Relation to Environmental and Occupational Causes of Disease and Death.

Scientific Reporting

Public interest in and demand for accurate, up-to-date information on eye disorders and their treatment increased markedly this year, as reflected in the number of requests for printed material received by the Information Office, the volume of Congressional inquiries directed to the National Eye Institute, and the increase in requests for information and assistance from both print and broadcast media. A number of events in particular stimulated a sudden influx of inquiries. These included resurrection of media interest in a treatment for retinitis pigmentosa developed in the Soviet Union, reports of a new surgical procedure for correcting refractive errors, newspaper articles about marihuana as a possible treatment for glaucoma, and a television program about eye problems associated with aging.

The year was also marked by the addition to the staff in November 1978 of an Information Officer, Marsha Slavin Corbett. STRIDE trainee Karen Robinson also joined the staff as an information trainee in July 1979.

Scientific Communications

A major project in which the Information Office was asked to become involved was the NEI's consensus development conference on intraocular lens implantation. The NIH consensus development program brings together practicing physicians, biomedical research scientists, consumers, and others in an effort to reach general agreement on the safety and efficacy of a medical technology, whether drug, device, or procedure. In addition to helping to organize, prepare for, and run the conference, the Office also provided writing, editing, and secretarial support services during preparation of meeting and panel summaries. The Office also coordinated dissemination of information and summary reports to program participants and attendees, the medical and vision care community, the general public, and the press.

The Office continued its support of the NEI's clinical trials program by assisting in the preparation of written materials marking closeout of the Diabetic Retinopathy Study and start-up of the Early Treatment Diabetic Retinopathy Study. These included patient explanation brochures, informed consent forms, certificates of appreciation to study participants, and manuscripts being prepared for publication. To highlight further the importance of modern-day controlled clinical trials, the Office is preparing an exhibit on trials supported by NEI grants and contracts, for display at the American Academy of Ophthalmology meeting in San Francisco. The exhibit will feature 13 such studies now underway or in the planning stage.

A member of the Office also served on the Protocol Review Subpanel for clinical studies conducted in the NEI's own Clinical Branch.

As in previous years, the Information and Planning staffs worked together on the Institute Director's Opening Statement before the House and Senate Appropriations Committees and reviewed and edited the transcripts of these hearings.

Assistance was also provided to Dr. Kupfer in the preparation of speeches presented before the World Council for the Welfare of the Blind and at the dedications of the University of Minnesota's Harris Laboratories and the University of Chicago's Eye Research Laboratories.

The Office helped to plan for and participated in the Seventh Session of the U.S.-U.S.S.R. Joint Committee for Health Cooperation where plans were developed for implementing a new collaborative effort in vision research. The Office also handled publicity following the meeting and was involved in scheduling activities to welcome Soviet visitors to the United States and familiarize them with vision research conducted in this country.

Special arrangements, including a tour of NEI facilities, were also made for a visit by three leading ophthalmologists from the People's Republic of China, the first ophthalmologists from China to visit this country in over 20 years.

The Information Office responded to a number of requests for information on eye disease and vision research from other offices within HEW. In conjunction with the Planning staff, two Special Reports to Congress were prepared which discuss the contributions of vision research to progress in the treatment of diabetes and genetic diseases. Information was also provided to the Office of Medical Applications of Research, NIH, about research involving the use of marihuana. In an effort to develop good working relations between various Federal agencies with an interest in research into the possible medical uses of marihuana, the Office arranged for a meeting that included officials from the National Institute on Drug Abuse, the Food and Drug Administration, and the NEI.

Consumer Education

As part of NIH's observance of 1979 as the International Year of the Child, the NEI helped to plan and prepare an exhibit showing how research on childhood diseases contributes to a healthy adult population. One panel of the exhibit was devoted entirely to the prevention, treatment, and study of eye disorders affecting children. It featured a self-testing eye chart and was accompanied by brochures in Spanish and English with a home vision test for children. Two identical exhibits were built, and they have been shown at state and community fairs, museums, and Government buildings throughout the United States.

In cooperation with others at NIH, the Information Office arranged for Dr. Kupfer to appear on "Panorama," a local television show, to discuss eye disorders, eye care, and vision research. A program on eye care and treatment of eye disorders in the elderly, conceived of and arranged by the Information

Office, was broadcast several times to 261 public television stations across the country.

In response to public demand for information on marihuana as a possible treatment for glaucoma, surgical correction of refractive errors, and a Soviet treatment for retinitis pigmentosa, detailed fact sheets were prepared on these subjects and distributed.

Press Relations

The year 1979 was marked by increased interest in eye disorders and vision research on the part of the broadcast media. The Office assisted CBS News in filming a segment for the morning news show in which Dr. Kupfer and Dr. David Newsome appeared. In the segment on macular degeneration, research on this disorder being conducted at the NEI was discussed. The Office also provided background material and guidance to the producers of "The Body Human" for the program devoted to the eye and disorders of vision, which CBS broadcast nationwide in prime time. The Office assisted the producers of the "NOVA" science series in preparing for the filming of a segment on vision scheduled for public television and also contributed to planning and development of a WNET (New York) television series on the brain. A one-hour segment on vision is planned.

Other television and radio stations were assisted by the Information Office during the last year. Information was provided and/or interviews set up with stations in the following areas: California, Connecticut, Indiana, Massachusetts, Michigan, New York, Ohio, Washington, D.C., and Canada.

The Office continued to receive a large number of inquiries from the print media. Among the publications or news services receiving information or assistance during the year were: Associated Press, United Press International, Medill News, Wall Street Journal, National Enquirer, Newsweek, New York Magazine, Washington Post, New York Times, New York Daily News, Newsday, Milwaukee Sentinel, Detroit Free Press, Duluth News Tribune, Bergen Record, Waterbury Republican, U.S. News and World Report, Look, Good Housekeeping, Better Homes and Gardens, Popular Mechanics, Parent's Magazine, Redbook, Reader's Digest, Prevention, New West, Harvard Crimson, Science News, Science Trends, Journal of the American Medical Association, Medical World News, Worldbook Encyclopedia, and specialty publications.

In addition, the Office reviewed articles in advance of publication for Vogue, Current Health, Public Affairs Committee, Inc., and Women Doctors' Health Guide and Medical Encyclopedia. Assistance in obtaining or providing photographs was offered to Omni, Time, Encyclopedia Brittanica, Smithsonian, and Washington Hospital Center newsletter.

"Search for Health" columns on the NEI, cataract, retinal disorders, and diabetic retinopathy were distributed nationwide through an NIH feature service for weekly newspapers. Newly prepared press releases, announcements, and fact sheets were distributed to the press on the following subjects:

Early Treatment Diabetic Retinopathy Study, marihuana and glaucoma research, surgical correction of refractive errors, U.S.-U.S.S.R. cooperative agreement on vision research, appointment of new members to the National Advisory Eye Council, appointment of an Associate Director for the NEI, and appointment of an Information Officer. For the Journal of the American Medical Association's "From the NIH" section, two columns were prepared--one entitled "Study Identifies Four Factors in Diabetic Retinopathy Associated with High Risk of Severe Visual Loss," and one entitled "Update on Marihuana Research."

Public Inquiries

As noted above, the attention given alleged new treatments for eye disorders in the news media can generate large numbers of inquiries from the public. To expedite handling of these letters and telephone calls, the Information Office continued to develop fact sheets on subjects of current interest in order to explain exactly what is and is not known about various eye disorders and their treatment. These fact sheets can be used to provide accurate information in a very timely fashion, and they can be updated easily. As the selection of fact sheets and brochures on eye disorders increases, the number of public inquiries requiring a specially drafted letter decreases and so does the amount of time it takes to respond.

During the past year, approximately 650 letters from the public were received which required individual attention and response. In addition, 33 written Congressional inquiries and 13 additional letters controlled through the NIH Executive Secretariat were answered. At least 1,400 telephone calls were handled by our information specialists during the year. Of these calls, approximately 70 were from Congressional offices. An additional 1,000 calls were screened by the secretarial staff and either handled routinely by them or referred elsewhere.

Publications

The Office distributed the following number of publications during the year:

Consumer Information

Cataract	4,948
Corneal Diseases	2,656
Diabetic Retinopathy	2,897
Diabetic Retinopathy Study	3,060
Diabetic Retinopathy Vitrectomy Study	2,835
Early Treatment Diabetic Retinopathy Study	310
Glaucoma	3,156
Know Your Eyes	3,060
Macular Degeneration	2,847
Refractive Errors	2,719
Retinal Detachment	2,971
Retinitis Pigmentosa	2,939

Professional and Scientific

Statistics on Blindness in the Model Reporting Area, 1969-1970	30
Vision Research Program Planning	5
Support for Vision Research	15
Summary and Critique of Available Data on the Prevalence and Economic and Social Costs of Visual Disorders and Disabilities (Westat Report)	25
Vision Research--A National Plan: 1978-1982 (total sets of Volumes One, Two, and Three)	365
TOTAL	35,178

Special

The Office, as in past years, drafted the annual Presidential proclamations for Save Your Vision Week and White Cane Safety Day. Several other Presidential messages were also prepared at the request of the White House.

During the past year, the Office served in an advisory capacity to other NEI offices with regard to the public information and press relations aspects of major policy issues and decisions, the writing and editing of scientific manuscripts, the preparation of brochures and audiovisual materials, and the handling of Freedom of Information requests and Congressional inquiries received by the Institute.

The Information Office continued to serve as a liaison between the Institute and the vision care community, fostering effective working relationships with organizations such as the American Academy of Ophthalmology, American Association of Ophthalmology, Better Vision Institute, Eye Care, Inc., Friends of Eye Research, Rehabilitation, and Treatment, National Retinitis Pigmentosa Foundation, and Research to Prevent Blindness, Inc.

INTRAMURAL RESEARCH

Clinical Branch

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1978 - September 30, 1979

REPORT OF THE CLINICAL DIRECTOR

Elmer J. Ballintine, M.D.

Because of the encroachment of Ambulatory Care Research Facility construction, the NEI Clinical Branch was obliged this year to vacate its outpatient clinic and several laboratory modules.

The clinic and the supporting photographic and psychophysical testing facilities were moved to the Clinical Center Inpatient Division, 13 West, where they were accommodated by reducing the number of NEI patient beds to 10 and occupying conference rooms and patients' solaria.

Despite the loss of space, the clinical programs have continued to expand so that there are now 15 approved active research protocols. The clinical facilities for psychophysical and electrophysiologic examinations have been expanded and consolidated under the supervision of one senior scientist who has also implemented programs to develop new techniques and to standardize and determine the statistical characteristics of the ones used at present.

The Section on Retinal and Ocular Connective Tissue Diseases has established a laboratory and clinical program for studying the defects of collagen, basement membrane, and proteoglycan metabolism in ocular diseases.

In the past, investigators have attempted to describe dystrophic and other pathologic processes in the cornea by detection of the products of enzymatic digestion of isolated corneal layers. In these cases, it is usually impossible to decide how the molecular fragments were distributed among the macromolecules of the intact cornea.

The methods developed in the ocular connective tissue laboratory avoid these uncertainties by first incubating intact human corneal surgical specimens under conditions that permit them to incorporate radiolabeled precursors into structural macromolecules. The labeled macromolecules are then extracted by guanidinuim chloride solution and fractionated by molecular sieve chromatography. The isolated glycoconjugates are characterized by enzymatic degradation and isolation of the radiolabeled fragments.

Application of these methods to corneal buttons from patients with macular dystrophy showed that corneas with macular dystrophy synthesized chondroitin sulfate normally but their synthesis of keratan sulfate proteoglycan is deficient. The dystrophic corneas did synthesize an unusual glycoprotein not found in normal corneas.

Systematic study of diseased corneas, especially those having genetically determined dystrophies, should lead to identification of the underlying enzymatic defect.

A study of patients with gyrate atrophy of the retina has shown that their transformed lymphocytes have a deficiency of ornithine aminotransferase. The demonstration of this metabolic defect suggested that the condition might be treated by dietary restriction of arginine, the precursor of ornithine, and by supplementation of the diet with pyridoxine, a co-factor for the deficient enzyme.

One patient has had improvement in dark adaptation during 18 months of the arginine deficient diet. Supplementation with pyridoxine has produced lowered concentrations of ornithine in the serum in a small fraction of the subjects. These results are important and encouraging, for not only do they offer hope of successful treatment in this blinding disease, but the methods of study serve as a model for other more common retinal degenerations.

Techniques for using the Q-switched laser to produce iridotomies in monkeys have been developed and have been applied in the treatment of one patient. A clinical trial of their use in human disease requiring iridotomy is being planned. The refinement of the apparatus permits fine focusing of the laser beam on the treatment site and furnishes precise information defining the characteristics of each laser pulse as it is delivered to the ocular tissue. These developments prepare the way for a clinical trial of Q-switched laser trabeculectomy for the relief of simple glaucoma in man in the near future

Until recently, most investigations of uveitis were directed toward determining the cause, such as attempts to demonstrate a specific infectious agent, in the expectation that treatment could be directed toward removing or neutralizing it. Recent knowledge about the details of a variety of immune mechanisms has suggested that an initial inflammatory insult may produce a variety of immunologic consequences; that the manifestations of the uveitis depend more on the kind of immune process than on the instigating agent; and that methods for manipulating the immune processes might be more effective in mitigating the inflammatory lesions than an attack on the inflammatory agent.

Investigators in the Ocular Immunology Laboratory continue to demonstrate examples of these processes. They have shown that some patients with recurrent posterior uveitis caused by different primary agents have lymphocytes sensitized to a common retinal antigen. This suggests that immunity to a component of retina may be an important sustaining mechanism in chorioretinitis. As the details of these mechanisms are worked out, we expect to undertake clinical trials of therapeutic agents that modify specific components of the immune mechanism.

Investigators in the Ophthalmic Pathology Laboratory have established a system for allotting fresh human ocular tissues from autopsy eyes to investigators in the Clinical Branch and the Laboratory of Vision Research. One hundred and thirty human autopsy eyes were accessioned, seventy were processed for standard histopathology, and sixty fresh eyes were made avaliable to members of the Clinical Branch for research. These eyes were used for cell cultures of cornea, retinal pigment epithelium, and lens, and for immunologic studies. Forty surgical biopsy specimens were processed.

Seventy animal eyes and ocular tissues used for experimental projects by other investigators in the Clinical Branch were processed for standard histologic examinations. These included guinea pig eyes with experimental uveitis, vitamin A deficient rats, monkey trabecular meshwork, retinal pigment epithelial cell cultures tagged with immunoglobulins, and monkey laser iridectomies and trabeculectomies.

Transmission electron microscopy was performed on 40 specimens, and scanning electron microscopy on 30 additional specimens, for research conducted by members of the Clinical Branch.

There were 1,071 Clinical Branch outpatient visits and 100 inpatient admissions this year, and 20 surgical operations were performed. The clinical staff provided 679 inpatient and 768 outpatient consultations for other NIH Institutes at the Clinical Center.

The Clinical Branch continued to cooperate with other NIH Institutes in the pursuit of timely research opportunities.

In collaboration with investigators in the National Cancer Institute, a Clinical Branch investigator has continued to monitor patients under treatment for metastatic breast cancer for ocular metastases and ocular toxicity of anticancer drugs. These drugs have been remarkably free of deleterious ocular effects, but recently, five patients receiving high doses of tamoxifen had reduced visual acuity, subepithelial corneal opacities, macular edema, and refractile white deposits in the retina. Tamoxifen-treated patients are being studied to determine if lower doses will also eventually produce the lesions.

Recently, the Clinical Branch has furnished a pediatric ophthalmology representative to the Inter-institute Genetics Group.

Clinical Branch staff scientists continued to serve as consultants to the National Institute on Drug Abuse, Interagency Committee on New Therapies for Pain and Discomfort, the Food and Drug Administration's Subcommittee on Ophthalmic Drugs, and the International Vitamin A Consultative Group.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00150-06 CB

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Ocular Hypertension Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI
Other:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB	NEI
	Richard Weiblinger	B.S.	Biologist	CB	NEI

COOPERATING UNITS (if any)

Office of Biometry and Epidemiology, NEI

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with ocular hypertension are randomly assigned to treatment with topical pilocarpine in one or both eyes or to no treatment. The objectives of the study are: 1) to determine if treatment with pilocarpine to reduce intraocular pressure before visual field changes occur will reduce the number of ocular hypertensive subjects who eventually become glaucomatous, and 2) to determine if measurements of aqueous humor dynamics, the response to water loading of diurnal variation in intraocular pressure, serial stereophotographs of the optic disc, and measurements of visual fields help to predict which patients will eventually become glaucomatous.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 77 EI 38

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

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

Major Findings: There has been no indication that the course of ocular hypertension has been affected by treatment. One hundred fifty patients have been examined to determine eligibility, and sixty-five have been admitted to the study.

Significance to Biomedical Research and the Program of the Institute: Early, precise identification of patients who require treatment because they are in the early stages of the simple glaucoma remains an unsolved problem. The data being collected in this study will furnish a basis for establishing criteria for treatment more precisely than is now possible. There is at present no detailed knowledge of the progression of optic disc changes in ocular hypertension. The data being collected in this study, as well as the development of better instruments for the measurements in this study, will supply needed information in this field.

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

NEI Research Program: Glaucoma--Medical and Surgical Treatment of Glaucoma

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00099 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Search for Diabetic Retinopathy in Acromegaly

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI
Other:	Phillip Gorden	M.D.	Chief, Clinical and Cellular Biology Branch	DB	NIAMDD
	Scott Foxman	B.S.	Biologist	CB	NEI

COOPERATING UNITS (if any)

Clinical and Cellular Biology Branch, NIAMDD

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with acromegaly enrolled in NIAMDD Protocol No. 69 A 154 are examined ophthalmologically with special attention to visual field measurement to reveal cheosmatic defects, ophthalmoscopic examination to detect elements of diabetic retinopathy, and fluorescein angiography. Results have been collated with results of growth hormone assay, fasting blood sugar, and glucose tolerance testing. The results do not support the hypotheses that high serum concentrations of growth hormone predispose to diabetic retinopathy.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 69 A 154

Objectives: Several investigators have speculated that diabetic retinopathy may be related to an excess of growth hormone. In the past, attempts to find diabetic retinopathy in acromegalics has usually failed. One reason may have been that the retinal examination methods were not sensitive enough to detect early changes. Retinal fluorescein angiography has been shown to detect early retinopathy in some eyes where it had been missed by other methods of examination. The large group of acromegalic patients under observation in NIAMDD Protocol No. 69 A 154 and the retinal fluorescein angiographic facilities of the Clinical Branch made it possible to seek early retinal changes in patients with acromegaly.

Methods Employed: Standard clinical examinations.

Major Findings: Of 52 acromegalic patients, 44 each had satisfactory fluorescein angiograms, 4 or more ophthalmoscopic examinations, and at least one set of fundus photographs. Typical, early diabetic retinopathy was found in only one patient, and he had long standing diabetes. No elements of diabetic retinopathy were found among 29 patients with serum growth hormone elevated an average 5 times the normal range for an average of 10.5 years. These findings do not support the hypothesis that elevated growth hormone will cause the retinal vascular changes seen in diabetes.

Significance to Biomedical Research and the Program of the Institute: The prevention of diabetic retinopathy is a major objective of the NEI. The growth hormone hypothesis, if sustained, would suggest several therapeutic possibilities that might lead to clinical trials of pharmacologic agents which block growth hormone release or interfer with its action on target organs. This study, suggests that such attacks on diabetic retinopathy are not likely to be successful.

Proposed Course: Approximately five acromegalic patients remain to be examined. The results of the study are being prepared for publication after which the study will end.

NEI Research Program: Retinal and Choridal Diseases--Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00017-05 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Tissue Culture of Trabecular Meshwork

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI
Other:	Richard Weiblinger	B.S.	Biologist	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Slices of trabecular meshwork from normal monkey eyes and from surgical trabeculectomy specimens from human glaucomatous eyes are being grown in tissue culture. Attempts are being made to identify the tissue of origin of the resulting cellular growth and to grow trabecular endothelial cells selectively.

Differences between trabecular tissues from glaucomatous and normal human eyes with respect to details of growth and metabolic activity are being sought.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Much evidence indicates that in simple open-angle glaucoma, the obstruction to aqueous humor outflow lies within the trabecular meshwork and the inner wall of Schlemm's canal. The amounts of human trabecular tissue available for biochemical and physiologic study are insufficient for most in vitro research methods. Therefore, tissue culture techniques are being employed in the hope of developing a system in which the basic physiologic and biochemical abnormality present in open-angle glaucoma can be explored.

Methods Employed: Specimens of trabecular tissue are obtained from monkey eyes for preliminary studies. Some surgical specimens from patients undergoing trabeculectomy have been studied. More of these surgical specimens as well as controls from human autopsy eyes are being sought.

Specimens of trabecular meshwork are sectioned into small fragments under the dissecting microscope and placed in tissue culture medium. Phase contrast microscopy is used to observe growth and form of these cells. They are being further characterized by their histologic and histochemical properties. Methods and criteria for growing trabecular epithelial cells free of fibroblasts are being developed.

Major Findings: Trabecular meshwork from monkey and human eyes has been grown consistently in tissue culture, and the conditions for this growth have been determined. It has been possible to obtain some cultures without a significant fibroblastic contamination.

Significance to Biomedical Research and the Program of the Institute: The mechanism by which the resistance to aqueous humor outflow increases in open-angle glaucoma is at present unknown. This project may be able to define the physiologic and biochemical abnormalities of trabecular epithelium that are the fundamental causes of open-angle glaucoma.

Proposed Course: Metabolic studies of the cultured cells will attempt to demonstrate differences in how they synthesize collagen and mucopolysaccharide between normal cultures and those from human eyes that have a pressure elevation following administration of topical corticosteroids.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Primary Open-Angle Glaucoma)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00022-05 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Urokinase Central Retinal Vein Occlusion Trial

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI
Other:	Harvey R. Gralnick	M.D.	Chief, Hematology Service	CC	NIH
	Richard Weiblinger	B.S.	Biologist	CB	NEI
	Frederick Ferris	M.D.	Medical Officer	CB	NEI

COOPERATING UNITS (if any)

Office of Biometry and Epidemiology, NEI

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with recent complete occlusion of the central retinal vein are randomly assigned to treatment either with intravenous urokinase followed by heparin, heparin alone or intravenous fluids alone. The patients are then examined periodically for one year, and the effectiveness of treatment is judged by restoration of vision and the degree of protection achieved against the development of hemorrhagic glaucoma.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 75 E1 100

Objectives: To determine if treatment with thrombolytic agent (urokinase) plus anticoagulation with heparin, or treatment by anticoagulation with heparin alone, is effective in reducing the loss of visual acuity and the progression to hemorrhagic glaucoma that is a consequence of occlusion of the central retinal vein.

Methods Employed: Patients are examined according to a detailed plan to determine eligibility for the study. Eligible patients, if they agree to participate, are assigned by randomization to one of three treatment plans:

1) Twenty-four hours of continuous intravenous treatment with urokinase in an effort to resolve the occlusion of the central retinal vein. This is followed by two weeks of anticoagulation treatment with heparin to prevent reformation of venous obstruction.

2) Heparin anticoagulation alone.

3) Hospitalization and administration of intravenous fluids similar in volume to those used in the other treatment groups.

After the treatment period, the patients are examined periodically for one year to determine the rate at which hemorrhagic glaucoma occurs and the degree of restoration of vision to the eye.

Major Findings: Twenty patients have been examined to determine their eligibility and seven patients have been randomized to treatment. No trends have been observed.

Significance to Biomedical Research and the Program of the Institute: Occlusion of the central vein is a serious cause of visual disability, and one of its major consequences is hemorrhagic glaucoma, which almost invariably results in a blind, painful eye. In the past, treatment with anticoagulation has been advocated, but no convincing evidence of effectiveness has been published. With the development of an effective thrombolytic agent (urokinase), the possibility of dissolving the presumed cause of the obstruction, a thrombus in the central retinal vein, and the demonstration that urokinase is effective in thrombolytic disease in other sites support the decision to undertake this trial.

Proposed Course: Examination of published data on the course of occlusion of central retinal vein indicates that 75 patients will need to be recruited to demonstrate that a 50% improvement in vision is produced by the treatment. We will continue to recruit until 75 patients have been treated.

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

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00100-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Corpora Amylacea of the Optic Nerve and Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jose Avendano	M.D.	Visiting Scientist	CB	NEI
Other:	Merlyn M. Rodrigues	M.D.	Medical Officer	CB	NEI
	Joseph Hackett	B.S.	Biologist	CB	NEI
	Reginald Gaskins		Histologist	CB	NEI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Branch

SECTION
Section of Clinical Eye Pathology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

One hundred autopsy eyes, stained with hematoxylin eosin and periodic acid-Schiff were studied by light microscopy. In selected cases other special histochemical stains and enzyme digestion procedures were used. Twelve optic nerve specimens were studied by electron microscopy and stained with uranyl acetate, lead citrate and the modified Thiery method (PAS-thiosemicarbazide). Corpora amylacea were more common in eyes from older individuals and occurred in 93% of the cases, either in the optic nerve or retina. They measured 2 x 2 µm in diameter and stained positive for sulphated polysaccharide. By electron microscopy, the deposits were best demonstrated with the modified Thiery stain. Corpora amylacea were intracellular or extracellular structures with randomly oriented, elongated straight, branching 6µm thick filaments and a granular electron-dense background. The demonstration of coated vesicles and nematosomes in corpora amylacea are indicative of their neural origin.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The purpose of this investigation is to determine the nature of corpora amylacea (CA) in the optic nerve and retina using histochemical stains and electron microscopy and to report the incidence and localization of corpora amylacea in autopsy eyes.

Methods Employed: One hundred autopsy eyes were stained with hematoxylin eosin and periodic acid-Schiff (PAS) and studied by light microscopy. In selected cases the following stains were used: PAS before and after diastase digestion, alcian blue at pH 0.5, 1.5, and 2.5 before and after digestion with hyaluronidase, colloidal iron, and toluidine blue. PAS stains were also performed on frozen sections before and after digestion with chondroitinase ABC and mixed glycosidases. Other stains included Congo red, crystal violet, Von Kossa, Danielli tetrazonium, dimethylamino benzaldehyde dihydroxydinaphthyl disulfite with and without thioglycolate, chloromine T Schiff, and oil red O.

For electron microscopy, four optic nerves were excised from paraffin blocks, eight from autopsy eyes, and one from a surgically enucleated eye. Thin sections were stained with the modified Thiery (PAS-thiosemicarbazide) method, as well as with uranyl acetate and lead citrate. In order to assess the specificity of the modified Thiery stain, control specimens were prepared omitting the following steps: (1) the periodate; (2) the silver proteinate; and (3) the thiosemicarbazide. The osmium was removed with hydrogen peroxide. Unstained sections were also examined and photographed in order to determine the specificity of the modified Thiery stain. X-ray energy dispersive analysis was performed using the EDAX detector on unstained frozen sections.

Major Findings: Corpora amylacea were more common in eyes from older individuals. The CA were observed in 93% of the cases, either in the optic nerve or retina and measured 2-20 μm in diameter. They were most commonly seen in the inner plexiform layer of the posterior and equatorial retina as well as in the optic nerve head. Corpora amylacea are probably sulphated polysaccharide inclusions, since they stain positive with the following methods: PAS before and after diastase digestion (+++), colloidal iron (++), and alcian blue at pH 0.5, 1.5 and 2.5 before and after hyaluronidase digestion (+). The deposits showed metachromasia with toluidine blue. With electron microscopy, the CA were best demonstrated by the modified Thiery method and to a lesser extent by uranyl acetate and lead citrate stains. Corpora amylacea were usually present as intracellular structures with randomly oriented, elongated, straight, branching, 6 μm thick filaments and scattered electron dense areas. Peripheral mitochondria were present. Occasional larger corpora amylacea were extracellular. The CA probably arise as smaller bodies intimately intermixed with neurofilaments and enlarge to form larger bodies. In three different cases of corpora amylacea, irregular "coated" vesicular bodies and nematosomes were present centrally and peripherally. It is possible that these could represent earlier stages in

the development of corpora amylacea and suggest a neuronal rather than a glial origin of CA. The cells at the periphery of the CA failed to stain for glycogen, (unlike glial cells), appeared neuronal, and included some myelinated axons.

In ten cases of ocular corpora amylacea, the brain showed similar deposits, mainly localized to the subpial and subependymal regions and frequently displaying a perivascular distribution.

Significance to Biomedical Research and the Program of the Institute: This study of one of the structures associated with aging changes in the central nervous system provided insight into the nature and origin of corpora amylacea.

The latter apparently result from degeneration of neurons in the optic nerve and retina.

Proposed Course: No further investigation is planned.

NEI Research Program: Retinal and Choroidal Diseases

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00088-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

A Computerized Ophthalmic Citation System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Fred C. Chu	M.D.	Senior Staff Fellow	CB NEI
Other:	David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB NEI
	Douglas B. Reingold	M.A.	Biologist	CB NEI
	Deborah H. Young		Television Production Specialist	CB NEI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Branch

SECTION
Neuro-Ophthalmology Section

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have developed a set of computer programs to cross-index an in-office ophthalmic reference file. Citations can be retrieved within seconds.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Organizing previously collected ophthalmic citations by subject or any other criterion is tedious. Yet if structured well, such a system in many respects becomes an efficient, in-office diagnostic tool for relating current ophthalmic information to the variety of unusual patients examined in a clinical research environment. Such a system allows virtually instantaneous access to references already available in-office, minimizing the need for repetitious visits to copy references elsewhere.

Methods Employed: Reference citations are sequentially numbered and assigned various subject codes prior to entry into a computer. These codes and all authors' names are used as keys to sort citations by subject and author. The resulting cross-reference lists are then placed on microfiche for use.

The lists are completely updated by generating new microfiche whenever approximately 500 new references are added to the file. Between such updates, the last few hundred references can be cross-indexed and printed as a minor appendix.

The following computer programs are written in PL/I:

a. REFMAKE: tests input data and creates a SORT input file.
b. REFSORT: sorting routine
c. REFREAD: uses SORT output file and generates the cross-indexed citation lists on microfiche.

Major Findings: As used, the computerized retrieval system is a time-saving tool in the recovery of references collected in the office.

Significance to Biomedical Research and the Program of the Institute: This project gives bibliographic support to the other research programs in the Neuro-Ophthalmology Section.

Proposed Course: This program will be continued.

NEI Research Program: Sensory and Motor Disorders of Vision--Strabismus and other Oculomotor Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00086-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Contributions to Ophthalmic Pathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI
Other:	Toichiro Kuwabara	M.D.	Chief, Pathology Section	LVR	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION
Neuro-Ophthalmology Section

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The pathology resource in the Cogan collection has been used to document the recent history of ophthalmic pathology in America and to study radiation effects on the eye.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To utilize to the fullest extent the collection of pathologic material in the files of David G. Cogan, M.D.

Methods Employed: Retrospective reviews of histologic slides collected during the last 35 years.

Major Findings: A review of the developments in ophthalmic pathology and the current status of this discipline in America was prepared for the hemicentennial celebration of the Association for Research in Vision and Ophthalmology. Pathology of the eye has become a recognized subspeciality practiced in some cases, by full-time career investigators.

A collaborative study during the past year has demonstrated a special radiosensitivity of the human meibomian glands. Destruction of these and their replacement by scar tissue leads to chronic irritations of the eyes following sufficient radiation exposure.

As part of another study, an ophthalmic specimen with the chemical appearance of an optic nerve tumor was found to have a congenitally large scleral canal with secondary buckling of the overlying retina.

Significance to Biomedical Research and the Program of the Institute: Interpreting ophthalmic pathology helps us to understand the morphogenesis of the eye relative to external influences.

Proposed Course: This project is not being agressively pursued.

NEI Research Program: Corneal Diseases--Tumors and Other Lid, Conjunctival, and Orbital Problems

Publications:

Cogan DG: Ophthalmic pathology in America 1928-1978. Invest Ophthalmol Vis Sci, supplement, 75-79, April 1978.

Karp LA, Streeten B, Cogan DG: Radiation induced atrophy of the meibomian glands. Arch Ophthalmol 97:303-305, 1979.

Cogan DG: Coloboma of optic nerve with overlay of peripapillary retina. Br J Ophthalmol 62:349-350, 1978.

Cogan DG: Aging and the eye. Introduction and general comments, in Hands SS (ed): Biology of Special Senses in Aging. Michigan, Institute of Gerontology of University of Michigan (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00091-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Disorders of Vision with Cerebral Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Cogan	M.D.	Chief Neuro-Ophthalmology Section	CB	NEI
Other:	Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI
	Douglas B. Reingold	M.A..	Biologist	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION
Neuro-Ophthalmology Section

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Topographic localization of lesions in the cerebral cortices in 19 patients and their correlation to aspects of visual dysfunction have re-emphasized the importance of the nondominant parieto-occipito-temporal juncture in spatial judgment.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: General Consultations

Objectives: To investigate the utility of evaluating disorders of vision for the purpose of localizing brain lesions; to determine in man the visual effects of lesions in certain nonvisual cortical areas.

Methods Employed: Patients with visual disturbances resulting from cerebral lesions are systematically evaluated to determine the nature of their visual signs and symptoms. Key portions of the examination are videotaped for subsequent review. Patients are evaluated neurologically by the staff of the National Institute of Neurological and Communicative Disorders and Stroke to determine the nature and extent of lesions, for correlation with evaluation of higher visual function.

Major Findings: Disorders of vision may be significant signs for the topographic identification of brain lesions. An analysis of 19 cases of brain lesions which appear to involve the parieto-occipito-temporal junction of the nondominant hemisphere has confirmed previous clinical experience and added to the significance of this area for visual localization in space.

Significance to Biomedical Research and the Program of the Institute: Clinical neuro-ophthalmology provides the pragmatic basis for evaluating parallels between neuronal physiology of the visual system and the dimensions of visual experience in man.

Proposed Course: The future program will consist of adding more patients (unsolicited) to the present series as a back-log for subsequent evaluation.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders

Publications:

Cogan DG: Spatial dysgnosia. Am J Ophthalmol (in press).

Chu FC, Reingold DB, Cogan DG: Video: Tool of clinical investigation, in Confland D (ed): Third International Congress of Scientific Audio-Visual Techniques: Medicine and Video. Paris, Centre National de la Recherche Scientifique (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00089-01 CB

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Eye and Metabolic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI
Other:	Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI
	Toichiro Kuwabara	M.D.	Chief, Physiology Section	LVR	NEI
	W. Gerald Robinson	Ph.D.	Geneticist, Cell Biologist	LVR	NEI
	John Barranger	M.D.	Acting Chief, Clinical Investigation Service	DMN	NINCDS

COOPERATING UNITS (if any)

Development and Metabolic Neurology Branch, NINCDS

LAB/BRANCH

Clinical Branch

SECTION

Neuro-Ophthalmology Section

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dysfunctions of vision and eye movements occur in a number of the inborn errors of metabolism. In many instances these defects can be ascribed to intracellular accumulation of toxic metabolic substances. For a number of these conditions, we have identified abnormal storage materials in conjunctival specimens.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: General Consultations

Objectives: To evaluate the use of ophthalmic tissues in the diagnosis and elucidation of inborn errors of metabolism.

Methods Employed: Patients with inborn errors of metabolism are fully examined for visual or ocular motility disturbances. Pertinent findings are photographically recorded or videotaped. The ophthalmic observations in each patient are considered in light of the neurological and biochemical findings. In a number of these patients, conjunctival biopsies have been performed.

Major Findings: Apart from ocular motor disturbances in certain patients with Gaucher's and Niemann-Pick's disease (See project on Ocular Motor Disorders in Human Subjects, Project Number ZO1 EY 00020-05 CB), the ophthalmic manifestations have been documented in several metabolic diseases. Conjunctival biopsies have shown storage substances in connective tissue or perivascular cells in Tangier's disease, Niemann-Pick's disease, and ceroid-lipofuscinosis. Pingueculas, which have frequently been reported to be characteristic of Gaucher's disease, have been found to be inconstantly present and do not contain, in our material, the alleged Gaucher cell. A histopathologic study is being completed for the autopsied eyes of a patient with the Sjögren-Larsson syndrome. Reflectile bodies in the macula clinically appear to correlate with regional abnormalities in the retinal pigment epithelium.

Significance to Biomedical Research and the Program of the Institute: New observations in the study of inherited disorders add to our understanding of these conditions, especially as clinicopathologic correlations are made.

Proposed Course: This program will be continued since there are continuing referrals of patients with metabolic disorders to the NEI.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Chu FC, Kuwabara T, Cogan DG, Schaefer EJ, Brewer HB Jr: Ocular manifestations of Tangier disease. Arch Ophthalmol (in press).

Cogan DG: Updating the inborn errors of metabolism affecting the retina. Trans Pac Coast Otoophthalmol Soc Ann Meet 59:23-37, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00020-05 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Ocular Motor Disorders in Human Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI
Other:	Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI
	Dan Milder	M.D.	Visiting Scientist	CB	NEI
	Douglas B. Reingold	M.A.	Biologist	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION
Neuro-Ophthalmology Section

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.3	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Quantitative measurement of eye movements is fundamental to understanding ocular motor control. Our method of electro-oculography permits vestibular, optokinetic, and saccadic responses to be recorded visually, graphically, and electronically with computerized analysis of the results. Studies in the past year have emphasized the contrasting abnormalities in patients with cerebellar lesions and those with progressive supranuclear palsy. The former involves primarily the visually monitored systems (e.g. pursuit) whereas the latter involves primarily the saccadic system (e.g. volition). An incidental study has also revealed contrasting abnormalities in patients with Gaucher's disease and with a form of Niemann-Pick's disease. The former are significantly accompanied by a disturbance in horizontal movements (at times simulating congenital ocular motor apraxia) whereas the latter are accompanied by disturbances similar to progressive supranuclear palsy. In neither case is the pathologic basis understood.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 77-E1-140

Objectives: Control of eye movements is studied best in humans if isolated abnormalities can be identified. If these defects are quantitatively characterized and correlated to pathological material (as available), then (1) eye movement disorders have localizing value in neuro-ophthalmic diagnosis and (2) we approach a fundamental understanding of the eye movement control system.

Methods Employed: As in previous years, we have had a unique opportunity to study eye movement defects in selected patients with metabolic or neurological disorders affecting the central nervous system.*

We record eye movements by the technique of electro-oculography. The patient is seated on a rotatable Bárány chair and is presented visual targets to fix. The chair and the visual targets are controlled by a PDP-11 computer, while eye position signals are being simultaneously recorded online in the computer. The data is analyzed for eye movement waveform, velocity and latency.

Major Findings: Disturbances of eye movements in cerebellar disease are being recorded and cataloged for eventual analysis and interpretation. The cerebellum appears to be essential for: the maintenance of eccentric gaze; the monitoring of pursuit movements; and the stabilization of central gaze. But it plays only a minor role in saccadic movements. What is not apparent, and will require more data on patient subjects, is the topographic representation of these functions within the component part of the cerebellum.

By contrast with the cerebellar cases, patients with the parkinsinoid syndrome, progressive supranuclear palsy, have shown disturbances of the saccadic system chiefly affecting vertical movements but also horizontal movements. The saccades became slow with an impairment or elimination of the "corrective" phase of the vestibular and optokinetic responses.

In cooperation with the Metabolic Unit of the National Institute of Neurological and Communicative Disorders and Stroke, we have been able to confirm sporadic reports in the literature that certain patients, diagnosed as having Gaucher's disease, have a disturbance of horizontal eye movements which according to our observations may simulate congenital ocular motor apraxia. We have also been able to confirm, and to quantitate, the predominant disturbance of vertical movements in a group of patients who are customarily diagnosed as having a form of Niemann-Pick's disease.

*Continuation of Project No. Z01 EY 00020-04, "Parametric Studies of Eye Movement Disorders in Human Beings".

Significance to Biomedical Research and the Program of the Institute: Quantitation of ocular motor disturbances by electronic means aids in the diagnosing of lesions within the central nervous system and contributes to the knowledge of how the brain programs information on eye movements.

Proposed Course: The project will be continued. We have accumulated for review videotapes and electrophysiological recordings of more than 200 patients with eye movement abnormalities. Observations in selected patients will be added to the study.

NEI Research Program: Sensory and Motor Disorders of Vision--Strabismus and Other Oculomotor Disorders

Publications:

Chu FC, Reingold DB, Cogan DG, Williams AC: The eye movement disorders of progressive supranuclear palsy. Ophthalmology 86:422-246, 1979.

Chu FC, Reingold DB, Cogan DG: A maneuver to elicit vertical "doll's" eye movements. Am J Ophthalmol 87:742, 1979.

Reinecke RD: Downbeat nystagmus and strabismus, in Reinecke RD (ed): Proceedings of the Third Meeting of the International Strabismological Association. New York, Grune & Stratton, 1978, pp 155-158.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00087-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Parametric Studies of the Pupillary Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI
Other:	Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI
	Douglas B. Reingold	M.A.	Biologist	CB	NEI
	Deborah Young		Television Production Specialist	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION
Neuro-Ophthalmology Section

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pupillary dysfunction is an important criterion for evaluating neuro-ophthalmological disorders. In this past year we have had the opportunity to document some new observations of pupillary physiology: (1) hypersensitivity to adrenergics in patients with asthma; (2) hypersensitivity to parasympathomimetrics in two patients with progressive external ophthalmoplegia and (3) parasympathetic hypersensitivity in a patient who presented with spasm of accommodation.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 76-I-281

Objectives: To understand the abnormal physiology of pupillary responses in selected diseases thereby broadening our knowledge of the neuro-pharmacology of the pupil and underlying disorders.

Methods Employed: Patients referred to the neuro-ophthalmic service for pupillary dysfunction are studied with topical sympathetic and parasympathetic agents. Mydriasis and miosis are recorded by a video pupillography technique. The findings are evaluated in the context of the patient's neurological condition.

Major Findings: We have documented some abnormalities of pupillary physiology which have not been previously reported: (1) hypersensitivity to adrenergics in patients with asthma; (2) hypersensitivity to parasympathomimetics in two patients with progressive external ophthalmoplegia and (3) parasympathetic hypersensitivity in a patient who presented with spasm of accommodation.

Significance to Biomedical Research and the Program of the Institute: Abnormalities of pupillary function presumably have a neurological basis when the pupils are hypersensitive to pharmacological manipulation. Our observations during the past year support the hypothesis that there is derangement of the autonomic function in patients with asthma and progressive external ophthalmoplegia. In a patient with spasms of accommodation, there is a suggestion of systemic dysautonomia, too; this is presently under study by the neurology service.

Proposed Course: Further studies of the pupil will be done where indicated as part of a neuro-ophthalmic examination, and occasionally as a service to other Sections of NEI or other institutes.

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

Publications:

Henderson WR, Shelhamer JH, Reingold DB, Smith LJ, Evans R III, Kaliner M: Alpha-Adrenergic hyper-responsiveness in asthma. N Engl J Med 300:642-647, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00090-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

A System for the Analysis of Human Oculomotor Control

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI
Other:	Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI
	Douglas B. Reingold	M.A.	Biologist	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION
Neuro-Ophthalmology Section

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have developed a facility for the system analysis of eye movement control in humans. Methods have been developed for computerized derivation of parameters from analysis of eye movement waveforms, and normal values have been obtained for these parameters.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 77-EI-140

Objectives: To understand the oculomotor system and its disorders from a control systems viewpoint.

Methods Employed: Our approach in this project is to consider the oculomotor system as a control system, that is, to evaluate it in terms of its dynamic response to commands and disturbances. We have developed a testing facility to isolate the influences upon eye movements of individual sensory systems and their interactions. Patients and normal subjects are seated on a servo-controlled rotating platform. Visual stimuli are projected onto the inside surface of a spherical screen lowered into place around the subject. A scanner to provide a target for fixation and tracking studies, a panoramic projector to simulate the visual events that normally accompany head motion, and the rotating chair, are all under computer control. Eye movements are recorded by electro-oculography and are digitized on-line for computer analysis. With the computer, the data are conditioned, and individual rapid eye movements are identified. Parameters are derived to characterize oculomotor systems response and are archived so as to permit cross-patient analysis.

Major Findings: A facility has been designed and constructed for delivering precise visual and vestibular stimulation and for recording with minimum interference the evoked eye movements. Methods have been developed for deriving the velocity profile of ocular saccades from electro-oculographic data, minimizing the effects of noise and bandwidth limitation. Responses of the saccadic system during smooth eye movements have been modeled, and the model is currently being tested experimentally in normal subjects. Normal values have been derived for a set of parameters characterizing oculomotor performance.

Significance to Biomedical Research and the Program of the Institute: This project applies mathematical control system analysis for the clinical study of human ocular motility. This method provides a basis for extrapolating animal research to humans and for understanding the human eye movement control system.

NEI Research Program: Sensory and Motor Disorders of Vision--Strabismus and Other Oculomotor Disorders

Publications:

Reingold DB, Chu FC, Cogan DG, Leighton SB, McMinn WO: A computerized testing facility for the clinical study of versional eye movement control, in Greenfield RH (ed): Computers in Ophthalmology. New York, IEEE Press (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00063-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Blue-Cone Function in Color Vision Defects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Francisco M. de Monasterio	M.D., D.Sc.	Visiting Scientist	CB	NEI
Other:	Stanley J. Schein	M.D., Ph.D.	Guest Worker	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study blue-sensitive cone function in selected cases of color vision defects, with special emphasis on acquired defects. Subjects are examined with electrophysiological and psychophysical tests.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To characterize and document color vision abnormalities mediated by dysfunction of blue-sensitive cones or of their retinal pathways.

Methods Employed: Color vision is examined on the basis of a battery of psychophysical tests (increment thresholds, field and test spectral sensitivity of pi-mechanisms, spectral luminosity, chromagraph and other conventional color tests) and electrophysiological studies of cone responses.

Major Findings: Two varieties of acquired "blue-yellow" defects have been characterized to date. These defects differ in the spectral location of neutral points and in the color naming of short wavelengths; they also differ in the waveform of electroretinographic cone responses elicited with intense violet flashes on yellow backgrounds. Similar varieties of "blue-yellow" defects have also been observed in rare cases of congenital alterations of color vision.

Significance to Biomedical Research and the Program of the Institute: The results may help our knowledge of the mechanisms of acquired color vision defects which preferentially affect blue-sensitive cone function in cases of retinal insult or disease.

Proposed Course: Studies of blue-sensitive cone function in color vision defects will be continued.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders/(Psychophysical Function)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00059-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Electrophysiological and Psychophysical Evaluation of Retinal Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Francisco M. de Monasterio	M.D., D.Sc.	Visiting Scientist	CB	NEI
Other:	Ralph D. Gunkel	O.D.	Ophthalmic Physicist	CB	NEI
	Doris Collie		Health Technician	CB	NEI
	Mary Fuhrman		Health Technician	CB	NEI
	Patricia Christian		Health Technician	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to provide diagnosis or evaluation of toxic, inflammatory, degenerative, or congenital retinal disorders, and to conduct tests and experiments directed towards the clinical application and development of electrophysiological and psychophysical procedures for measuring visual function in patients of NEI's Eye Clinic and of other services in the NIH Clinical Center.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Diagnosis or evaluation of visual function in toxic, inflammatory, degenerative, and congenital visual disorders affecting the retina. Development of clinical procedures for the study of visual function.

Methods Employed: Commercially available and laboratory-developed instruments are used in measuring visual function in normal volunteers and clinical patients on the basis of electroretinography (single flash and averaged Ganzfeld, averaged Focal), visually evoked cortical potentials, electrooculography, sensory rod and cone thresholds, color vision testing, Stiles-Crawford effects, retinal image stabilization, visual perimetry and other psychophysical functions.

Major Findings: Psychophysical and electrophysiological evaluations were performed on about 200 patients for diagnostic purposes in collaboration with clinical associates and staff members of the NEI.

Present efforts are directed towards the development of a non-invasive system of retinal image stabilization for clinical procedures which would permit studies of focal electroretinography with very small stimuli at different retinal eccentricities, microperimetry, and psychophysical functions.

Significance to Biomedical Research and the Program of the Institute: The work has provided evaluations and diagnosis of retinal disorders in inpatients, outpatients, and referred patients of NEI's Eye Clinic at the NIH Clinical Center. Development of new research techniques and the application of new and existing research techniques to clinical procedures are expected to help improve the diagnosis of visual disorders and the understanding of physiopathological mechanisms of retinal disease.

Proposed Course: Electrophysiological and psychophysical studies of retinal disorders will be continued.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders (psychophysical Function)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00065-02 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Physiological and Anatomical Studies of the Visual System of Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Francisco M. de Monasterio	M.D., D.Sc.	Visiting Scientist	CB	NEI
Other:	Stanley J. Schein	M.D., Ph.D.	Guest Worker	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project aims to study the anatomical and physiological organization of neurons of the visual system of non-human primates that can serve as a model for the human visual system. The project gives emphasis to the chromatic and spatial properties and central projections of neurons of the retina, lateral geniculate body, striate cortex and extrastriate cortex of macaque monkeys.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the neural organization underlying the processing of visual data in retina and cortex, with particular emphasis on color.

Methods Employed: Intracellular and extracellular recordings from single neurons, intracellular staining with fluorescent dyes, extracellular recordings of mass responses; correlation of the distribution of single cell varieties and morphological cell types as seen by electron and light microscopy; autoradiography of the distribution of radionuclide-labelled neurons.

Major Findings:

I. Extracellular recording of retinal ganglion cells in the intact monkey:

The retina and (dorsal) lateral geniculate body (dLGB) of macaques contain a large variety of functionally distinct cell types. Many studies show two main functional groups of cell with antagonistic center-surround organization and properties resembling those of the X- and Y-cell systems described in the retina of the cat. Unlike the cat, however, the Y-cell system of macaques appears to be organized into two different cell varieties. Most Y ganglion cells of maques, type III cells, have antagonistic center and surround regions receiving input from the same cone types and thus show non-opponent-color responses, i.e. they respond to changes in stimulus luminance independent of wavelength. Type III ganglion cells have been more frequently encountered towards the peripheral retina. Other Y ganglion cells, type IV cells, have center and surround regions with different spectral sensitivities and thus show opponent-color responses, i.e. they are typically excited by mid-spectral and short wavelengths and inhibited by long wavelengths. Type IV cells have been more frequently encountered in the central retina. These two cell types are primarily found in the magnocellular layers of the dLGB, where they show similar spectral differerences.

Whereas little is known about the functional role of type IV cells, type III cells are generally thought to subserve luminance-information processing because (i) their responses to different wavelengths can be equalized by adjusting stimulus attenuation, and (ii) the averaged spectral sensitivity to large stimuli of some of these non-opponent-color cells resembles the photopic luminosity function of the CIE.

We found differences between the spectral sensitivity of foveal and perifoveal type III ganglion cells, differences due to the variation with retinal eccentricity of the spectral properties of the surround of these neurons. Because of this variation, the sensitivity of foveal type III cells is narrower than the CIE photopic function at the long wavelengths and resembles that of type IV cells, while the sensitivity of perifoveal type III cells resembles, on the average, the CIE function. These results show that type III and IV neurons do not represent two separate Y-cell types, but rather regional varieties of a single Y ganglion cell system which has a reduced long wavelength sensitivity in the foveal area. Many studies, using different techniniques, have reported measurements showing a similar reduction of red sensitivity in (foveal)

macaque luminosity functions. These functions have a shape similar to that of functions of protanomals but narrower than those of normal trichromats at the long wavelenghts. These similarities suggest that both types of Y-cells contribute to the processing of luminance-information in macaques.

II. Intracellular recordings and staining of single retinal cells in the arterially-perfused eyecup:

To understand the anatomic organization responsible for the physiological functioning of the primate retina, we are applying intracellular recording and staining to identify functional cell types. We have had considerable experience with these techniques. With new methods for microelectrode fabrication, we have performed preliminary studies aimed at impaling smaller retinal cells such as bipolar cells. We plan to couple the intracellular studies with the 2-deoxy-glucose activity labeling method (see below). We are replicating the published results of a study that allows high resolution activity labeling. Our most important result of these trials was the recognition of the limitations of that technique. We are looking forward to modifications of this technique, which should overcome these limitations.

III. Extracellular recording from single units in the intact monkey cortex:

Our initial work along these lines was to bring the necessary techniques and skills into operation in the laboratory. The required areas of expertise include not only cortical extracellular single unit recording, but also neurosurgery for placement of cortical lesions and histology suitable for architectonic analysis. The cortical studies focus on the processing of color in the lateral geniculate body (LGB), striate cortex and potentially in the extrastriate cortical area referred to as V4. Preliminary extracellular unit recordings were begun, but intensive work along these lines will not occur until early in the next fiscal year. The physiology required us to set up an extensive optical system to permit use of chromatic adaptation, a system including a modified fundus camera, Xenon arc lamp, interference filters and associated optics and electronics. The recording system, including the Faraday cage, signal averager, tape recorder, and associated electronics, was placed into operation. Finally, we have obtained the hardware required for the semi-chronic preparation.

We plan the dual approach of studying lesioned as well as normal animals. The necessary surgical skills were obtained during the year and suitably lesioned animals were produced. We experimented with numerous histological staining procedures and settled on two for routine use in the architectonic analysis required to delimit V4 and its components.

IV. Physiological anatomy of the color pathway in the cortex of the intact monkey:

All of the techniques which were brought to the useful state for extra-cellular cortical unit recording (unit recordings, surgery, and histology)

contribute to this study. Using psychophysical procedures for blue cone isolation, we were able to provide conditions under which only the blue cones were modulated. We also established conditions under which only rods would be activated. These conditions were used in conjunction with the 2-deoyglucose method to mark the blue cone and the rod specific pathways in LGB, striate cortex, and beyond.

Significance to Biomedical Research and the Program of the Institute: Understanding the organization of the visual system of non-human primates is valuable for the understanding of the human visual system, which at present can only be studied by indirect methods. Radionuclide-label studies appear to be one of the most promising approaches in this direction, because autoradiographic studies can be substituted by non-invasive methods of mapping the distribution of a positron-emitter nuclide.

Proposed Course: Both extracellular and intracellular recordings from single cells of the monkey visual system, as well as neuroanatomical studies of the system, will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaptation; Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders

Publications:

de Monasterio FM: Spectral interactions in horizontal and ganglion cells of the isolated and arterially-perfused rabbit retina. Brain Res 150:239-258, 1978.

de Monasterio FM: Macular pigmentation and the spectral sensitivity of retinal ganglion cells of macaques. Vision Res 18:1273-1277, 1978.

de Monasterio FM: Properties of concentrically-organized X and Y ganglion cells of the retina of macaques. J Neurophysiol 41:1394-1417, 1978.

de Monasterio FM: Center and surround mechanisms of opponent-color X and Y ganglion cells of the retina of macaques. J Neurophysiol 41:1418-1434, 1978.

de Monasterio FM: Properties of ganglion cells with atypical receptive-field organization in the retina of macaques. J Neurophysiol 41:1435-1449, 1978.

de Monasterio FM: Signals from blue cones in "red-green" opponent-colour ganglion cells of the macaque retina. Vision Res 19:441-449, 1979.

de Monasterio FM: Assymetry of ON- and OFF-pathways of blue-sensitive cones of the retina of macaques. Brain Res 166:39-48, 1979.

de Monasterio FM, Schein SJ: Protan-like spectral sensitivity of foveal Y ganglion cells of the macaque retina. J Physiol (Lond) (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00061-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Retinal Function in Posterior Uveitis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Francisco M. de Monasterio	M.D., D.Sc.	Visiting Scientist	CB	NEI
Other:	Robert Nussenblatt	M.D.	Senior Staff Ophthalmologist	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Abnormalities of retinal function at the level of rods and cones or their pathways are being documented by electrophysiological and psychophysical studies of patients with posterior uveitis of suspected immunological origin.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 79 EI 49

Objectives: To understand the retinal physiopathology of posterior-segment uveitis and chorio-retinitis of suspected immunological origin.

Methods Employed: Retinal function is assessed by electroretinography (single flash and averaged Ganzfeld responses, focal responses), electrooculography, sensory dark-adaptation thresholds, visual perimetry and color vision tests in cases of ocular toxoplasmosis, par planitis, Behcet's disease, ocular sarcoid, Vogt-Kayanagi-Harada's syndrome, ocular histoplasmosis and other inflammatory diseases affecting the posterior segment of the eye.

Major Findings: Studies of 25 cases of posterior uveitis indicate that diffuse and central involvement of the retina produces early electroretinographic waveform changes of rod and, most especially, cone responses. These changes, primarily involving responses mediated by signals from red- and green-sensitive cones, are accompanied by reduction or extinction of responses presumably mediated by signals from blue-sensitive cones. These alterations, which appear to be an accurate diagnostic criteria to detect inflammatory activity of immune origin, are accompanied by relatively typical, though unspecific, color vision defects of central vision. The observed waveform changes seem to represent a quasi-pathognomonic sign of diffuse central posterior uveitis, as they are not seen in other cases of uveitis with either a localized or diffuse peripheral distribution or in other commonly observed disorders of the central retina. However, they have been occasionally observed in some rare cases (e.g. Tangier's disease). Present efforts are being directed to the characterization of these alterations at the retinal cell level through the development of a non-human primate model which should permit physiological and anatomical studies.

Significance to Biomedical Research and the Program of the Institute: The detected electrophysiological signs should serve to study the clinical evolution of the cases with diffuse central uveitis with retinal involvement using comparatively simple tests. Characterization and localization of disordered retinal function may elucidate some of the physiopathological processes of immunological retinal disease.

Proposed Course: Studies of retinal function in posterior uveitis will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00050-03 CB

PERIOD COVERED

October 1, 1978,, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Aqueous Humor Flow Measurement by Fluorophotometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Lessie McCain	R.N.	Clinical Technician	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0.025	0.025

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aqueous humor flow in humans is measured by determining the rate of loss of fluorescein from the eye after iontophoresis into the cornea in normal volunteers and in patients with ocular hypertension or glaucoma.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 77 EI 104

Objectives: This project is designed to measure directly aqueous humor flow in humans. This will be compared to calculated aqueous humor flow. The symmetry and reproducibility of measurements of aqueous humor flow in the two eyes of normal volunteers and of patients with either ocular hypertension or glaucoma are to be studied; medication effects will be assessed.

Methods Employed: A cylindrical piece of polyacrilamide gel is saturated with fluorescein solution. The gel is touched to the cornea, and fluorescein is deposited due to a small current provided by a dry cell battery. A photomultiplier tube with appropriate filters, mounted on a slit lamp biomicroscope, measures the total amount of fluorescein in the eye as well as the aqueous concentration. Illumination is provided by a chopped light source. The photomultiplier tube signal is fed to a tuned amplifier. The rate of loss of fluorescein from the eyes as a function of time yields the flow rate of aqueous humor.

Major Findings: FY 79 was the second year of this project. For most of the year, the project was inactive due to an unavoidable need to relocate the laboratory, and to personnel turnover.

Studies of the rate of turnover of aqueous humor in a group of patients with ocular hypertension have been started. Study of these individuals is important because they appeared to have higher than normal flow during an earlier study in which the flow was evaluated indirectly, by calculation from the Goldmann equation. Their true facility was nearly normal and their elevation of intraocular pressure appeared to be accounted for mainly by increased aqueous humor flow.

Significance to Biomedical Research and the Program of the Institute: The aqueous humor flow rate is a primary determinant of the intraocular pressure. An accurate, safe, reproducible, noninvasive, direct determination of the flow in humans under normal and pathological conditions could lead to increased understanding of glaucoma and hypotony.

Proposed Course: The studies will continue, emphasizing aqueous flow in ocular hypertension and comparison to calculated flow.

NEI Research Program: Glaucoma--Optic Nerve and Vision Changes in Glaucoma

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 000154-06 CB

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Experimental Glaucoma in the Rhesus Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Douglas E. Gaasterland M.D. Senior Staff Ophthalmologist CB NEI
Other: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this investigation is to study the morphology, physiologic function, and pharmacologic behavior of the eye of the rhesus monkey in its normal state compared to its state when experimental glaucoma has been induced by argon laser photocoagulation of the trabecular meshwork.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study physiologic function, pharmacologic behavior, and morphology of the monkey eye after induction of glaucoma by argon laser photocoagulation of the trabecular meshwork. To compare observations to normal control eyes.

Methods Employed: Circumferential argon laser photocoagulation of the rhesus monkey trabecular meshwork causes sustained elevation of intraocular pressure to the range of 30 to 55 mmHg, the pressure range found in many humans with open-angle glaucoma. This is in contrast to the acute short duration, very high pressure elevation (more than 65 mmHg, up to 95 mmHg) seen in most animal models for glaucoma. Outflow facility is evaluated by perfusion. Aqueous flow is determined by turnover of radioiodinated serum albumin injected into the anterior chamber. Retinal and optic nerve function can be studied by autoradiography and morphologically to evaluate evidence of altered axoplasmic flow. The retina can also be studied in cross section or by preparing whole-mounts of the tissue. Additional studies of the effect of less than circumferential argon laser photocoagulation have been started.

Major Findings: In FY 79, this project has been hampered by the laboratory being moved, the argon laser undergoing major repairs, and personnel turnover.

Preliminary results indicate that coagulation of one-half or less of the circumference of the trabecular meshwork is not sufficient to induce appreciable rise of intraocular pressure; coagulation of two-thirds of the circumference causes intermittent elevation of the intraocular pressure which is insufficient to cause clinical alteration of the optic nerve from normal. The pressure in these eyes fluctuates between high normal and about 30 mmHg.

Significance to Biomedical Research and the Program of the Institute: This experimental glaucoma is the best model available for human chronic open-angle ("simple") glaucoma. Using this model allows close examination of the retina and optic nerve changes, with the promise of additional insight into the mechanism of loss of visual function in the patient with glaucoma.

Proposed Course: The project will continue with immediate emphasis upon effects of treating less than the entire circumference of the trabecular meshwork.

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

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00046-03 CB

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Laboratory Studies of Aqueous Humor Dynamics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Douglas E. Gaasterland M.D. Senior Staff Ophthalmologist CB NEI
Other: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various projects have been carried out to clarify intraocular fluid movement in rhesus monkeys. The effects of an artificial solution to substitute for aqueous humor upon the endothelium of isolated corneas were studied and found to be similar to the effects of pooled rhesus aqueous humor during storage periods of up to eight hours. This solution is now in use for ocular perfusion to determine total facility. An in vivo calibration of displaced volume during corneal applanation has been performed and compared with a similar in vitro calibration. This information is relevant to the calibrations used for human applanation tonography.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project is designed to examine the physiology of intraocular fluid movement under varied experimental conditions.

Methods Employed: Standard methods of cannulation and perfusion with noninvasive and invasive pressure measurements and with subsequent determination of volumes and flow by weight changes, dilution, or turnover techniques have been used.

Major Findings: Progress has been slowed in FY 79 by the laboratory being moved and unavoidably shut down for six months, and by personnel turnover.

The solution to substitute for aqueous humor previously described was tested by comparing corneas stored for up to eight hours in either the artificial aqueous humor solution, glutathione-bicarbonate-Ringer's solution (GBR), or in situ at room temperature in enucleated eyes. The density of endothelial cells was not affected by the storage method, but the cells stored in GBR consistently had a smaller nucleus. Corneas stored for the same period in normal saline showed stromal edema with loss of endothelial cells and swelling of remaining cells. These studies were performed using the vital stain, trypan blue, and by examination of flat mounts of endothelium after standard histologic stains.

The volume of aqueous humor displaced during applanation of the cornea was studied in living monkeys and in a series of monkey eyes after enucleation. The relation of the area of applanation (determined from photographic records) to the ratio of the applanating force intraocular pressure during applanation, was determined and compared to the Imbert-Fick Law prediction. The area was also related to the volume displaced, and the actual volume displaced was compared to the volume calculated for flattening of a spherical segment of the same base area and curvature as the eyes studied. Application of the observations to the procedures used for human applanation tonography indicates that the calibration from in vitro human eyes is about 30% low in predicting the total facility in living eyes. This conclusion gives weight to the possibility that clinical indentation tonography is fairly accurate--the error of the method is no more than about 50% low and is insufficient to account for the observed discrepancy between measured and calculated human aqueous humor flow.

Significance to Biomedical Research and the Program of the Institute: The studies are elucidating normal dynamics of aqueous humor, as well as abnormal dynamics in experimentally induced situations, mimicking clinical problems. It is hoped that these studies will yield information applicable to understanding and treating glaucoma and hypotony.

Proposed Course: Similar studies will be continued, emphasizing inflow and outflow studies and aqueous humor composition.

NEI Research Program: Glaucoma--Hydrodynamics of the Eye

Publications:

Pederson JE, Gaasterland DE, MacLellan HM: Anterior chamber volume determination in the rhesus monkey. Invest Ophthalmol Vis Sci 17: 784, 1978.

Bartels SP, Pederson JE, Gaasterland DE, Armaly MF: Sites of breakdown of the blood-aqueous barrier after paracentesis of the rhesus monkey eye. Invest Ophthalmol Vis Sci (in press).

Gaasterland DE, Pederson JE, MacLellan HM, Reddy VN: Rhesus monkey aqueous humor composition and a primate ocular perfusate. Invest Ophthalmol Vis Sci (in press).

Rodrigues MM, Gaasterland DE: Current concepts in the pathology of the glaucomas (anterior segment), in Nicholson D (ed): Update in Ophthalmic Pathology. New York, Masson Publishing Co (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00168-04 CB

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Laser Surgery for Glaucoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Charles Bonney	D.V.M., Ph.D.	Visting Scientist	CB	NEI
	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI
	Carl Kupfer	M.D.	Director		NEI
	Robert Bonner	Ph.D.	Physicist	BEIB	DRS

COOPERATING UNITS (if any)

Biomedical Engineering and Instrumentation Branch, DRS; Armed Forces Radiobiology Research Institute

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.35	0.35	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The high energy and power of lasers offer a tool for noninvasive alteration of anterior intraocular tissue. Specifically, iridotomy and trabeculotomy are possible. This has importance for glaucoma patients because of the potential improvement of surgical outcome and reduced surgical morbidity. The aim of this project is a systematic evaluation of laser effects in simian (rhesus) eyes and the application of promising systems and procedures to human glaucoma eyes under controlled conditions.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To develop workable laser systems for anterior segment surgery and to apply these systems to the normal monkey eye. To study the physiological and morphologic effects of laser energy upon monkey eyes. To apply favorable laser systems under controlled conditions to the treatment of glaucoma in human.

Methods Employed: Instruments are being developed to meet the unique requirements of ophthalmic application. Standard laboratory physiologic and histopathologic (including SEM and TEM) techniques are employed to study laser effects.

Major Findings: In FY 79, the fourth year of this project, considerable attention has been given to the further development of instrumentation for the Q-switched ruby laser. On-line energy monitoring for each pulse has now been attained. A coaxial aiming beam using a helium-neon laser is being developed. An articulating arm delivery system is needed to allow interfacing with either a slit lamp or operating microscope; this is in process. Energy is measured with a ballistothermopile system or a dielectric photodetector system, and is now reproducible with 10%. Spot size is recorded upon special light-sensitive paper. The energy density of pulse applications is calculated.

The chronologic effects upon monkey corneal epithelium and endothelium and the iris away from the aiming site after laser treatment to create an iridotomy have been extensively studied. For most observed changes, histopathologic information is now available. Epithelial lesions are induced at a threshold of about 10 J/cm^2 and heal within 48 hours. Endothelial lesions appear to have a lower threshold and heal more slowly, if at all. In one eye after a pulse of 150 mJ, a local anterior cortical lens opacity was induced. This is rare and not been observed in about 100 other eyes. After treatment monkey irides often develop a streak of subtle hyperpigmentation to the pupil margin, starting at about three days. This is thought to be along the path of the expanding acoustic pressure wave which develops at the target site.

One aphakic patient with a thick, dense-brown iris has been treated in an attempt to create an optical iridectomy to relieve her correctopia and poor vision. Three sessions, 10 to 14 days apart, with two Q-switch ruby laser pulses per session have not been sufficient to open the iris.

In two monkeys, treatment of the trabecular meshwork through the new glass gonioscopy lens at a level of 100 to 120 mJ per pulse created a hole from the anterior chamber to the canal of Schlemm in each of three application sites per eye. Unfortunately, there was associated bleeding and local iridodialysis. Perfusion of the treated and untreated eyes of each monkey showed no improvement of outflow facility in the treated eyes; the facility determined for the control eyes reproduced the value obtained for the same eyes approximately one year previously. Histopathologic examination of these acute specimens verified the observations made clinically. This treatment caused

pitting of the gonioscopy lens; probably secondary to non-linear phenomena.

Significance to Biomedical Research and the Program of the Institute: Conceivably, a physically-noninvasive laser system for anterior segment surgery might replace conventional invasive operative procedures for some types of glaucoma. This possibility is still being investigated.

Proposed Course: The project will be continued to expand experience with argon and Q-switch ruby laser effects on iris and anterior chamber angle in monkeys and patients.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Primary Open-Angle Glaucoma/Primary Angle-Closure Glaucoma/Secondary Glaucomas)

Publications:

Bonney CH, Gaasterland DE: Low-energy, Q-switched ruby laser iridotomies in macaca mulatta. Invest Ophthalmol Vis Sci 18:278, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00143-06 CB

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Radioiodinated Chloroquine Analog for Diagnosis of Ocular Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI
	Carl Kupfer	M.D.	Director		NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this clinical investigation has been to assess the value of systemically administered I-125 labeled chloroquine analog for the detection of ocular melanoma. Patient enrollment terminated 30 June 75, after the 36th patient was accepted. Follow-up examination of some patients continue. This is expected to yield information on the clinical course of diagnosed and treated melanoma patients, of diagnosed melanoma patients who refused treatment, and of patients with lesions which may or may not be ocular melanoma. The course will be compared to the results of the radioactive tracer testing.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 76 EI 370

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

Methods Employed: During this year, several follow-up clinical examinations have been performed, and information concerning other patients has been obtained by mail.

Major Findings: Many of the 36 patients in this study are seen intermittently for follow-up examination; others correspond. The patient who developed metastatic disease in spring 1978, four years after enucleation, died in the spring, 1979; the cause of death was metastatic disease. Two patients who have refused enucleation continue to be seen with slowly growing lesions and no clinical evidence of metastatic disease five and six years after diagnosis. In both, visual function remains good. One of these patients is now 84 years old; the other is in his 50's. None of the lesions originally thought to be benign has had a change of diagnosis.

Significance to Biomedical Research and the Program of the Institute: Continued follow-up information concerning the course of the enucleated patients and the other patients is important because the registry of melanoma patients created by this project serves as an information resource concerning course of disease.

Proposed Course: The intermittent examinations of this small group of patients will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Tumors

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00030-08 CB

PERIOD COVERED
October 1, 1978, to September 30,1979

TITLE OF PROJECT (80 characters or less)

Studies of Parameters of Intraocular Pressure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Carl Kupfer	M.D.	Director		NEI
	Lessie McCain	R.N.	Clinical Technician	CB	NEI
	Roy Milton	Ph.D.	Head, Section on Biometry	OBE	NEI

COOPERATING UNITS (if any)
Normal Volunteer Office, CC, NIH; Pharmaceutical Development Service, CC, NIH; Biomedical and Engineering Instrumentation Branch, DRS, NIH

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.65	0.1	0.55

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this continuing study of the parameters of intraocular pressure, young and old normal volunteers and patients with glaucoma and ocular hypertension participate. There is interest in determining the actual values of the parameters in eyes not affected by medications and in determining the acute and chronic effects of antiglaucoma medications alone and in combination upon the parameters in normal and in diseased eyes. Two recent studies have emphasized receptor pharmacodynamics.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 75 EI 114

Objectives: To evaluate parameters of intraocular pressure in normal eyes and eyes with ocular hypertension or glaucoma before and after anti-glaucoma medications.

Methods Employed: Replicate studies are done upon experienced human participants. Seven parameters are determined before and after medication: intraocular pressure, episcleral venous pressure, total facility, true facility of outflow, pseudofacility, aqueous flow, and ocular rigidity. Acute drug effects are emphasized. Chronic drug effects are studied by use of the Ocusert system for pilocarpine and in patients receiving monocular treatment in the ocular hypertension protocol of Dr. Ballintine (Project No. ZO1 EY 00150-06 CB).

Major Findings: Studies in other laboratories have shown that rats and monkeys chronically treated with pilocarpine or carbachol develop desensitization of the parasympathetic (muscarinic) receptors. The amount depends upon the amount of exposure. In our laboratory this has been tested during FY 1979 by measuring the magnitude of the effects of 2% pilocarpine drops administered bilaterally to the eyes before, and at one and two weeks after monocular treatment three times a day with 1% pilocarpine. Four young volunteers participated in this study. The parameters outlined above as well as refractive error and accomodation were evaluated during each session of measurements. The results show considerable scatter, with no clearly recognizable inability of the chronically treated eye to respond to the acute pilocarpine treatment. It is concluded that 1% pilocarpine topically, three times a day for two weeks is not sufficient to cause desensitization in young normal human volunteers. To be investigated is whether pilocarpine administered more frequently, e.g. by the Ocusert delivery system, would cause desensitization in humans, as it did in monkeys.

Six volunteers received 2% epinephrine bitartrate in single doses to one eye on the first day and to both eyes on the second day. Intraocular pressure was monitored for six hours after treatment on each day. No "paradoxical" response (rise) of pressure was found in the "experienced" eyes. Such a paradox has been observed in the eyes of rabbits.

Ten volunteers underwent repeated indentation tonometry. Each day two tonograms were performed with a one-hour interval between studies. Each volunteer participated in two test days. On one of the test days for one of the tonograms (order was determined by flip of a coin) the volunteer was prepared by inflation of a pediatric blood pressure cuff about the neck to a pressure of 25-30 mm Hg. The cuff was in place and inflated during the tonogram so that episcleral venous pressure would be elevated. Without the neck cuff, the mean values for total facility are symmetric and reproducible. With the neck cuff there is symmetry, but the total facility is about 20% lower than when the cuff is not present. The mean values are reproducible between test days provided the cuff is not on the neck and inflated. There was moderate individual variation; reproducibility was very good for some of the volunteers, but not all.

Studies of isolated cat eyes have indicated that low doses of atropine cause relaxation of muscles served by receptors on the downstream side of the ciliary processes; this results in slower formation of aqueous humor due to decreased capillary pressure and therefore decreased ultrafiltration. Atropine eyedrops were diluted to 0.01%, 0.001%, and 0.0001%. In preliminary studies the pressure was monitored for six hours after treatment of one eye of volunteers. The intraocular pressure rose from 12 to 14 mm Hg after 0.01% atropine; the effect was about complete at 1 hour, in full force at 3 hours, and going away at 6 hours. After 0.001% atropine nothing happened to the intraocular pressure. After 0.00001% atropine, surprisingly, in all three volunteers tested intraocular pressure dropped by about 2mm Hg compared to a minimal change in the untreated (control) eye. This will require additional testing, performed in a masked trial.

Significance to Biomedical Research and the Program of the Institute: Study of patterns of alteration in the parameters of intraocular pressure caused by glaucoma medications allows clearer understanding of their mechanisms of action. Studies of these parameters more clearly define the difference between normal and abnormal. The measurements can be extrapolated to more basic physiologic functions, yielding insight to the function of the human eye. This information is unique in ophthalmic research.

Proposed Course: The project will be continued, emphasizing medication effects upon parameters.

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

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00077-02 CB

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Treatment of Neovascular Glaucoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with rubeosis iridis and neovascular glaucoma are being recruited. Those with salvageable vision are invited to join this prospective, randomized study of whether cyclocryotherapy or cyclodiathermy is better for the treatment of this disease. Outcome will be judged by assessing preservation of visual function; adequate control of intraocular pressure, with or without medications; and control of discomfort. It is estimated that approximately 40 non-diabetic and 40 diabetic patients will be needed for this project.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 78 EI 17

Objectives: To determine whether one of two methods for ciliary body ablation, cyclodiathermy or cyclocryotherapy, is better for treatment of neovascular glaucoma.

Methods Employed: Patients who are eligible to join the study, and who give informed consent to join, are randomly assigned to receive one of the two methods of treatment. Follow-up is aimed at identifying adequacy of treatment and complications.

Major Findings: Four patients have entered the trial. None has had rubeosis and neovascular glaucoma due to diabetes. The random assignment of these patients has resulted in two being treated with cyclodiathermy and two being treated with cyclocryotherapy. In all, the progression of the elevation of intraocular pressure has been interrupted with one treatment. All have become comfortable. Visual status assessment is continuing, as well as evaluation of other follow-up parameters.

Significance to Biomedical Research and the Program of the Institute: This study has potential for indicating the proper management of these difficult secondary glaucoma patients.

Proposed Course: The study will be continued to allow gathering of additional data.

NEI Research Program: Glaucoma--Medical and Surgical Treatment of Glaucoma

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00006-08 CB

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Research in Methods of Evaluating Visual Processes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ralph G. Gunkel	O.D.	Ophthalmic Physicist	CB NEI
Other:	David G. Cogan	M.D.	Medical Officer	CB NEI
	Fred C. Chu	M.D.	Senior Staff Fellow	CB NEI
	Douglas Reingold	M.S.	Biologist	CB NEI
	David A. Newsome	M.D.	Senior Staff Ophthalmologist	CB NEI
	Donna M. Putt	R.N.	Ophthalmic Nurse	CC

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose and intent of this project is to conduct tests, research, and experiments directed toward the use, improvement, and development of clinical procedures and instruments for measuring functions or properties relating to optics and physiology of the eyes. This has consisted primarily of subjective measurements of visibility and chromaticity thresholds, which provide our best estimate of the functional efficiency of the rods and cones in the retina. In cases of corneal, lens, or vitreous abnormality, transparency of the media is involved, but usually the sensitivity of the endorgans is measured as an index of the genetic, degenerative, toxic, or pathological condition of the retina.

PHS-6040
(Rev. 10-76)

Project Description.

Objectives: To discover and utilize the most effective and least traumatic methods for quantitating and evaluating changes in the eye or its adenexae brought about by disease, toxic materials, or degenerative processes. Objective methods are vigorously sought, but are not often attainable. The goal of the project continues to be to provide information which will contribute toward the maintenance or restoration of normal visual function wherever possible.

Methods Employed: Conventional ophthalmic instruments and those developed here are used in measuring rod and cone thresholds and other ocular functions in clinical patients. These psychophysical tests were done on 450 patients during the past year. This number includes normal controls, NEI patients, and referrals from other NIH Institutes.

Major Findings: Dark adaptation studies are neglected by most practitioners, but they can be very important in differential diagnosis as well as in counseling.

Parents frequently report that their children are misunderstood, if not actually abused at school because of undiagnosed defects in their color vision. Currently available color tests are helpful in screening, but they are not specific and are not used often or carefully. The Chromagraph previously developed and described here shows exactly which colors are seen at a given saturation level, so that it is most helpful in efforts to understand problems related to color and color coding. In several specific cases, a careful analysis and explanation of defective color vision has been helpful to young students, their parents, and their teachers.

A reportedly high incidence of deuteranomaly among manic depressive patients has not been confirmed.

Very frequent coincidence of a yellow color defect with macular degeneration has been discovered as well as a large number of orange or red defects at certain stages in multiple sclerosis. These and other findings are scheduled to be reported at the American Academy of Ophthalmology Annual Meeting in November.

Significance to Biomedical Research and the Program of the Institute: Sensitive measurements of cone and color thresholds may be our best index for measuring toxic reaction to chemotherapy in the treatment of arthritis, systemic lupus erythematosus, breast cancer, and some other diseases. Medications and dosage are frequently modified according to our findings. The validity of these concepts is being questioned and studied.

Threshold measurements provide our best means for monitoring progress of the degenerative retinopathies, and although no effective therapy has been proven, it is useful to have records on individual patients going back more than 20 years.

Proposed Course: The project will be continued with special emphasis on the correlation of subtle color defects with ocular or systemic pathology and the confirmation of tests for early damage from toxic medications.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaptation

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 0079-02 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mechanism of Action of Vitamin A on Corneal Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John R. Hassell	Ph.D.	Research Biologist	CB	NEI
Other:	David A. Newsome	M.D.	Senior Staff Ophthalmologist	CB	NEI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experiments are being conducted using corneas obtained from normal animals and vitamin A deficient animals as well as human donors. The corneas were radioactively labeled in organ culture, the epithelia were harvested, and glycoconjugates synthesized by the epithelia were isolated and characterized by biochemical procedures. The results indicate that vitamin A stimulates the synthesis of a major epithelial glycoprotein of high molecular weight.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Although vitamin A has been shown to inhibit keratinization of corneal and various other epithelia, the mechanism by which vitamin A acts to maintain a normal epithelium is not well understood. The purpose of this study is to determine the biochemical basis for the vitamin A mediated changes in corneal epithelium.

Methods Employed: Corneas were excised and radioactively labeled in organ culture. Vitamin A was either administered to the animal prior to excision or added to the culture medium. The epithelium was then harvested and the epithelial glycoconjugates separated and characterized by DEAE-cellulose chromatography, molecular sieve chromatography and gel-electrophoresis.

Major Findings: Vitamin A stimulates ^{3}H glucosamine incorporation into a glycoprotein. The molecular weight of this glycoprotein can be estimated at $0.5 - 1.0 \times 10^{6}$. Analysis of the glycopeptides derived from this glycoprotein showed that ^{3}H glucosamine incorporation was stimulated in only one of the five glycopeptide types. ^{14}C leucine labeling of the glycoprotein was unchanged. These findings suggest that, in affecting epithelial differentiation, vitamin A alters the glycosylation of the glycoprotein.

Significance to Biomedical Research and the Program of the Institute: Xerophthalmia, which can progress to keratomalacia, is a human corneal disease which is thought to arise, in part, from vitamin A deficiency. This disease involves the keratinization of the corneal epithelium and can lead to blindness. The knowledge gained from this study is expected to indicate the biochemical processes of epithelial differentiation that are directly regulated by vitamin A and thereby permit more effective use of vitamin A as a therapeutic agent. Furthermore, this approach may allow the development of diagnostic procedures that will be useful in clinically evaluating human epithelial diseases.

Proposed Course: We will attempt to determine the functional role of the high molecular weight glycoprotein in inhibiting keratinization and the mechanism by which vitamin A regulates its synthesis. Antibodies against this glycoprotein will be prepared for use in clinical diagnosis and basic research.

NEI Research Program: Corneal Diseases--Dry Eyes and Tear Abnormalities, Epithelial Disorders, and Drug Delivery

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00085-02 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The HLA and ABO Antigens and Immunologic Studies in Cogan's Syndrome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	David G. Cogan	M.D.	Senior Staff Ophthalmologist	CB	NEI
	Kamal K. Mittal	Ph.D.	Research Microbiologist	BB	DBBP
	Barton Haynes	M.D.	Staff Fellow	LCI	NIAID
	Anthony Fauci	M.D.	Senior Physician	LCI	NIAID

COOPERATING UNITS (if any)

Bureau of Biologics, Food and Drug Administration
Laboratory of Clinical Investigation, NIAID

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.7	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this protocol is to determine the phenotype frequency of the HLA and ABO antigens as well as to explore the possibility of altered immune response in patients with Cogan's syndrome.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 77 EI I 138

Objectives: To determine the HLA and ABO antigens in patients with Cogan's syndrome. To determine in vitro immunologic studies on serum, blood, or separated mononuclear cells.

Methods Employed: Patients having Cogan's syndrome are examined according to a standard set of procedures to confirm the diagnosis. Blood specimens are analyzed to HLA and ABO antigens and a prescribed battery of in vitro immunologic studies.

Major Findings: Patients with Cogan's syndrome do not have a specific HLA type.

Significance to Biomedical Research and the Program of the Institute: To determine the immunologic basis of an eye disease.

Proposed Course: Continue follow-up one year.

NEI Research Program: Corneal Diseases--External Ocular Infections and Inflammatory Diseases

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00018-05 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Ophthalmologic Screening for Tamoxifen Toxicity to the Eye

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Marc Lippman	M.D.	Head, Medical Breast Cancer Section	MB	NCI

COOPERATING UNITS (if any)

National Cancer Institute

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to perform a prospective study to monitor the effects of tamoxifen (an antiestrogen used in breast chemotherapy) upon the eye in order to establish the minimum level at which ocular changes can be detected.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine in patients placed on tamoxifen the minimum level at which ocular changes are noted.

Methods Employed: All NCI metastatic breast carcinoma patients placed on tamoxifen are examined opthalmoscopically. In addition, psychophysical testing including color vision testing, cone thresholds, and dark adaptation are performed. When appropriate, fundus photographs are taken. Patients are reevaluated periodically, depending upon the total dosage achieved.

Major Findings: Ocular toxicity of tamoxifen has been discovered in five patients on high-dose tamoxifen for prologned periods.

Significance to Biomedical Research and the Program of the Institute: If the safe minimum level of tamoxifen can be recognized, then patients on this drug will not need to be monitored until such a dosage is reached.

Proposed Course: The project will continue for one additional year.

NEI Research Program: Retinal and Choroidal Diseases--Special Areas of Future Interest (Toxic and Environmental Disorders)

Publications:

Kaiser-Kupfer MI: Role of the ophthalmologist in the therapy of breast carcinoma. Trans Ophthalmol Soc UK 98:184, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00083-02 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Pathogenesis of Gyrate Atrophy and Trial of Pyridoxine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Muriel I. Kaiser-Kupfer M.D. Senior Staff Ophthalmologist CB NEI
Other: David Valle M.D. Assistant Professor, The Johns Hopkins School of Medicine

COOPERATING UNITS (if any)

Department of Pediatrics and Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with gyrate atrophy of the retina are examined systematically to confirm the diagnosis. Skin fibroblasts grown in tissue culture are assayed for ornithine aminotransferase activity. Other enzymatic activities related to ornithine metabolism such as ornithine decarboxylase activity will be measured. The results will be examined for correlation with the presence of homo- or heterozygosity for the disease trait. Patients will be given pyridoxine to see if the serum concentration of ornithine can be reduced, and if so, the patient will be classified as a "responder," and treatment with pyridoxine will be continued. Nonresponder and responder patients will be placed on a low arginine, low protein diet with supplemental amino acids and observed for an arrest or improvement of their disease.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 78 EI 01

Objectives: To determine the biochemical processes responsible for the elevated serum ornithine and the retinal lesion that occurs in gyrate atrophy of the retina. To determine which patients respond to pyridoxine treatment with a decrease in serum ornithine concentration. To determine if treatment of "responders" with pyridoxine and dietary manipulation will arrest the progress of the retinal atrophy.

Methods Employed: Patients suspected of having gyrate atrophy of the retina are examined according to a standard set of procedures to confirm the diagnosis. Serum ornithine concentration is measured periodically. Punch biopsies of the skin are grown in tissue culture, and their enzymatic activity related to ornithine metabolism is measured.

Major Findings: Patients with gyrate atrophy of the retina have been shown to have a deficiency of ornithine aminotransferase. A small percentage of patients with gyrate atrophy have a 30%-50% decrease of serum ornithine while on pyridoxine therapy. A single patient has been followed for 18 months on a low protein, low arginine diet and has been found to show an improvement in dark adaptation on this regime with lowered serum ornithine levels.

Significance to Biomedical Research and the Program of the Institute: Gyrate atrophy of the retina is the first of the genetically determined isolated severe retinal degenerations for which a specific biochemical concomitant defect has been demonstrated. The study will guide and test the efficacy of treatment for this blinding eye disease and serve as a model for the investigation of other genetically determined retinal degenerations.

Proposed Course: This project will be continued for three more years to further assess the knowledge of reduced ornithine in halting the chorioretinal degeneration.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Valle D, Kaiser-Kupfer MI, Del Valle L: Gyrate atrophy of the choroid and retina: Deficiency of ornithine aminotransferase in transformed lymphocytes. Proc Natl Acad Sci USA 74:5159-5161, 1977.

Valle D, Brusilow SW, Walser M, Kaiser-Kupfer MI: Hypoammonemia in gyrate atrophy of choroid and retina. Pediatr Res 12:513, 1978.

Valle D, Walser M, Brusilow SW, Kaiser-Kupfer M: Gyrate atrophy of the choroid and retina: Amino acid metabolism and correction of hyperornithinemia with an arginine deficient diet. J Clin Invest (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00011-05 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Pigment Dispersion With and Without Glaucoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Carl Kupfer	M.D.	Director		NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 220205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to compare patients having pigment dispersion syndrome with and without glaucoma. The data acquired may enable a determination of the risk of patients with pigment dispersion syndrome to develop glaucoma as well as add to understanding of the pathology of the disease state.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 76 EI 189

Objectives: To compare patients having pigment dispersion with and without glaucoma by documenting and following the clinical features and course of their disease and by evaluating the patient's performance on a variety of diagnostic tests. To determine the presence of abnormal aqueous humor dynamics using provocative testing in patients having pigmentary dispersion with and without glaucoma. To compare pigment dispersion with and without glaucoma with respect to possible genetic markers. To determine whether pupillary responses to light stimulation are abnormal in cases having iris transillumination.

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

Complete family history with detailed pedigree
Best corrected visual acuity with manifest refraction
Slit lamp examination
Visual field examination (Goldmann I_{2e} and I_{4e})
Applanation Goldmann tension (app)
Photography of iris transillumination
Goniophotography

At the next visit, the following examinations are performed:

Static perimetry
Base-line tonography and water-drinking tonography one hour later
Fasting blood sugar when indicated

At the third visit, the following examinations are performed:

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

At the fourth visit, pupillography is performed.

Major Findings: Patients may have pigment dispersion syndrome for as long as 20 years without developing glaucoma.

There may be a hereditary predisposition in some cases, as seen in a mother and daughter, two brothers, and a brother and sister.

Steroid testing and PTC taste testing do not appear to show any particular categorization of these patients. Recent evidence has indicated that HLA antigens in patients with pigment dispersion are also not significantly different than those in the normal population.

It may be noted that whether filtering procedures are performed or not, pigment may be lost from the trabecular meshwork in time.

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

Proposed Course: This project will be continued for three more years to continue to obtain data to further understand the knowledge about pigment dispersion syndrome.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Developmental Glaucoma/Secondary Glaucomas)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00062-03 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Progressive Essential Iris Atrophy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Carl Kupfer	M.D.	Director		NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.56	.44	.12

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients are being recruited with progressive essential iris atrophy with or without associated corneal disease. Information is being gathered to evaluate the clinical features and course of the disease process, to investigate aqueous humor dynamics in both affected and unaffected eyes, and to attempt to find genetic markers such as HLA and ABO antigens or physical correlates with the disease process.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 76 EI 219

Objectives: The objectives of the study are to develop a panel of patients with progressive essential iris atrophy and to study these patients to determine factors which may aid in understanding the pathophysiology of the disease process and to study the natural history of this disease. Measurements of aqueous humor dynamics, assessment of genetic markers such as HLA and ABO antigens and physical correlates, and iris fluorescein angiography to determine the role of the vasculature will be carried out.

Methods Employed: During the course of the evaluation the following procedures are performed:

Complete family history with detailed pedigree
Best corrected visual acuity with manifest refraction
Slit lamp examination
Visual field examination (Goldmann I_{2e} and I_{4e})
Photography of iris and iris transillumination
Gonioscopy and goniophotography
Iris fluorescein angiography and photography
Baseline tonography
A complete medical and dental evaluation
Dilated ophthalmoscopic examination
Stereophotographs of the optic nervehead

Major Findings: Histopathologic and electron microscopic study of iris and trabecular meshwork tissue has not indicated any clues to the pathogenesis of the disease process.

An ultrathin corneal contact lens is useful in certain patients to prevent recurrent rupture of corneal bullae.

Significance to Biomedical Research and the Program of the Institute: These data may contribute to an understanding of pathophysiologic factors involved in the rare entity of progressive essential iris atrophy. In addition, a careful study of the progression of the disease from the earliest signs will clarify the significance of corneal involvement and the status of outflow channels which may add to the understanding of the mechanism of glaucoma.

Proposed Course: The project will continue for four more years in an effort to obtain more data regarding the pathophysiology of this process.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Developmental Glaucoma/Secondary Glaucomas)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00060-03 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Visual Function and Ocular Pigmentation in Albinism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Muriel I. Kaiser-Kupfer M.D. Senior Staff Ophthalmologist CB NEI
Other: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.15	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with hypomelanotic disorders such as ocular albinism, oculocutaneous albinism, Chediak-Higashi Disease, Hermansky-Pudlak Syndrome and iris transillumination defects are being recruited to determine visual function and to evaluate its course over time. Family members are evaluated to attempt to determine factors which may identify the heterozygous state.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 76 EI 207

Objectives: The objectives of the study are to relate the level of visual function to the amount of ocular pigmentation, especially iris and retinal pigmentation; to correlate the amount of nystagmus with visual acuity and iris pigmentation; to determine whether ocular pigmentation, visual acuity, and nystagmus change with age; and to identify the heterozygous state in family members.

Methods Employed: The following examinations are performed:

Complete family history with detailed pedigree
Best corrected visual acuity at near and distance with refraction
Slit lamp examination
Psychophysical testing including D-15 and Munsell 100
hue, rod and cone thresholds
Dilated ophthalmoscopic examination
Hair bulb incubation
Photography to document hair color, eye colorcolor, iris
transillumination, disc, and macula

Examination of family members includes:

Best corrected visual acuity
Slit lamp examination of iris
Photography of iris transillumination
Fundus examination when vision not corrected to 20/20

Major Findings: Examination of patients and family members indicates that the finding of transillumination of the iris may be seen in the absence of recognized albinism. The pattern appears to be punctate and may be present in a diffuse manner or limited to the 6 o'clock sector.

Significance to Biomedical Research and the Program of the Institute: These data may allow identification of the carrier state in albinism which would be of importance in genetic counselling. In addition, it may be possible to determine whether the development of the fovea is abnormal in albinism, and if this is the cause of the decreased visual acuity in albinism or whether decreased visual acuity is secondary to hypopigmentation, and the resultant light-scatter and glare. In addition, it will be possible to ascertain whether visual acuity improves with age and if this is correlated with changes in pigmentation.

Proposed Course: This project will be continued for five more years in order to obtain additional data.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00084-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Anterior Chamber Anomalies Associated with Glaucoma or Ocular Hypertension

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carl Kupfer	M.D.	Director		NEI
Other:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

With recent embryological research indicating the role of the neural crest in contributing to all connective tissues anterior to the lens epithelium, the group of developmental anomalies of the anterior chamber with glaucoma or ocular hypertension are being reviewed.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 77 EI 119

Objectives: The objective of this study is to determine whether congenital and/or developmental anomalies of the anterior chamber are related to faulty migration or terminal differentiation of neural crest tissue.

Methods Employed: Patients of all ages with congenital and/or developmental anomalies of the anterior chamber are being examined clinically to determine involvement of cornea, trabecular meshwork, iris stroma, and ciliary body. When intractable glaucoma is present that cannot be controlled with medication, surgery will be performed and the specimens examined histologically.

Major Findings: It appears that in this group of anomalies of anterior chamber development there are pathological changes in one or several tissues derived from neural crest. These include corneal stroma, corneal endothelium, anterior iris stroma, Descemet's membrane, and trabecular meshwork endothelium.

Significance to Biomedical Research and the Program of the Institute: A better understanding of the pathogenesis of these glaucomas may help in improving diagnosis and treatment.

Proposed Course: Patients with other anomalies of the anterior chamber including congenital cataracts will be examined for abnormalities in tissue derived from neural crests.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Developmental Glaucoma)

Publications:

Kupfer C, Kaiser-Kupfer M: New hypothesis of developmental anomalies of the anterior chamber associated with glaucoma. Trans Ophthalmol Soc UK 98:213-215, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00013-08 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Study of Pharmacodynamics of Various Agents Affecting the Intraocular Pressure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Frank J. Macri Ph.D. Pharmacologist CB NEI
Other: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The physiology and pharmacology of aqueous humor production and intraocular pressure are being studied by the use of an enucleated, arterially perfused eye. Although it is generally assumed that all drugs applied to the cornea exert their effect on intraocular receptors, data obtained during current studies suggest a reevaluation of these concepts may be in order. We have found that when PGE_2 is administered in the arterial circulation, no direct effect on the eye is apparent. However, the topical application of PGE_2 causes a marked increase in the rate of aqueous humor formation and of IOP. A more provocative indication for the involvement of corneal receptors is the ability of cold air applied to the cornea to cause a lowering of both functions.

The mechanism by which timolol causes a lowering of IOP in humans is still an enigma. We have found that timolol is a competent B-adrenergic blocking agent in the enucleated eye. Yet, though we have no difficulty in demonstrating this type of activity, we are still unable to demonstrate an ocular hypotensive action in this eye preparation.

PHS-6040
(Rev. 10-76)

Project Description:

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

Methods Employed: Studies were performed, in situ, on eyes of anesthetized cats and on enucleated arterially perfused eyes. In the latter, the perfusate is channeled throught 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 ^{125}I tagged serum albumin.

Major Findings: We reported in last year's summary that the application of cold air to the cornea of the enucleated, arterially perfused cat eye induced a lowering of the rate of aqueous humor production and a decline of intraocular pressure. The finding was so unusual and unexpected that it appeared that the response might have been caused by some abnormality in the isolated eye preparation. The experiments were repeated on the living cat eye with almost identical results. Cold air applied to the cornea of the eye caused a marked decrease in the rate of aqueous humor production; however, the intraocular pressure remained little affected.

We also reported last year that the intra-arterial administration of some prostaglandins had no direct effect on the rate of aqueous humor formation or on the intraocular pressure. However, administration of the PGs by this route caused only a decrease in the formation rate of aqueous humor, but only after the inflow rate had been stimulated by acetylcholine. There is a general unanimity of published results indicating that when the PGs are applied to the cornea of the living eye, the eye pressure increases and the protein content of the anterior chamber becomes elevated. It has been assumed that the amount of fluid formed in the anterior chamber is also increased. With the prospect in mind that the lack of effect of PG on the eye might be due to a marginally functioning enucleated eye, it was decided to test the PGs once again by applying it directly to the cornea. Under this condition the eye pressure became elevated and the rate of aqueous formation increased--findings identical to those observed in the living animal.

The mechanism by which timolol causes a decrease in the eye pressure of humans is still an enigma to us. We have found that timolol has strong β-adrenergic blocking properties in the enucleated eye in that it can completely block the ocular hypotensive action of d,ℓ-isoproterenol but is without effect in inhibiting the similar action of ℓ-epiniphrine or that of ℓ-norepinephrine.

Studies have begun to determine the physiologic status of the enucleated arterially perfused eye. Using protein distribution ratios (aqueous humor/plasma) as a criterion, no differences could be discerned between the enucleated eye and eyes in situ.

Significance to Biomedical Research and the Program of the Institute: We have evidence of the possible presence of corneal receptors which can influence the rate of aqueous humor production. Cold air applied to the cornea lowers the IOP and the rate of aqueous humor formation. This is an immediate and easily reversible response. PGE_2 administered via the arterial supply causes a decrease in the rate of aqueous humor inflow and of IOP. The topical application of PGE_2 to the cornea, however, causes the inflow rate and the IOP to become elevated. The concept of the presence of local corneal receptors which can influence the eye certainly is not well documented but is suggestive enough that an effort should be made to study this point further.

It has generally been assumed that timolol lowers IOP in patients due to its well-known β-adrenergic blocking properties. We have been able to demonstrate this type blockade in the enucleated, arterially perfused eye but have been unable to demonstrate a parallel decrease in IOP or of aqueous humor production. We believe that other activities of timolol should be examined so that its pharmacologic activity on the eye can be explained in a more rational manner.

Proposed Course: The principal investigator will assume a university position before the next year. This project will not be continued.

NEI Research Program: Glaucoma--Hydrodynamics of the Eye

Publications:

Macri FJ, Cevario SJ: The formation and inhibition of aqueous humor production: A proposed mechanism of action. Arch Ophthalmol 96:1664-1667, 1978.

Macri FJ, Cevario SJ: Clonidine: Effects on aqueous humor formation and intraocular pressure. Arch Ophthalmol 96:2111-2113, 1978.

Ross KS, Macri FJ, Kupfer C: The effect of carotid artery ligation on aqueous humor formation in the Rhesus monkey (Macaca mulatta). Exp Eye Res 27:687-690, 1978.

Helal J Jr, Macri FJ, Cevario SJ: Timolol inhibition of aqueous humor production in the cat. Gen Pharmacol (in press).

Macri FJ, Cevario SJ: Inhibition of aqueous humor flow by application of cold air to the cornea. Exp Eye Res (in press).

Macri FJ, Cevario SJ, Helal J Jr: Timolol inhibition of isoproterenol action I effects on aqueous humor production and IOP. Exp Eye Res (in press).

Macri FJ, VanAlphen GWHM: The effects of prostaglandins on aqueous humor dynamics. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00097-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemistry and Biology of Normal and Pathologic Retinochoroidal Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Newsome	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	John R. Hassell	Ph.D.	Senior Staff Fellow	CB	NEI
	Jeffrey Gross	B.S.	Microbiologist	CB	NEI

COOPERATING UNITS (if any)

Hazleton Laboratories
Section on Retinal and Corneal Metabolism, Laboratory of Vision Research, NEI

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.85	0.35

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations are being conducted into aspects of specialization of the primate and human retina into the macula, as well as into tissue-tissue interactions which are necessary for the maintainance of a normally functioning retinochoroidal complex. The macula-specific concentrations of various cyclic nucleotides have been investigated by radioimmunoassay in normal and diseased human as well as rhesus monkey retinas, pigmented epithelia, and choroids. By performing controlled experiments with rhesus monkey retinochoroidal tissues, the time course of stability of the stable pool of cyclic nucleotides in these tissues was determined and used as a basis for interpreting the human studies. Certain enzymes such as tyrosinase have also been measured in geographically specific areas of the retinochoroidal complex. The sensitivity of retinal-pigmented epithelial and choroidal cells to immunoglobulin labeled erythrocytes in normal Irish setter pups and those with inherited rod-cone displasia was tested. The specificity of the ingestion of radioactively labeled photoreceptor outer segments was also examined by cell culture techniques using liquid scintilation counting and electron microscopic detection of phagosomes for confirmation.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Although the functional and anatomical specialization of the macula is well-known and easily observed, the cellular and tissue mechanisms which provide for this specialization are not well understood. A major goal of this project is to define, using a variety of techniques including histochemical, biochemical, tissue culture and histological methods, those functions which create the special visual ability of the primate and human macula. We also want to investigate the interactions of the various tissue layers in the retinochoroidal complex that serve to provide metabolic support, one layer for the other, as well as to look for possible alterations in various levels of metabolically important compounds which may explain disease processes. To this end a multidisciplinary approach including a variety of techniques is brought to bear on the important problems concerning these metabolic functions. Emphasis is placed on the maintenance of integrity of the choriocapillaris/Bruch's membrane/pigmented epithelium subportion of the complex.

Methods Employed: Biochemical techniques include a modified radioimmunoassay for cyclic nucleotides, as well as a modified adaptation of a standard radioactive water-release assay for tyrosinase. Other techniques employed include microdissection, organ tissue and cell culture specimens, electronmicroscopy, both transmission and scanning, in vitro labeling of photoreceptor outer segments and their subsequent separation and collection by differential centrifugation, and light microscopy. Immunological techniques included the incubation of control and variously sensitized sheep erythrocytes with pigmented epithelium cells that had been collected either fresh or resuspended from cultures.

Major Findings: The cyclic nucleotides, cyclic GMP and cylic AMP, were found to be present in lower concentrations in the central macula region of the human neural retina than in more peripheral retinal areas. This pattern followed the gross distribution of rod photoreceptor cells. Surprisingly there was an inverse gradient of cyclic GMP concentration observed in the retinal pigmented epithelium; levels in the central pigmented epithelium were fourfold higher than in pigment epithelium cells from the periphery. In two specimens of diabetic retina, cyclic AMP but not cylic GMP was found to be several-fold higher than in the normal specimens.

Tyrosinase activity, measured in cultured retinal pigmented epithelium cells from the submacular region, as well as from more peripheral regions of the retina, was found to persist in cultures of those cells derived from the more peripheral regions. The presence of this activity seemed to correlate with the preservation of more differentiated morphology in culture.

Freshly harvested, or more usually, cultured, pigmented epithelial cells from normal or age-matched, rod-cone displastic Irish setter pups were exposed to controlled, unsensitized sheep erythrocytes and sheep erythrocytes sensitized with immunoglobulin G. Positive binding was assessed by quantitating rosette formation and revealed that a significant number of pigmented epithelium cells from normal animals formed Ig G-dependent rosettes. This binding was trypsin insensitive. Displastic dog cells had three to fourfold lower binding than those

cells from normal animals. Cultured normal conjunctival fibroblasts, not known to be phagocytic in vivo, showed no binding. Neither pigmented epithelial cell type formed rosettes with unsensitized erythrocytes. The maximal binding observed with pigmented epithelial cells was approximately one-fourth that observed with peripheral blood monocytes from normal or displastic animals.

Significance to Biomedical Research and the Program of the Institute: The macula region falls victim to a variety of blinding disease processes which seem to have a predilection for this specialized central retinal area. Knowledge gained about cellular and molecular mechanisms which provide for the specialization of this fine-vision area is crucial, not only to our understanding of the normal functioning of the macula, but also to pathological processes. Indeed, certain biochemical alterations in animal models of human retinal disease have indicated that cyclic nucleotide levels may even be causally related to certain types of retinal degenerative disease.

Surface properties of specialized phagocytes, as well as the particles they anteriorize, influence the regulation of phagocytosis. Monocytes and other leucocytes from several species have been shown to possess immunoglobulin and complement-dependent surface receptors. Our demonstration that canine retinal pigmented epithelial cells also bind Ig G-sensitized particles and that this binding is altered in cells from animals with rod-cone displasia indicates that certain immune systems may play an important role in regulating pigmented epithelium phagocytosis of shed photoreceptor outer segment discs. Because abnormalities of photoreceptor-renewal processes, have been shown to play a role in certain animal models of human disease, a greater understanding of the possible role of the immune system in these critical processes could be crucial to advancing our understanding of and developing possible treatments for various degenerative processes.

Proposed Course: Observations of cyclic nucleotide and other biochemical parameters in diseased human ocular tissues will be continued as specimens become available. Attempts will be made to increase the number of potential donors to the Clinical Branch pathological eye tissue program, as well as to develop sources, through departments of Ophthalmology at academic institutions, to obtain human material for study.

Investigations of the possible role of the immune system in the regulation of phagocytosis by the pigmented epithelium will be continued and expanded in animal models, probing particularly the relationship between exposure of pigmented epithelium to shed outer segments and the initiation of phagocytic activity.

Studies are also being initiated to investigate the sources and methods of turnover and renewal of Bruch's membrane. It is expected that these investigations in the retinochoroidal complex will be crucial to our understanding of the functioning of the pigmented epithelium as a selective barrier for nutrients and other vital metabolic factors for the photoreceptor outer segments.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Newsome DA, Fletcher RT, Chader GJ: Human retinal cyclic nucleotides vary by area. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00098-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Clinical and Laboratory Studies in Macular and Tapetoretinal Degenerations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Newsome	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Helen H. Hess	M.D.	Medical Officer (Research)	CB	NEI
	Ann Rahe	A.B.	Biologist	CB	NEI

COOPERATING UNITS (if any)

Bureau of Biologics, Food and Drug Administration

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Clinical investigations are underway to determine the early natural history of senile macular degeneration. Drusen, and serous and hemorrhagic detachments of the retina and retinal pigmented epithelium, as well as choroidal neovascularization, are considered manifestations of senile macular degeneration and will be studied by serial recordings of the anatomical appearance and visual functioning of eyes at high risk of developing disease. These results will be compared with those obtained from fellow eyes with more advanced disease, as well as a matched group of normal eyes. Clinical chemical studies of serum levels of hormones such as cortisol, thyroid hormones, and of trace metal ions will also be conducted. The metabolism of trace metal ions will be explored further by quantitating the inexcretion in urine over a 24-hour period. By emphasizing investigations in affected family clusters, and using various genetic markers such as HLA antigens to segregate the affected from the nonaffected members of family clusters, it is hoped that those factors which are materially associated with the appearance and/or progression of various retinal degenerative conditions will be elucidated.

Project Description:

Protocol Number: 79 EI 16

Objectives: Senile macular degeneration has been extensively studied in its more advanced forms and its devastating effects on vision are well known. However, little is known of the early natural history of the disease, particularly the relationship of anatomical findings such as drusen and retinal pigmented epithelial detachments to the presence and progress of the disease. It has also been suggested, but not well substantiated, that senile macular degeneration is a dominantly inherited form of retinal dystrophy, casting doubt on the theory that the pathogenesis of the disease is simply linked to degenerative changes associated with aging. A variety of studies have been published which indicate that alterations in the metabolism of copper in the body has a signigicant effect on pigmentary and perhaps other retinal degenerations. Other published experimental evidence has indicated a significant influence of various hormones, such as thyroid hormones, on the integrity and differentiation of retinal pigmented epithelial cells in vitro. This project was designed to bring multiple disciplines to bear in a broad-scale investigation of this complex family of retinal degenerative disease processes, with particular emphasis on understanding the sites at which the disease processes are initiated, the interrelationship of the various tissues in the retinochoroidal complex as the disease advances, and learning more about the possible genetic linkage of senile macular disease.

Methods Employed: Patients will be recruited into the study by referral from an ophthalmologist. Patients will be admitted into the study according to the official protocol, clinical research project 79 EI 16. Patients will be subjected to a routine ophthalmological examination, complemented with blood and urine studies, genetic typing, electrophysiological and psychophysical studies, fluorescein angiography when indicated, and serial visits to follow the progress of the disease through time. Normal age and sex-matched controls are used for companion studies when indicated.

Major Findings: A significant number of patients with pigmentary tapetoretinal degeneration have been found to have a cluster of elevated serum values of various hormones and trace metals. These alterations seem to run in a distinct pattern within certain families that can be segregated according to their genetic typing. In the course of the study, a family with a neverbefore described type of pigmentary macular retinal degeneration was discovered and thoroughly evaluated. These findings shed further light on the progression of changes in the retinal vasculature and changes in the retinal pigmented epithelium layer in this disease. The 24-hour urinary excretion of zinc was found to be elevated in certain patients with retinitis pigmentosa, but not in others. Twenty-four hour urinary copper excretions have generally been within normal limits.

Significance to Biomedical Research and the Program of the Institute: Senile macular disease is the leading cause of legal blindness in the United States and the United Kingdom and perhaps more frequently, the cause of

disability or impairment less severe than legal blindness. There is no effective treatment at present for this disease nor is there for all tapetoretinal degenerations, which, taken as an aggregate, form the leading cause of untreatable blindness in the world. Some of the processes which appear to constitute the disease picture, such as neovascularization, do occur in other parts of the body. Knowledge gained from this study should be instrumental in understanding the progression and the possible inheritance of these diseases and should contribute to devising studies of more effective modes of treatment. In addition, basic facts which may be learned about certain processes involved in these diseases could have wide applicability to various tissues and organ systems in the body.

Proposed Course: Patients will continue to be recruited into this study during the coming year and will be evaluated thoroughly and followed for a period of three to five years. The recruitment goal is 100 patients. Because of positive findings in the clinical studies, laboratory experiments to evaluate the importance of these observations and their possible effect on ocular tissues will be performed both with animals and with cultured cells. By combining a clinical and laboratory approach, it may be possible to learn information which will point to possible therapeutic trials. Special attention will be given to improving the collection of pathological human material for study and to expanding the already active tissue donor program among the patients under study here.

NEI Research Program: Retinal and Choroidal Diseases--Macular Diseases

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00057-01 CB

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Ocular Connective Tissues Macramolecules and Their Function in Vision

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Newsome,	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	John R. Hassell	Ph.D.	Research Biologist	CB	NEI
	Jeffrey Gross	B.S.	Microbiologist	CB	NEI
	Ann Rahe	B.A.	Biologist	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The structure and function of the major connective tissue macromolecules, collagen and proteoglycan, are being examined in animal models and human diseases. Samples of cornea, sclera, trabecular meshwork, and choroid are either radiolabeled in organ culture or cells derived from these tissues are grown and labeled in cell culture. The macromolecular products synthesized by these cultures are characterized using biochemical procedures. Alterations in the normal composition of collagens and/or proteoglycans may accompany or even be the basis for certain visually disabeling diseases.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The extracellular matrix of connective tissues consists of an orderly network of collagen fibers, proteoglycans and glycoproteins. The presence, interaction, and arrangement of these structural macromolecules is crucial to the normal function of these tissues, such as the optical clarity of cornea and the outflow rate of the trabecular meshwork. The purposes of this study include characterization of the collagens, proteoglycans, and glycoproteins normally present in the cornea, sclera, trabecular meshwork, and choroid and the determination of the alterations that occur in these macromolecules in certain ocular diseases.

Methods Employed: Either ocular connective tissue samples will be radiolabeled in organ culture or cells derived from these tissues are grown and labeled in cell culture. The biosynthetically labeled products are characterized using molecular sieve chromatography, DEAE-cellulose chromatography, CMC-cellulose chromatography, and gel electrophoresis, as well as with specific enzymes, such as collagenase, chondroitinase, keratanase, glycosidases, papain, and pepsin. Chemical characterization, in terms of amino acid and carbohydrate analysis, are also conducted.

Major Findings: The keratan sulfate proteoglycan and the chondroitin sulfate proteoglycan, the two major proteoglycans of the corneal stroma, were isolated from monkey corneas and characterized. Normal human corneas also contain these two proteoglycans. However, corneas from patients with corneal macular dystrophy contain only the chondroitin sulfate proteoglycans and not the keratan sulfate proteoglycan. Macular dystrophy corneas do contain an unusual glycoprotein not found in normal corneas. Stromacytes from macular corneas synthesize the normal proportion of collagen types. The unusual glycoprotein in macular corneas may represent the accumulated material which produces the corneal clouding.

Stromacytes from normal corneas synthesize type I collagen as the major collagenous product. Stromacytes from patients with granular dystrophy synthesize equal amounts of types I and III collagen. Granular corneas contain normal amounts of the two stromal proteoglycans. The abnormal ratio of type I to III collagen may be responsible for corneal clouding in granular dystrophy.

Significance to Biomedical Research and the Program of the Institute: Connective tissue is by far the predominant tissue of the eye. It is likely that alterations in the quantity or quality of the macromolecules which comprise these tissues will be the basis of certain blinding and visually disabling ocular diseases.

Proposed Course: This study may provide information that will allow the formulation of testable therapeutic modalities. The project will continue by utilizing appropriate animal models and human material. Antibodies to purified glycoconjugates will be prepared for use in clinical and basic research.

NEI Research Program: Corneal Diseases--Corneal Edema, Dystrophies, and Inherited Disorders

Publications:

Newsome DA: Embryology and biology of the ocular surface. Int Ophthalmol Clin 19:53-72, 1979.

Newsome DA, Foidart JM, Kratchmer JH, Hassell JR, Rodrigues MM, Katz SI: Detection of specific corneal collagen types in normal and keratoconus corneas. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00093-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cataracts in Juvenile Guinea Pigs with Allergic Encephalomyelitis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Nussenblatt	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Sanford Stone	Ph.D.	Head, Immunology Unit	OSD	NIAID
	Fran Cross		Microbiologist	OSD	NIAID

COOPERATING UNITS (if any)

Department of Pathology, Albert Einstein College of Medicine, New York 01461
Office of the Scientific Director, NIAID

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.3	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Allergic encephalomyelitis is a central nervous system disease of immunologic origin. Juvenile strain 13 guinea pigs developed cataracts during severe allergic (autoimmune) encephalomyelitis syndromes produced actively or by transfer of living lymph node cells from sensitized strain 13 donors. These lens changes were manifested bilaterally within a two-week period of active sensitization or transfer of sensitized cells. The morphologic in vivo appearance of these cataracts is similar to both the galactosemic induced and tryptophan deficiency cataract models. A better understanding of the etiology of these lesions not seen before in this entity in guinea pigs will help in understanding cataract formation in systemic disease.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To investigate the etiology of cataract formation in young inbred animals which develop an acute autoimmune neurologic disease.

Methods Employed: Induction of allergic encephalomyelitis in juvenile strain 13 guinea pigs is accomplished in one of two ways. The first method for immunization of these animals is the injection of guinea pig spinal cord in complete Freund's adjuvant into multiple nuchal sites. A second method is the induction of the disease in strain 13 adults or juveniles with the subsequent transfer of immunologically active cells to the histocompatible juvenile animals.

A careful evaluation of each animal is standardly done, watching for evidence of weight loss, urinary incontinence, hind-limb wasting, and cataracts.

Major Findings: The majority of juvenile strain 13 animals which were recipients of transfers of lymph node cells from histocompatible juvenile or adult donors using donor-recipient ratios of 4:1 showed bilateral cataracts. A large number of those actively immunized also manifested the same lesions. The opacities are first located in the cortex and have a doughnut appearance, with fully opacified lenses being the end result. These lesions did not appear in nonhistocompatible guinea pig recipients.

Significance to Biomedical Research and the Program of the Institute: Cataract formation in guinea pigs has never been reported before with induction of this well-known immunologic model. This cataract model could provide an understanding as to how systemic diseases may alter the ocular environment so as to induce lenticular opacities.

Proposed Course: Biochemical studies will be done to identify the basis for the lens changes. Emphasis will be placed on attempts at preventing the induction of these cataracts during the disease.

NEI Research Program: Cataract--Congenital, Metabolic, and Genetic Cataracts

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00092-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

HLA, ABO, and B-cell Alloantigens and Ocular Inflammatory Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Nussenblatt	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Kamal K. Mittal	Ph.D.	Research Microbiologist	BB	FDA

COOPERATING UNITS (if any)

Bureau of Biologics, FDA

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with ocular toxoplasmosis, pars planitis, sarcoidosis, Behcet's disease, chorioretinitis of unknown origin, and iridocyclitis of unknown etiology are being studied to determine the phenotype frequency of the HLA, ABO, and B-cell alloantigens. Since the B-cell alloantigens or DR antigens are thought to play a role in the immunologic response to antigens, these findings will complement other immune uveitis studies being simultaneously carried out.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 79 EI 48

Objectives: To determine whether ocular inflammatory disease manifests specific HLA or B-cell alloantigens more frequently than the average population.

Methods Employed: Heparinized blood samples from patients are subjected to microcytotoxic tests to determine the HLA and B-cell antigens. The ABO system is evaluated utilizing an anti-sera method.

Major Findings: No test results will be available until a sizeable and statistically representative sample has been studied.

Significance to Biomedical Research and the Program of the Institute: The role of HLA and B-Cell alloantigens in the immune response is only beginning to unfold. This study will indicate if these alloantigens play a role in the ocular immune response.

Proposed Course: This study will continue in order that sizeable populations of various ocular immune entities will be studied.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00075-02 CB

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Immune Functions in Ocular Diseases of Obscure Etiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Nussenblatt	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI
	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
	Sandra Bornstein		Technician	CB	NEI

COOPERATING UNITS (if any)

Laboratory of Vision Research, NEI
Department of Ophthalmology, University of Louisville, Louisville, Kentucky

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.5	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro cellular immune functions are being studied in a single masked method in patients with ocular toxoplasmosis, presumed ocular histoplasmosis, pars planitis, Behcet's disease, ocular sarcoid, and chorioretinitis of unknown origin. Crude ocular antigens as well as the purified uveitogenic soluble antigen (S-antigen) of the retina are being used in a lymphocyte microculture technique in order to evaluate the presence of cellular immune memory to ocular tissues. Immune memory is also evaluated by the production of lymphokine in a capillary migration system. A subgroup of patients have been identified as having this immunologic memory. Other studies concentrate on the presence of suppressor cell activity and functioning of macrophages in these patients. These results shed light on the basic mechanisms of uveitis and may be used as a guide for specific immunologic therapy.

PHS-6040
(Rev. 10-76)

Project Description:

Protocol Number: 79 EI 49

Objectives: The objective of this study is to investigate several immunologic factors in ocular inflammatory disease and how they may relate to the course and chronicity of this disease. The identification of groups with specific immunologic alterations provide us with a more rational approach to therapy.

Methods Employed: The ophthalmic examination of all patients includes slit lamp examination, visual field tests, electroretinogram, and fluorescein angiography. Lymphocyte cultures are prepared using the microculture technique, where the immune cells are tested against various crude ocular extracts, as well as purified human and bovine S-antigen, in order to assess evidence of cellular immune memory which is considered to be the in vitro equivalent of the anamnestic response in vivo. The capillary migration system is used to evaluate migration inhibition of macrophages, a test considered as an in vitro equivalent of lymphokine production in vivo. Suppressor cells from patients during latent and active ocular disease are induced in the laboratory by using Conconavalin A, with their suppression capabilities tested in vitro in the presence of fresh responder cells and mitogens. Suppressor cell activity is also evaluated by the use of suboptimal doses of Conconavalin A in culture, as reported by Bresnihan and Jasin (J Clin Invest 59:109, 1977). Macrophage activity is studied by examining their production of lymphocyte activating factor.

Major Findings: A subpopulation of patients with ocular inflammatory disease manifested a positive "memory" response to the S-antigen. Positive responders appear to be those with active or inactive retinal lesions, and patients with various diseases were found to respond. It therefore appears that similar immune groups are present in different clinical entities.

Some patients with posterior uveitis respond to crude retinal extracts but not to the S-antigen, indicating the possible role of other retinal antigens still to be purified.

Significance to Biomedical Research and the Program of the Institute: Uveitis is the cause of five percent of legal blindness in the United States. This is the first time that patients' immune cells have been shown to manifest cellular immune memory to a purified retinal antigen.

The grouping of patients with uveitis on the basis of specific immunologic functions or alterations may provide a more rational basis upon which to develop specific immunotherapy. Elucidation and treatment of inflammatory conditions of the eye are major interests of the NEI.

Proposed Course: This continuing study will focus on the posterior uveitic entities in order to investigate further the role of the S-antigen

in each of these, and what, if any, role abnormal suppressor cell activity may play.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

BenEzra D, Nussenblatt R: Ocular manifestations of Behcet's disease. J Oral Pathol 7:431, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00094-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Immune Mechanisms in Experimental Autoimmune Uveitis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Nussenblatt	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI

COOPERATING UNITS (if any)

Department of Ophthalmology, University of Louisville, Kentucky

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Guinea pig strain 13 animals, immunized at a site distant to the eye with the Soluble antigen (S-antigen) of the retina in complete Freund's adjuvant will develop ocular inflammatory disease. Depending on the antigen immunizing dose, the ocular lesions can vary from an iridocyclitis to a panuveitis. Lymph node cells or nonadherent T-cells obtained from peritoneal exudate cells from immunized animals manifested significant cellular immune responses whether measured by the lymphocyte culturing technique or by evidence of the production of migration inhibition factor (MIF) of macrophages. In vivo lymphoproliferative responses to the S-antigen can be suppressed by histocompatable Concanavalin A (Con A) induced suppressor cells, indicating a possible role for in vitro immunoregulation.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This study is designed to elucidate the basic immunologic mechanisms of this laboratory model for uveitis and how this model may be altered or regulated.

Methods Employed: One group of strain 13 guinea pigs is immunized with purified S-antigen in complete Freund's adjuvant in one hind footpad. Evidence of ocular inflammatory disease is monitored via slit lamp and ophthalmoscopic examinations. After three weeks, lymph node cells are collected and used for several cellular immune studies. Lymphocyte cultures are prepared in microtiter plates and are stimulated with S-antigen. In some cultures concanavalin A induced, mitomycin-C treated, and α-methyl mannoside washed isogenic suppressor cells are added. Other lymph node cells from immunized animals are mixed with isogeneic macrophages in order to demonstrate the release of migration inhibition factor in the presence of S-antigen. Other groups of strain 13 animals receive "protecting" doses of suppressor cells before and after the immunization process. Antibodies are evaluated by gel diffusion techniques, and eyes are taken for histology.

Major Findings: Immunized animals develop obvious clinical anterior and posterior uveitis which is confirmed by histology. Animals with ocular disease manifest significant cellular immune memory responses when measured by lymphoproliferative and macrophage inhibition techniques.

Concanavalin A-induced suppressor cells appear able to suppress the in vitro lymphoproliferative response normally seen when immunoactive cells from immunized animals are stimulated with S-antigen. Initial evidence suggests that in vivo therapy with suppressor cells may alter the clinical response of immunized animals.

Significance to Biomedical Research and the Program of the Institute: Experimental autoimmune uveitis is the first uveitis model utilizing a purified retinal antigen. The mapping out of its immune mechanisms may lead to an improved understanding of human ocular inflammatory disease. Immunoregulatory models developed in this system may be utilized in future human clinical trials.

Proposed Course: To describe fully the underlying immune events in this disease, and to develop a successful protocol dealing with either specific or nonspecific suppression of the disease.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00078-02 CB

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemistry and Morphology and Human Control Dystrophies and Degeneration

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Merlyn M. Rodrigues	M.D.	Medical Officer	CB	NEI
Other:	David Newsome	M.D.	Senior Staff Ophthalmologist	CB	NEI
	John S. Hassell	Ph.D.	Staff Fellow	CB	NEI

COOPERATING UNITS (if any)

Department of Ophthalmology, University of Iowa

LAB/BRANCH

Clinical Branch

SECTION

Section on Clinical Eye Pathology

INSTITUTE AND LOCATION

National Institutes of Health, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Human corneal dystrophies and degenerations, which have been clinically documented, are studied as keratoplasty specimens with histochemical stains, scaning and transmission electron microscopy, and immunologic techniques in an attempt to elucidate pathogenetic mechanisms. This approach has provided insight into cell-cell relationships in the normal and diseased states. Cell cultures performed in selected cases are examined by scanning and transmission electron microscopy. Tissue and cell culture studies have demonstrated in vivo proliferation of corneal cells including epithelialization of the endothelial layer in corneas of three patients with posterior polymorphous dystrophy. Two patients with benign monoclonal gammopathy have shown abnormal extracellular corneal stromal deposits. In a patient with macular corneal dystrophy intracellular and extracellular accumulation of fibrogranular material was observed in the corneal stroma, Descemet's membrane, and corneal endothelium. The presence and production of collagen, glycoconjugates, and collagenase has been investigated with immunofluorescent, electrophoretic, and chromatographic methods.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The study attempts to combine detailed, clinical and genetic studies of patients with human corneal diseases, particularly corneal dystrophies, in order to obtain further insight into the mechanisms of corneal opacification.

Methods Employed: Corneal specimens from transplant patients were divided into portions and used separately for light, scanning, and transmission electron microscopy. These data provided insight into the morphological appearance of the cells and the extracellular materials of the corneal layers. Other portions of the surgical specimens were placed into tissue and cell culture to allow examination of the morphology and biosynthetic activities of the cells of the three corneal layers. Indirect immunofluorescence has shown the range of collagen types present in normal and abnormal tissue. Column chromatography and electrophoresis have provided information about the collagen and glycoconjugate biosynthetic patterns of abnormal tissues.

Major Findings: All four corneal buttons from patients with hereditary posterior polymorphous dystrophy had an admixture of epithelial-like and endothelial cells on the posterior surface of Descemet's membrane which is normally lined by a monolayer of endothelial cells. The epithelial-like cells were characterized by numerous microvillous projections, prominent desmosomal cell junctions, intracytoplasmic filaments, and scant mitochondria. The adjacent endothelial cells displayed gap junctional complexes, numerous mitochondria with horizontal disposition of cristae, and prominent Golgi. Cells cultured from the corneal endothelium exhibited a similar admixture of cells with epithelial-like and endothelial characteristics. Corneal tissue with lattice dystrophy stained positive for amyloid with Congo red and displayed dichroism. Immunofluorescence and biochemical studies are in progress to characterize the type of amyloid present. In a patient with macular corneal dystrophy, corneal buttons were obtained from both eyes, examination revealed abnormal accumulation of glycosaminoglycan in the corneal stroma as well as in Descemet's membrane and corneal endothelium. The deposits were composed of fibrillogranular material and stained positive with stains for glycosaminoglycan. In the case of Schnyder's corneal crystalline dystrophy, cholesterol and lipid aggregates were demonstrated in the subepithelial layer and the superficial stroma. This patient appears to represent a rare sporadic example of this entity since the usual autosomal dominant inheritance was lacking. Keratoconus specimens had the same range of collagen types as normal cornea, with predominantely type I collagen. Type III collagen was detected only in scarred areas. Radioactive labeling experiments on cultured cells from these corneas have demonstrated an elevated production of collagenase compared with the normal. In two patients with benign monoclonal gammopathy, Kappa light chains of immunoglobulin were present in the serum and urine, and stromal crystals showed deposits of immunoglobulin in the cornea. In both patients deposits of immunoglobulin were present in the corneal stroma.

Significance to Biomedical Research and the Program of the Institute: The mechanisms of opacification and destruction of the cornea in a variety of human diseases must be understood for the improved diagnosis and classification of these entities. This may also lead to a more rational basis for the appropriate treatment of these visually disabling processes. A thorough knowledge of the genetic component of these disorders, if any, will aid in more effective and complete genetic counseling.

Proposed Course: Patient material will be entered into this combined study as it becomes available. Emphasis will be placed on elucidating pathogenetic mechanisms in hereditary posterior polymorphous dystrophy, keratoconus, and lattice and granular dystrophies. The use of immunological techniques will be expanded to a wider variety of specimens.

NEI Research Program: Corneal Diseases--Corneal Edema, Dystrophies, and Inherited Disorders.

Publications:

Rodrigues M, Krachmer J, Miller S, Newsome D: Bilateral corneal crystalline deposits in benign monoclonal gammopathy. Arch Ophthalmol 97:124, 1979.

Krachmer J, Rodrigues M: Posterior keratoconus. Arch Ophthalmol 96:1867, 1978.

Waring G, Rodrigues M, Laibson RP: Corneal dystrophies, I. Dystrophies of the Bowman's layer, epithelium and stroma. Surv Ophthalmol 23:71, 1978.

Waring G, Rodrigues M, Laibson PR: Corneal dystrophies, II. Endothelial dystrophies. Surv Ophthalmol 23:147, 1978.

Rodrigues M, Waring G: Anterior and posterior corneal dystrophies, in Klintworth G, Garner A (eds): Pathobiology of Ocular Diseases. New York, Marcel Dekker Co. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00096-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Clinicopathologic Studies of Human Ocular Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Merlyn M. Rodrigues	M.D.	Medical Officer	CB	NEI
Other:	Jose Avendano	M.D.	Visiting Scientist	CB	NEI
	Patricia Donohoo	M.S.	Biologist	CB	NEI
	Joseph Hackett	B.S.	Biologist	CB	NEI

COOPERATING UNITS (if any)
Wills Eye Hospital, Philadelphia
Department of Ophthalmology, Louisville

LAB/BRANCH
Clinical Branch

SECTION
Section of Clinical Eye Pathology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with localized ocular diseases or with ocular manifestations of systemic diseases are examined clinically, and photographic documentation is made of significant findings. The biopsy specimens or autopsy eyes from these patients are examined by scanning and transmission electron microscopy and histochemical stains. Studies are performed on patients with corneal and conjunctival viral infections, glaucoma, retinitis pigmentosa, vitreoretinal membranes, cataract, and laser-induced ocular lesions. Histological studies are also performed on normal human cornea, iris, and trabecular meshwork and include scanning and transmission microscopy of tissue specimens as well as of tissue cultures.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Studies of the morphology of tissue specimens as well as cell cultures from normal and abnormal ocular tissues are essential for further insights into possible pathogenetic mechanisms of disease. The utilization of immunohistochemical methods and histochemical stains are also helpful in the diagnosis of certain conditions.

Methods Employed: Specimens are obtained from patients at the National Eye Institute as well as from other ophthalmic centers in the United States. In most instances, specimens are processed by appropriate techniques for cell culture, histology, histochemistry, and electron microscopy. Selected specimens are frozen for special immunologic studies. In other cases, routine histopathology is performed.

Major Findings:

1. Histologic studies of selected normal human ocular tissues

Scanning and transmission electron microscopy were performed on normal human cornea, iris and trabecular meshwork obtained from eye bank and autopsy eyes. Cell cultures of the iris and trabecular meshwork were also examined by the same methods.

Three book chapters were prepared for a text book of histology. These included scanning and transmission electron microscopy of the normal human cornea, iris, and developmental abnormalities of the cornea.

II. Diseases of the cornea and conjunctiva

A. Studies on rapid techniques in the detection of ocular viral infection

We utilized rapid techniques for the detection of viruses in conjunctival and corneal specimens from patients with epidemic keratoconjunctivitis (EKC), herpes simplex keratitis, rubella, vaccinia, and molluscum contagiosum. These techniques included direct and indirect immunofluoescence and negative stain electron microscopy.

We obtained 50 conjunctival specimens from patients with EKC; positive immunofluorescence was obtained in 22 cases. The latter were from subjects with early onset of infection (up to one week). Cultures and typing disclosed that 20 of these infections were type 13, one type 3, and one type 1. Electron microscopy revealed typical icosahedral viruses measuring 65 to 70 nm in diameter. In three patients with herpetic dendritic keratitis, positive immunofluorescence was demonstrated; all were herpes simplex hominis type I. Scrapings from corneal dendritic lesions revealed virions measuring 100 nm in diameter with intact capsids.

A child with ocular rubella had unilateral cataract and microphthalmos. Smears of the cataractous lens revealed positive immunofluorescence. Scrapings from eyelid lesions of molluscum contagiosum revealed brick-shaped virions measuring 250 x 200 nm by negative stain electron microscopy. Vaccinia lid lesions showed virions with mulberry and capsule forms, of the same size as molluscum virions.

B. Corneal lesions associated with Darier's disease

Patients with Darier's disease (hyperkeratosis follicularis) have unusual peripheral, deep epithelial grouped opacities. Electron microscopy of corneal biopsies from two patients revealed irregular and moderate edema of the corneal epithelium with subepithelial granular material. The attachment complexes of the basal epithelium to Bowman's layer were absent.

III. Secondary glaucoma

A. Chandler's syndrome

Cases of Chandler's syndrome were characterized clinically by unilateral glaucoma, mild iris stromal atrophy, corneal endothelial dystrophy, and elevated intraocular pressure. They were examined by slit lamp microscopy and gonioscopy and had photographic documentation of the significant changes. Scanning and transmission electron microscopy of trabeculectomy and iridectomy specimens disclosed a downgrowth of degenerated corneal endothelium and Descemets membrane across the inner uveal meshwork. The iris stromal changes were minimal and the corneal endothelial extension across the trabecular meshwork disclosed a moderate increase of microvilli, cytoplasmic blebs, and filopodial processes. Descemet's membrane was irregularly thinned and closely adherent to the inner uveal meshwork.

B. Glaucoma associated with endothelialization of the trabecular meshwork in two of the cases of posterior polymorphous dystrophy

The cells lining the trabecular meshwork disclosed features of epithelial-like cells with desmosomal junctions, scant mitochrondria and numerous microvillous projections. These cells were a direct extension from the corneal endothelium which also exhibited similar features.

C. Pseudoexfoliation glaucoma

This patient had unusual iris transillumination defects that differed from those described with this entity. The enucleated eye showed deposits of basement membrane-like material on the lens, iris, ciliary epithelium, in the conjunctival and iris vessels, as well as in the anterior chamber. Scanning and transmission electron microscopy revealed 10 nm filaments of basement membrane-like material. These were most abundant in the iris and ciliary epithelium.

IV. Studies on ocular lesions associated with systemic diseases

Light and electron microscopy were performed on lesions from patients with midline granuloma, Bechet's disease, and allergic conjunctivitis. Vitreoretinal membranes were examined in culture from bases of retinal detachment, some associated with massive periretinal proliferation and others from patients with diabetic retinopathy. Scanning and transmission electron microscopy of the cell cultures disclosed cells of glial origin and others derived from retinal pigmented epithelium.

Significance to Biomedical Research and the Program of the Institute: These studies were directly concerned with mechanisms involved in secondary glaucoma and corneal and conjunctival diseases, and ocular manifestations of systemic diseases.

Proposed Course: These projects will continue in the next fiscal year.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Secondary Glaucomas); Corneal Diseases--External Ocular Infections and Inflammatory Diseases; Retinal and Choroidal Diseases

Publications:

Rodrigues MM, Lennette DA, Arentsen, JJ, Thompson, C: Methods for rapid detection of human ocular viral infections. Ophthalmology 86: 452, 1971.

Blackman HJ, Rodrigues MM, Peck G: Corneal lesions in Darier's disease. Ophthalmology (in press).

Rodrigues MM, Gaasterland D: Current concepts in the pathology of the glaucomas (anterior segment), in Nicholson D (ed) Ocular Pathology Update, New York, Masson Publishing Co. (in press).

Rodrigues MM, Phelps C, Krachmer J, Cibis G, Weingeist T: Glaucoma secondary to endothelialization of the anterior chamber angle: A comparison of posterior polymorphous dystrophy and Chandler's syndrome. Arch Ophthalmol (in press).

Rodrigues MM, Streeten B, Spaeth GL: The spectrum of Chandler's syndrome. A clinico-pathologic study. Proc. XXIII Int Congr Ophthalmol (in press).

Rodrigues MM, Waring G, Hackett J, Donohoo P: Histology of the normal human cornea, in Duane TD and Jakobiec F. (eds): Histology of the Eye. Hagerstown, Harper and Row (in press).

Rodrigues M, Hackett J, Donohoo P: Histology of the normal human iris, in Duane TD, Jakobiec F (eds): Histology of the Eye. Hagerstown, Harper and Row (in press).

Waring G, Rodrigues M: Developmental anomalies of the cornea, in Duane TD, Jakobiec F (eds): Histology of the Eye. Hagerstown, Harper and Row (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00095-01 CB

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Collagen and Immunoglobulins in the Human Trabeculum in Glaucoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Merlyn M. Rodrigues	M.D.	Medical Officer	CB	NEI
Other:	Stephen I. Katz	M.D.	Medical Officer	DB	NCI
	Jean-Michel Foidart	M.D.	Visiting Scientist	LDBA	NIDR
	Joseph Hackett	B.S.	Biologist	CB	NEI

COOPERATING UNITS (if any)
Dermatology Branch, NCI
Laboratory of Developmental Biology, NIDR

LAB/BRANCH
Clinical Branch

SECTION
Section of Clinical Eye Pathology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The significance of immunologic mechanisms is being evaluated in primary open-angle glaucoma. We used light and electron microscopy to demonstrate the presence of type IV collagen, fibronectin, and noncollagenous protein in the human trabecular meshwork using immunofluorescence and immunoperoxidase. Factor VIII antigen, a marker for vascular endothelium, was demonstrated in the conjunctival blood vessels of controls, but was absent in Schlemm's canal and the trabecular meshwork of both glaucomatous and nonglaucomatous eyes. We did not detect immunoglubulins IgG, IgM and IgA or C_3 component of complement.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Investigation of the outflow pathway in primary open-angle glaucoma is essential for a better understanding of the pathophysiology of this disease. The availability of human trabeculectomy specimens provides an opportunity to examine earlier stages of the disease than is possible in eyes enucleated for glaucoma or in autopsy eyes.

Methods Employed: Trabeculectomy specimens were excised from patients with primary open-angle glaucoma and frozen in 10% glycerin. Controls included eye bank and autopsy eyes obtained within six hours postmortem. Frozen sections were prepared for immunofluorescence and immunoperoxidase and examined by light and electron microscopy. Collagen types I-IV were used as antigens; the identity and purity of each was confirmed by aminoacid analysis of the proteins, by CM cellulose chromatography, and by polyacrylamide gel electrophoresis. Fibronectin was purified by affinity chromatography on a gelatin sepharose 4B column. Antibodies were prepared by immunizing rabbits with subcutaneous injections of 2.5 to 5 mg of antigen dissolved in PBS and emulsified with an equal volume of Freund's complete adjuvant. Booster injections of the same amount of antigen emulsified in incomplete Freund's adjuvant and were given two weeks later. Antibodies were purified by immunoabsorption and examined for cross-reactivity to other types of collagen by indirect immunofluorescence, micropassive hemagglutination assay and enzyme linked immunosorbent assay.

Major Findings: All our trabeculectomy specimens showed marked reaction to antibodies to type IV collagen, laminin, and fibronectin using immunofluorescence and immunoperoxidase stains. Fibronectin, a cell surface glycoprotein, and noncollagenous protein (laminin) are also components of basement membranes. An attempt is being made to quantitate possible differences between the glaucomatous eyes and the nonglaucomatous controls. There was absence of stain for procollagen II and III. Immunofluorescent stains for immunoglobulins IgA, IgM, IgG and C_3 component of complement were negative. The endothelium of Schlemm's canal and the trabecular endothelium failed to stain with factor VIII antigen in contrast to the marked stain with factor VIII observed in conjunctival vessels or collector channels in the control autopsy eyes, since superficial sclera and conjunctival tissues were not excised in the trabeculectomy specimens.

Significance to Biomedical Research and Program of the Institute: This project is directly related to structural and possible immunologic aspects of the aqueous outflow pathway in human glaucoma and in nonglaucomatous controls.

Proposed Course: No further investigation is planned.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Primary Open-Angle Glaucoma).

Publications:

Rodrigues M, Katz SI, Foidart J, Spaeth GL: Collagens and immunoglobulins in the human trabecular meshwork in glaucoma. Ophthalmology (in press).

Laboratory of Sensorimotor Research

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1978 - September 30, 1979

REPORT OF THE CHIEF, LABORATORY OF SENSORIMOTOR RESEARCH

Robert H. Wurtz, Ph.D.

Since this is the first annual report from the Laboratory of Sensorimotor Research, it is appropriate to outline the rationale and goals of the laboratory. The rationale for such a laboratory is that clinical progress can best come in an environment where the clinic can take advantage of the techniques and ideas developed in the basic research laboratory. With the organization of the Laboratory of Sensorimotor Research we now have the opportunity to extend the analysis of the visual and oculomotor systems developed in our studies of the behaving monkey to clinical problems, particularly those studied within the neuro-ophthalmology program of the NEI.

The goal of our research is to investigate the sensorimotor organization of the primate visual and oculomotor systems with a multidisciplinary approach which includes physiological, behavioral, psychophysical, and anatomical methods. Classical neurophysiology has made great advances by studying the responses of neurons in the anesthetized animal both to external stimuli and to electrical stimulation. However, as research has progressed, it has become apparent that study of the brain mechanisms related to movement requires the analysis of events occurring in the brain of a behaving animal. An important methodological advance has been the integration of the computer into the laboratory not only for data analysis but to facilitate the actual performance of physiological and psychophysical experiments. These techniques have enabled us to investigate how the monkey brain analyzes the visual world and uses this analysis for the generation of visually-guided movement. Work in the laboratory has centered on analysis of the discharge patterns of single cells in the visual and oculomotor systems, but we now hope to expand our program to include more neuroanatomical analysis at the cellular level and more psychophysical analysis at the behavioral level. This latter development will facilitate the translation of data and concepts from the laboratory to the clinic.

Research in the laboratory this year concentrated on two general problems: the modulation of visual processing by eye movements and the visual initiation of eye movement.

One of the fundamental modulations of visual processing that the brain must perform is the selection of some stimuli from a continuing barrage of sensory stimulation for further analysis or as the target for a saccadic eye movement. In the awake monkey, free to move its eyes, we have been able to determine at the level of single neurons the outline of such a selection process, which is expressed as a more vigorous or enhanced response to a visual stimulus when the monkey uses the stimulus as the target for a saccadic eye movement. In the superior colliculus in the brainstem and the frontal eye field area of the frontal cortex, the enhanced response is specifically related to the initiation of saccadic eye movements, but in the

occipital lobe, in striate and prestriate cortex, the enhanced response is associated with increased alertness. Such enhanced responses now have also been found in the posterior parietal cortex and in the pulvinar nucleus of the thalamus but with significant differences. In the parietal cortex the enhancement effect is related to the monkey's use of the stimulus regardless of how he responds to it, suggesting that the response is related not to specific movements but to a more general attentional mechanism. In the pulvinar nucleus this enhancement is still related to eye movements but it is less specific than that seen in the superior colliculus. The pattern that emerges from these studies is that the brain organizes responses to the external world at least partially by modulating neuronal responses to stimuli in the external world. Thus, areas concerned with eye movement show enhanced responses to stimuli that will be targets for eye movements. Areas concerned with visual attention show enhancement to stimuli whenever the animal attends to the stimuli. Areas concerned with visual analysis independent of behavior show modulation more related to the alertness of the animal than to the specifics of behavior. Much of the processing that precedes behavioral responses is thus concerned with the analysis and selection of stimuli relevant to the behavior, and the distinctions between sensory and motor blur as the processing becomes more remote from either sensory input or movement output.

Another type of modulation of visual processing occurs as the eye sweeps over the visual scene during saccadic eye movements. We have found that the brief stimulation occurring during saccadic eye movements is attenuated by a visual "masking" effect of the stimuli falling on the receptive field of a cell both before and after a saccade. This seems to be the primary mechanism present in the visual pathway to the striate cortex. In the pathway to the superior colliculus there is, in addition, a corollary discharge, that is, a discharge from the oculomotor system that indicates the occurrence of an eye movement. This corollary discharge reduces the sensitivity of the cells to the visual stimulation produced by saccadic eye movements and is the first to be found in the primate visual system.

Visual processing itself has been studied in the thalamus and in the cerebral cortex. Initial studies of the pulvinar nucleus of the thalamus have shown that the activity of some cells here share the lack of stimulus specificity (spots of light being adequate stimuli) seen in the superior colliculus while others require specific stimulation (oriented lines or directional movement) as in striate cortex. The posterior parietal cortex receives visual input from both the pulvinar and the striate cortex. Behavioral experiments have indicated that the striate projection may be significant in maintaining the visual properties of parietal cortex, and a few clinical reports have described patients from whom the striate cortex has been surgically removed who have a significant visual capacity in the affected field. The residual properties of parietal cortex in the presence of unilateral striate lesions have been studied in the monkey. Initial results indicate that there is a small visual area of posterior parietal cortex in which visual properties are unaffected by striate lesions, although the bulk of parietal cortex is no longer responsive to visual stimuli in the hemifield contralateral to the striate lesion. Anatomical studies have also been started which are aimed at delineating the areas of

prestriate cortex receiving direct inputs from the striate cortex, and these studies have so far revealed the organization of retinotopic maps in three prestriate areas.

The other line of work within the laboratory is on the initiation of eye movements. The frontal eye fields have long been suspected on the basis of clinical evidence to be involved in the initiation of eye movements, and electrical stimulation of the frontal eye fields easily induces saccades. Previous single cell studies have shown that neurons in the frontal eye fields do not discharge before all eye movements although some visual neurons have an enhanced response to stimuli that are the targets for eye movements. Recent experiments in the laboratory have shown that electrical stimulation in the frontal eye fields results in eye movements that are predictable from the visual properties of the cells in the area being stimulated. This result suggests that the enhanced visual response might be an intermediate stage in the initiation of saccadic eye movements, possibly via the connections of this area to the superior colliculus. The frontal eye fields do not participate in the genesis of all eye movements but may instead provide a transfer mechanism from the visual system to the oculomotor system.

The role of the superior colliculus in the generation of visually-guided saccades has been undisputed. Somewhat puzzling, however, has been the lack of a clear deficit in the guidance of saccades following ablation of the superior colliculus. This year we have found a striking deficit in a monkey's ability to make saccades to the peripheral visual field following the ablation of the colliculus. While cortex and colliculus may jointly guide movements in the central visual field, the colliculus is required for such guidance to peripheral targets.

From this brief summary of work in the laboratory, it must be clear that the brain organizes behavior in large part by analyzing the sensory environment, yet beyond the periphery of the nervous system it is difficult to speak separately of sensory and motor functions. There is an interdigitation of sensory and motor events, and even neurons with classically sensory properties such as those in the superior colliculus are subject to the effects of movement, and cells with properties important in the genesis of movement, such as those in the frontal eye fields, have some aspects of their discharge which depend upon the sensory environment. A defect in this interrelation of sensory and motor is frequently also present in clinical problems of oculomotor dysfunction, and our studies of the mechanism of visual behavior may well yield therapeutic and diagnostic strategies for coping with such disorders.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00053-01 LSR

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Modulation of Visual Processing by Saccadic Eye Movements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert H. Wurtz	Ph.D.	Chief	LSR	NEI
Other:	Barry J. Richmond	M.D.	Senior Staff Fellow	LSR	NEI
	Stuart J. Judge	Ph.D.	Visiting Fellow	LSR	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We investigated how cells in the visual pathways of the primate respond to stimuli during rapid or saccadic eye movements. In the striate cortex only about half of the supragranular cells responded to stimuli as the eye swept the receptive field of a cell across the stimulus while nearly all infragranular cells responded. Visual masking by stimuli falling on the receptive field of a cell before and after a saccade was effective in reducing the response of the cell to the stimulus during the saccade. Forward masking was more prolonged than backward masking. A similar masking effect was found in the monkey superior colliculus. In addition a corollary discharge was found in the superior colliculus. The visual masking effect in striate cortex appears to be closely related to lack of perception during saccadic eye movements while the corollary discharge to the superior colliculus is probably most closely related to the initiation of saccades.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: One of the most remarkable facts about human vision is that in spite of the jerk-like or saccadic eye movements made from one part of the visual field to another our visual experience remains clear and stable. We do not experience a transient blurring of the visual scene as the eye moves rapidly across it. Two explanations for this lack of transient interruption in our vision have been put forward. The first, most recently emphasized in psychophysical experiments by Campbell and Wurtz, argues that the lack of perception during eye movements results from visual masking, that is, the clear image before and after a saccadic eye movement eliminates the blurred image during an eye movement. The second explanation relies on a corollary discharge notion, that is, at the same time the brain sends signals to move the eyes, it also signals the channels of visual processing to ignore the visual input that results from the movement of the eyes. This year we have completed a series of experiments designed to investigate the physiological mechanisms which might be the basis for the relative lack of vision during eye movements.

Methods Employed: We used awake rhesus monkeys trained to fixate on a point of light on a screen in front of them. During the time that the monkey was fixating we found the location of the visual receptive field of a particular cell--the location of the stimulus necessary to activate the cell under study. We next had the monkey make saccadic eye movements across a visual stimulus to determine whether the cell was responsive during eye movements. Alternatively, while the monkey fixated, we swept the stimulus rapidly across the receptive field of the cell.

Major Findings: We studied cells in the two major visual pathways extending from the retina to the brain, that to striate cortex and that to the brainstem. In the first system, we have found that many cells in the striate cortex respond to stimulation during rapid eye movements even though the stimulus is only briefly on the receptive field of any particular cell. What is particularly striking is that only about half of the cells above the granular layer (the layer that receives most of the input fibers) respond to stimuli during rapid eye movement whereas nearly all of the cells below the granular layer respond to such stimulation. This is significant because of the different outputs from these cells to other parts of the brain. The supragranular cells project to prestriate cortical areas; infragranular cells project primarily to subcortical areas.

Our subsequent experiments, which were concentrated on the supragranular cells, showed that a visual masking effect was the salient factor controlling the response of striate cortex neurons during eye movements. A stimulus falling on the receptive field before or after a rapid or saccadic eye movement proved to be effective in reducing the response of that cell to the visual stimulation resulting from the sweep of the receptive field across a stimulus during a saccadic eye movement. We then had the monkey fixate and demonstrated that the same masking effect occurred when we projected stimuli successively on the receptive field just

as would occur if the monkey made a saccade across the receptive field. The time course we found for the masking effect was much longer in the forward direction than in the backward; a stimulus 200 msec before the brief stimulation of a receptive field produced effective masking of that brief stimulation, but a stimulus only 50 msec after the brief stimulation had little if any effect on the response of the cell to the brief stimulation.

The probable physiological mechanism for the forward perceptual masking effect seems clear: the response to the brief stimulation during a saccade is attenuated by the preceding masking stimulus. However, our previous psychophysical experiments in man had shown that some backward masking was present even when there did not seem to be any reduction in the response to the brief stimulation in the monkey. This indicates that the mechanism of backward masking differs from that of forward masking. In our experiments we had been struck by how difficult it was to distinguish the response of cells to the brief stimulation from the response to the subsequent masking stimulus. Our hypothesis on the mechanism of backward masking is that the nervous system has the same problem. Backward masking in our experiments probably results from a confounding of the neural response to the brief stimulus and to the masking stimulus rather than from an attenuation of the response to the brief stimulation.

The second visual pathway projects primarily to the superior colliculus which lies on the roof of the brainstem. We found that the visual masking effects on the response of cells in the superior colliculus were similiar to those effects seen in the striate cortex.

We had previously shown that another factor operates on the cells within the superior colliculus: an extravisual input occurs following a saccadic eye movement and decreases the sensitivity of superior colliculus cells, thus reducing their response to visual stimulation during an eye movement. We had previously argued that this extravisual input was a corollary discharge arising from the central nervous system rather than feedback from the periphery since it was present even when proprioceptive input from the eye muscles was blocked by injecting anesthetic into the orbit. This argument assumed that no other proprioceptive input would be correlated with an attempted saccadic eye movement. However, if our monkeys were attempting to make head movements correlated with attempted eye movements (which could not be made because the monkey's head was restrained), this attempted head movement could produce proprioceptive feedback. We determined whether this feedback was a factor by recording attempted head movements with a strain gauge attached to the monkey's head holder. We found that when the monkey made saccades to visual targets there was indeed an attempted head movement, but when the monkey made saccades spontaneously in the light no such attempted head movement occurred. Since the suppression we saw in the superior colliculus was present when the monkey made such spontaneous eye movements, we conclude that proprioception from attempted head movement could not be the source of the suppression in the superior colliculus.

Significance to Biomedical Research and the Program of the Institute: We have found a physiological basis for both of the mechanisms which have been proposed to reduce the stimulation of the visual system during saccadic eye movements. In both the striate cortex and the superior colliculus there is a prominent visual masking effect which serves to reduce the brief and degraded stimulation occurring during an eye movement. This factor is likely to be the prominent physiological mechanism underlying the lack of visual perception during saccadic eye movements; it is consistent with psychophysical experiments showing that visual masking is important in eliminating perception during eye movements. On the other hand, the superior colliculus is probably most important for the initiation of saccadic eye movements rather than visual perception. The corollary discharge to the superior colliculus allows the system to be very sensitive to stimulus movement in the environment, which is a particularly potent stimulus for the initiation of saccadic eye movements, while reducing the sensitivity of the system to stimulus movement produced by the saccades themselves. Such modulation of a visual pathway by a corollary discharge is the first to be found in the primate visual system.

This understanding of how the primate visual system might use different physiological mechanisms to eliminate input during saccadic eye movements is essential to the understanding of the normal functioning of the human visual system. Since different mechanisms exist in the two visual pathways, this difference might itself be an aide in the diagnosis of visual-motor dysfunction.

Proposed Course: Although this project was largely completed during this year, one aspect will be investigated in the future. Cells in the frontal cortex of the monkey discharge after saccadic eye movements, and we intend to investigate whether the suppression effect observed in the superior colliculus is derived from frontal cortex.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders

Publications:

Wurtz RH: The primate superior colliculus and visually guided eye movements, in Chivers DJ, Herbert J (eds): Recent Advances in Primatology, Vol. I Behaviour. New York, Academic Press, 1978, pp 980.

Campbell FW, Wurtz RH: Saccadic omission: Why we do not see a grey-out during a saccadic eye movement. Vision Res 18:1297-1303, 1978.

Wurtz RH, Goldberg ME, Robinson DL: Behavioral modulation of visual responses in the monkey: A neurophysiological approach to attention, in Progress in Physiological Psychology and Psychobiology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00055-01 LSR

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Visual and Oculomotor Functions of the Primate Superior Colliculus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joanne E. Albano	Ph.D.	Postdoctoral Fellow	LSR	NEI
Other:	Robert H. Wurtz	Ph.D.	Chief	LSR	NEI
	Helen C. Healy	B.A.	Psychology Technician	LSR	NEI
	Mortimer Mishkin	Ph.D.	Research Psychologist	LN	NIMH
	Stephen G. Lisberger	Ph.D.	Staff Fellow	LNP	NIMH

COOPERATING UNITS (if any)

Laboratory of Neuropsychology, NIMH
Laboratory of Neurophysiology, NIMH

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of the superior colliculus in the visual guidance of saccadic eye movements has been investigated. Rhesus monkeys are first trained and tested on several tasks which allow measurement and control of visual and oculomotor responses to stimuli in the central and peripheral visual field. After collection of normative data, the monkeys receive unilateral lesions of the superior colliculus. Following a period of postsurgical recovery without visual experience, monkeys are retested on the visuomotor tasks in order to assess the effect of the brain damage and to study the course of recovery. The monkey's postlesion ability to detect stimuli is impaired, but this recovers within several days of visual experience. The ability to make saccades to peripheral targets is permanently impaired as is opto-kinetic nystagmus. Pursuit eye movements remain normal.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The primate superior colliculus has long been thought to play an important role in the visual guidance of eye movements. Much of this evidence has come from neuroanatomical and physiological studies linking the superior colliculus with lower brainstem regions known to be critically involved in generating rapid or saccadic eye movements. However, behavioral experiments have failed to uncover a vital role of the colliculus in the initiation of saccades. Previous studies have concentrated on eye movements made to the central visual field with small or incomplete lesions of the colliculus, and these studies have allowed postsurgical visual experience before testing. The goal of this project is to examine the normal visual and oculomotor behavior of the primate in response to visual stimuli in the central and peripheral visual field and to examine the consequences of complete colliculus removal and subsequent recovery.

Methods Employed: Rhesus monkeys are trained on three computer-controlled tasks: visual detection, visual guidance of saccadic eye movements, and saccadic eye movements from nonprimary orbital positions. The visual detection task requires that the monkeys signal that they have detected the occurrence of a small stimulus (0.2° in diameter) while maintaining fixation on a central spot of light. The saccade tasks require that the monkeys make a saccadic eye movement from a fixation point to a 0.2° visual target. In addition, the monkeys are tested on their ability to track a small moving visual stimulus (smooth pursuit) and on optokinetic nystagmus.

Eye movements have been recorded using the electro-oculogram during performance of these tasks although the magnetic search coil method is now being used. This eye movement information, the monkeys' responses and stimulus presentations are displayed and stored in digital form by an on-line computer. After prelesion testing, the monkeys receive large, unilateral surgical ablations of the superior colliculus. This is accomplished by sectioning the corpus callosum and ablating the colliculus by subpial suction. The monkeys are denied visual experience during the postsurgical period preceding postlesion testing by insertion of translucent contact occluders.

Major Findings: I. Visual detection:

On the first day of testing following removal of the contact occluders, the monkeys with ablations fail to detect stimuli that appear in the peripheral visual field contralateral to the side of the lesion. This deficit recovers quickly. During the next few days of testing, detection of visual stimuli gradually improves for the more central targets first and the most peripheral targets last until nearly complete recovery occurs after four to ten days of visual experience.

II. Visually-guided saccadic eye movements:

After recovery from the visual deficit the monkey's ability to make visually-guided eye movements to targets throughout the visual field is retested. Typically, the normal animal will make a single saccade to acquire nearer targets for foveal viewing. Targets appearing further in the periphery than about 15-25° are usually acquired with two saccades: an initial saccade which falls short of the target by less than 5° and a second saccade which brings the eye on target or nearly on target. After ablation of the colliculus the initial saccade to more peripheral targets falls short of the average prelesion values and the target is not acquired by a second saccade. The recovery of this deficit is variable from 28 days in one animal and until sacrifice at 6 months in another.

III. Saccadic eye movements made from nonprimary eye positions:

Normal animals fixate a peripheral target and make saccades directed either toward more peripheral targets or toward more central targets. Animals with collicular lesions fail to fixate and saccade to targets appearing more than about 30° from primary eye position.

IV. Smooth pursuit:

The ability to make smooth pursuit eye movements is preserved following colliculus lesions.

V. Optokinetic nystagmus:

This ocular reflex is severely disrupted by colliculus lesions. In the two animals tested the optokinetic response was weak or absent for drum rotations toward the side of the lesion but vigorous for drum rotations toward the side of the intact superior colliculus. This deficit was observed for drum rotations of 30°, 60°, 90°, or 120°/sec.

Significance to Biomedical Research and the Program of the Institute: Knowledge of the role of neural structures, particularly those in the brainstem, in the mediation of visual-oculomotor abilities is a prerequisite to understanding sensory and motor disorders that occur in man. Previous work in this laboratory had shown that either the striate cortex or the superior colliculus was adequate for the guidance of saccadic eye movements. The present experiments show that this is not true for the peripheral visual field; absence of the superior colliculus leads to striking deficits in the accuracy of peripherally-directed saccadic eye movements. The results of this project indicate that the superior colliculus plays an important role in visually-guided eye movements, and these results also emphasize the importance for distinguishing between central and peripheral visual fields in analyzing visual-oculomotor deficits in man.

Proposed Course: Using a more accurate eye movement measurement technique, the magnetic search coil, we will concentrate upon collection of accurate quantitative data of visually-guided eye movements to stimuli in the central and peripheral visual field. We will also test for any visual-motor deficits resulting from section of the corpus callosum.

NEI Research Program: Sensory and Motor Disorders of Vision--Strabismus and Other Oculomotor Disorders

Publications:

Wurtz RH, Albano JE: Visual-motor function of the primate superior colliculus, in Annual Review of Neuroscience, Vol 3 (in press).

Wurtz RH, Goldberg ME, Robinson DL: Behavioral modulation of visual responses in the monkey: A neurophysiological approach to attention, in Progress in Physiological Psychology and Psychobiology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00049-01 LSR

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cerebral Cortical Mechanisms for Eye Movements and Visual Attention

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael E. Goldberg	M.D.	Research Medical Officer	LSR	NEI
Other:	M. Catherine Bushnell	Ph.D.	Guest Worker	LSR	NEI

COOPERATING UNITS (if any)

Armed Forces Radiobiology Research Institute
Neurology Department, Georgetown University

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are being conducted to investigate the mechanisms by which the cerebral cortex controls eye movements in the primate. Single units are recorded in the frontal eye fields and inferior parietal lobule while the monkeys perform a series of tasks involving visual fixation, saccadic eye movements, and peripheral attention or reaching without eye movements. Discharge patterns of single neurons are correlated with the monkey's movements and the presentation of visual stimuli. Various neuron types are then stimulated electrically through the recording electrode to determine the effect on eye movements. We found that the frontal eye fields have activity specifically related to visually guided eye movements, while the inferior parietal lobule has activity related to general visual attention.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Although clinical evidence has long indicated that the cerebral cortex has some control over eye movements, the nature of that control is not understood. Two cortical areas, pre-arcuate cortex, also called the frontal eye fields, and the posterior parietal cortex, are thought to be important in the cerebral control of eye movements. Electrical stimulation of the frontal eye fields in animals causes the eyes to move toward the visual field opposite the side of the brain stimulated, and pathological seizure activity in humans also drives the eyes to the opposite side. These facts imply that activity in the frontal eye fields is important in initiating eye movements, yet the only frontal eye field cells found to be related to eye movements discharge during or after saccades, and therefore cannot participate in the initiation of these movements. Conversely, although clinical evidence has implicated the posterior parietal cortex in the general process of visual attention, physiological evidence has linked this area with the initiation of eye movements. To reassess the roles of the frontal eye fields and posterior parietal cortex in the control of eye movements, we are using physiological recording and microstimulation of cortical neurons in monkeys performing a variety of visual tasks.

Methods Employed: A digital computer was used for behavioral control, data acquisition, and on-line analysis of monkey behavior, eye movement, and neuron discharge time patterns. Monkeys were trained on four tasks: fixate a spot of light and ignore other stimuli; make a saccade from the fixation point to the peripheral stimulus; reach out and touch the peripheral stimulus without making an eye movement; respond to the peripheral stimulus by releasing a lever. Activity of single neurons in frontal eye fields and posterior parietal cortex was recorded while the monkeys performed their tasks. Sites of certain neurons were stimulated through the microelectrode while the monkey was at rest and while he performed tasks. Current levels were low enough to preserve the microelectrode and occasionally even the cell.

Major Findings: The majority of the neurons in both areas have visual receptive fields. Roughly half of these neurons show a greater rate of discharge in response to a visual stimulus that the animal uses for some aspect of its behavior than to a neutral stimulus; the activity of the visual neuron is behaviorally enhanced. This enhancement is spatially specific: it occurs when the animal intends to use the stimulus in the receptive field, but not when the animal intends to use a stimulus outside the receptive field. The nature of this enhancement differs between the parietal and the frontal cortices. Responses of parietal neurons are enhanced when the animal uses the stimulus for any behavior: as the target for an eye movement, the target for a hand projection without an eye movement, or the cue for a bar release. Frontal neurons, on the other hand, give enhanced discharge when the stimulus is the target for an eye movement, but not when the monkey responds to the stimulus in other ways.

Low threshold microstimulation at the sites of visually active neurons in the frontal eye fields results in the generation of eye movements toward the receptive field of the neuron. In contrast, stimulation at sites of frontal eye field cells that discharge after eye movements in the posterior parietal cortex does not result in eye movements at these current levels. The current threshold at which eye movements can be induced from the frontal eye fields depends not only on the location of the electrode, but also on the animal's behavioral state. When the animal is fixating a spot of light, the threshold is twice that seen when the animal is resting or making spontaneous eye movements in a dimly lit familiar environment.

These experiments indicate that the parietal and frontal visual areas have related, but significantly different, roles in the genesis of visually-guided behavior. The neurons in the parietal cortex respond whenever a visual stimulus is important to the animal, regardless of the strategy with which the animal responds to the stimulus. This property makes the posterior parietal cortex a good candidate as a center for visual attention, but a less good candidate as a command center for any specific movement. Conversely, the enhanced discharge of frontal eye field neurons might serve to command visually guided eye movements, especially since the electrical stimulation which induces eye movements may mimic the enhanced discharge of visual neurons being stimulated. The experiments also indicate that not all eye movements are the same: visually-guided eye movements are preceded by activity in frontal and parietal cortices, but other eye movements which have the same motor parameters are not preceded by this activity.

Significance to Biomedical Research and the Program of the Institute: These experiments have begun to show how the cerebral cortex contributes to the generation of visually-guided eye movements and attention by demonstrating the existence of a general attentive mechanism in the posterior parietal cortex and a specific oculomotor mechanism in the frontal eye fields. This information can lead to the increased understanding of the mechanisms by which patients with cerebral disease manifest disorders of visual attention and ocular motility, and may lead to the development of treatment which can compensate for such deficits.

Proposed Course: A more detailed analysis of the frontal eye fields will be performed to answer the following questions. What is the relationship of neurons in the frontal eye fields to neurons in the superior colliculus that discharge before eye movements? What is the relationship of these neurons to combined eye and head movements per se? Finally, we will want to know more about the actual parameters of the eye movements induced by frontal stimulation during visual behavior. To do these experiments, we will attempt to develop techniques for finer measurement of eye movements using the magnetic search coil system and techniques for recording and analyzing information from several single neurons.

NEI Research Program: Sensory and Motor Disorders of Vision--Strabismus and Other Oculomotor Disorders

Publications:

Goldberg ME, Robinson DL: The superior colliculus, in Masterton RB (ed): Handbook of Behavioral Neurobiology. New York, Plenum Press, 1978, pp 119-164.

Goldberg ME, Robinson DL: Behavioral modulation of visual response of neurons in monkey superior colliculus and cerebral cortex, in Thompson RH (ed): Proceedings of USA-USSR Conference on Goal Directed Behavior (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00047-01 LSR

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Visual Processing in Brains Following Cortical Ablation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael E. Goldberg	M.D.	Research Medical Officer	LSR	NEI
Other:	M. Catherine Bushnell	Ph.D.	Guest Worker	LSR	NEI
	Leslie G. Ungerleider	Ph.D.	Senior Staff Fellow	LSR	NEI
	Mortimer Mishkin	Ph.D.	Research Psychologist	LN	NIMH

COOPERATING UNITS (if any)

Laboratory of Neuropsychology, NIMH
Neurology Department, Georgetown University
Armed Forces Radiobiology Research Institute

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Rhesus monkeys are made hemianopic by total removal of the striate cortex in one hemisphere using subpial suction under direct vision. After the animals recover from surgery, they are trained on a series of visuomotor tasks, and the activity of single neurons in posterior parietal cortex both ipsilateral and contralateral to the lesion is studied. An initial series of experiments has shown that the parietal association cortex receives some visual input in the absence of the striate cortex, but that this visual input is concentrated in a limited region of the posterior parietal cortex rather than spread diffusely as in the normal cortex. This indicates that both the extrageniculate and the geniculostriate visual systems project to the posterior parietal cortex but that the recipient areas are anatomically separate.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Information from the retina can reach the parietal cortex in two ways: first, via the lateral geniculate nucleus of the thalamus, then through the striate cortex; second is via the superior colliculus, then through various posterior thalamic nuclei. Processing in these pathways is very different: the geniculostriate system is concerned with fine details of visual stimuli, such as orientation, color or direction of movement; the extrageniculate system is insensitive to stimulus detail, but modulates its activity by the importance of the visual stimulus to the animal's behavior. Neurons in the parietal cortex more closely resemble those of the extrageniculate system than those of the geniculostriate system, yet behavioral evidence from lesion studies indicates that there is a powerful visual contribution from the striate to the posterior parietal cortex. This project was designed to elucidate the different contributions of two visual pathways to the visual functioning of posterior parietal cortex by studying the visual properties of posterior parietal cortex in a brain in which a striate cortical lesion had been made.

Methods Employed: Rhesus monkeys underwent unilateral striate ablation. After recovery from surgery they were trained to perform a number of visuomotor tasks including visual fixation, visually-guided tracking and saccadic eye movements, visually-guided hand reaching, and response to the flickering of a peripheral stimulus without an eye movement toward the stimulus. Finally, responses of single neurons were recorded in the parietal cortices ipsilateral and contralateral to the striate lesion.

Major Findings: Initial results indicate that neurons in the posterior parietal cortex contralateral to the striate lesion were normal except that the representation of the ipsilateral field (i.e. that visual field contralateral to the striate lesion) was sparser than expected. In the posterior parietal cortex ipsilateral to the striate lesion there was a markedly reduced representation of the contralateral field (i.e. that visual field contralateral to the striate lesion). This representation was limited to an area of the cortex approximately three millimeters in diameter, whereas in the normal posterior parietal cortex visual activity is spread over several square centimeters of the cortical surface. Within this small active region all cell types typical of parietal cortex have been found, even a few neurons with directional selectivity for stimulus movement. As in the normal parietal cortex, discharge frequency of some visual neurons was greater when the stimulus was behaviorally significant to the animal than when the stimulus was neutral.

Significance to Biomedical Research and the Program of the Institute: The initial results of this project are quite surprising: there seem to be two independent visual representations in the posterior parietal cortex. One requires an intact striate cortex, another smaller one does not. Therefore, although normal posterior parietal cortex does not utilize the stimulus specificity typical of the striate cortex, some visual activity in the parietal cortex is dependent on input from the striate cortex. The

anatomic separation of input to the posterior parietal cortex may imply that an area which had been thought to be unitary has, in fact, separate components with different inputs and perhaps different outputs. The existence of redundant visual pathways implies that patients with damage in their geniculostriate pathways still have visual information available to their visual association cortices, encouraging the search for new strategies of rehabilitation.

Proposed Course: The posterior parietal cortex of striate lesioned monkeys will be searched over a wider area for the existence of other small extrastriate visual projections. Retrograde transport anatomic methods will be used to determine the afferent projections to the nonstriate recipient visual area. The eye movements of monkeys with striate lesions will be studied using the magnetic search coil method for recording eye movements. A clinical protocol will be developed to study the visual and visuomotor capibilities of patients described as hemianopic.

NEI Research Program: Sensory and Motor Disorders of Vision--Rehabilitation

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00051-01 LSR

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Measurement of Eye Position with Implanted Magnetic Search Coils

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Stuart J. Judge	Ph.D.	Visiting Fellow	LSR	NEI
Other:	Barry J. Richmond	M.D.	Senior Staff Fellow	LSR	NEI
	Fred C. Chu	M.D.	Staff Associate	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A method was developed for recording eye position in monkeys. The magnetic search coil technique of Robinson was used but the implantation of the coil was modified. Instead of implanting the coil behind the insertions of the extraocular muscles and risking a subsequent strabismus, the coil was inserted in front of the muscle insertions. This method is surgically simpler, and does not produce a strabismus. It is now used routinely in this laboratory for analysis of the visual and oculomotor systems.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Accurate and reliable information about the position of the eyes is of crucial importance to the success of the principal task of this laboratory, namely probing the visual and oculomotor systems in the awake behaving (and misbehaving) monkey. We sought to develop a radically better method of measuring eye position than that used to date (implanted electro-oculogram electrodes).

Methods Employed: We adopted the magnetic search coil method which has been popular among those interested in the peripheral oculomotor system since its introduction by Robinson in the 1960s. The monkey is placed in the center of high frequency alternating magnetic fields in spatial and temporal quadrature. Phase locked amplification is then used to separate the voltage in a search coil wound on the globe under the insertions of the extraocular muscles into components related to the vertical and horizontal position of the eye. Wider use of this method by those interested in vision and visuomotor integration has been discouraged by the tendency of the monkeys to display a strabismus.

To avoid the occurrence of strabismus we have developed a procedure for implanting a preformed coil on the globe in front of the insertions of the extraocular muscles. The essential feature of our surgical approach is to make a ring incision through the conjunctiva and Tenon's capsule at the limbus, and carry the incision back by blunt dissection until the extraocular muscles can just be seen. The preformed coil is then placed on the globe and the conjunctiva is sutured together to hold the coil in place. A small loop of the twisted lead from the coil is left in a pouch between Tenon's capsule and the conjunctiva on the lateral side of the globe, so that the eye can rotate without restriction by the lead running from the coil to a connector on the skull.

Major Findings: The monkeys recover from the surgery within a week and never display a strabismus detectable by comparing the corneal reflexes in the two eyes. We used the signal from the search coil to measure precisely the position of the operated eye while the monkey was doing a fixation task binocularly, monocularly with the operated eye, or monocularly with the unoperated eye. The operated eye is aligned to within 6 to 12 minutes of arc of the same position whether the animal is viewing monocularly with that eye or binocularly. Since 6 to 12 minutes is the size of Panum's fusional area for the human subject, it is reasonable to suppose that our monkeys have normal binocular alignment of the eyes. With fixation at our standard distance of 57 cm, the monkeys display a slight exophoria of 1/5 to 3/4 of a degree. The small exophoria may well be a consequence of the short viewing distance.

We have also examined the range of eye movement in animals implanted with coils. Within 30 degrees of primary position eye movements are not restricted. In some animals there is some suggestion of a limitation of movement to angles 35 degrees or more from the primary position, but it is

difficult to be sure that the inaccuracy of these larger movements is really due to limitation of eye movement produced by the presence of the coil rather than the animal's disinclination to make very eccentric fixations.

Significance to Biomedical Research and the Program of the Institute: Considerable interest has been aroused among visual and oculomotor physiologists by this simple improvement of a well-known method. The technique is not only advantageous over the older method in not producing a strabismus, but is also much simpler and quicker to execute. It has already been used successfully by workers in several other laboratories and has been found to be as successful in cats as in monkeys.

Proposed Course: The new method will be routinely used to implant coils in all laboratory animals, allowing accurate and reliable measurement of eye position without disrupting the functioning of the visual system by diplopia. In some animals, coils will be implanted in both eyes to allow the investigation of vergence eye movement.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00045-01 LSR

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Visual and Eye Movement Properties of Neurons in the Pulvinar Nucleus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David Lee Robinson	Ph.D.	Research Psychologist	LSR	NEI
Other:	William Keys	Ph.D.	Guest Worker	LSR	NEI

COOPERATING UNITS (if any)

Behavioral Sciences Department, Armed Forces Radiobiology Research Institute
Neurology Department, Georgetown University

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experiments are being conducted on the visual and eye movement properties of neurons in the pulvinar nucleus of awake monkeys. Many pulvinar cells respond to all types of visual stimuli. The response of some of these cells is enhanced when a monkey is going to use a stimulus as the target for a saccadic eye movement. The enhancement is spatially nonselective; it occurs prior to eye movements into as well as outside of the receptive field. This enhanced response encodes no data on the direction or amplitude of the subsequent eye movement; this aspect of the response is similar to that seen in visual cortex. The presence of an enhanced visual response is, however, eye movement specific since it is not present when the monkey shifts its attention to a stimulus but makes no eye movement to it. Enhancement under these conditions is similar to that seen in the superior colliculus. An enhanced visual response from the pulvinar indicates that a visually guided eye movement is about to occur. Such data may be used by perceptual portions of the visual system in stabilizing the visual scene during eye movements.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Visual information reaches the cerebral cortex via two major pathways. One is through the lateral geniculate nucleus of the thalamus to the striate cortex; the other is through the superior colliculus on the roof of the midbrain, then through the pulvinar nucleus in the thalamus and finally to striate and prestriate cortices. Although the visual properties of cells in the pathway to the striate cortex and to the superior colliculus have been extensively analyzed, the pulvinar nucleus has been largely unexplored. Furthermore, in the awake monkey both the striate cortex and the superior colliculus show alterations in visual processing which are dependent upon the monkey's level of arousal or intended use of the stimuli. The source and manner in which such modulations affect the pulvinar nucleus are also unknown. The present studies are designed to analyze visual processing in the pulvinar and to evaluate the modulation of these visual properties by eye movements and attention.

Methods Employed: The activity of single neurons in the pulvinar nucleus was recorded from awake, trained rhesus monkeys. The monkeys learned to fixate a spot of light on a tangent screen in order to obtain water reinforcement. While the animal fixated, other lights were flashed onto the screen to study the visual properties of cells. To study behavioral properties of these cells, the animal was trained to make a saccadic eye movement from one light to another. This same animal also learned to reach out and touch a panel illuminated by a visual stimulus; in addition, it could attend to a peripheral stimulus without making a movement toward it. This combination of tasks allowed us to test any cell for its visual properties, eye movement relations, attentional activity, and relation to visually-guided eye and hand movements. The control of the animal's tasks and the on-line analysis of data were performed by a digital computer.

At the conclusion of these experiments, the monkey's brain was perfused and sectioned to reconstruct the location of the cells. This allowed for the correlation of physiological properties with anatomical location in the brain.

Major Findings: Most of the neurons isolated in the pulvinar respond to visual stimuli. The cells tend to have receptive fields which are larger than those in the striate cortex and superior colliculus. One group of cells responds well to any type of visual stimulus which enters their receptive field. They respond equally well to large and small stimuli of different shapes and have no directional preference in terms of stimulus movement. A second class of cells responds well to all shapes of stationary stimuli but responds only to stimuli moving through their receptive fields in particular directions. The final group of visual cells responds only to elongated stimuli which are rather precisely positioned and oriented in the receptive field.

For many pulvinar cells, the response to the onset of a visual stimulus is enhanced or facilitated when the monkey is going to make a saccadic eye movement to the stimulus. The enhanced response is time-locked to the onset of the stimulus, and no change of activity occurs in these cells when the same eye movement is made spontaneously. Thus, this enhancement effect is a modulation of the visual response and not a discharge related to the generation of the eye movement. This modulation is spatially nonselective; it occurs on those trials when the animal makes a saccade to a stimulus outside of a receptive field as well as into it. In this respect the enhancement is similar to that seen in the striate cortex, a major afferent to the pulvinar. This suggests that the information encoded in the enhanced discharge of a pulvinar cell tells little about the direction or amplitude of the impending eye movement.

The enhanced response requires an eye movement; it is not present when the monkey attends to a stimulus but makes no movement to it. In this respect the enhancement is like that seen in the superior colliculus. When a pulvinar neuron fires intensely in the enhanced condition, it signals various regions of the brain that there is a visual stimulus present and that a visually-guided eye movement is going to occur. It does not indicate the direction or amplitude of that eye movement. These properties appear to be a hybridization of the major afferents to the pulvinar from the superior colliculus and striate cortex.

Significance to Biomedical Research and the Program of the Institute: The processes by which the brain takes the continually changing pattern of retinal stimulation and converts this into a percept of a stable visual world are unknown. Furthermore, the methods and strategies the brain uses to select some visual stimuli as opposed to others are unknown; this selection process must underlie visual attention and the generation of eye movements. It is entirely possible that different brain centers are specialized for each of these functions. The experiments outlined here are directed toward delineating functional systems of the brain for such active visual processes. Understanding of these basic brain mechanisms will enable more accurate diagnosis of visual-motor and perceptual deficits in man.

Proposed Course: Future experiments will attempt to clarify the role of the pulvinar in active visual behavior. Receptive field experiments will be directed toward determining how the functional properties of the pulvinar are related to other visual structures. Behavioral experiments will study how the pulvinar responds to the stimulus movement caused by eye movements. Cells in the striate cortex and superior colliculus prevent such responses by visual and nonvisual mechanisms respectively. Study of this process in the pulvinar will illustrate how these afferents influence the pulvinar. These experiments should indicate what the contribution of the pulvinar is to visual behavior.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders

Publications:

Robinson DL, Goldberg, ME, Stanton, GB: Parietal association cortex in the primate: Sensory mechanisms and behavioral modulations. J Neurophysiol 41:910-932, 1978.

Robinson DL, Goldberg, ME: Sensory and behavioral properties of neurons in posterior parietal cortex of the awake, trained monkey. Fed Proc 7:2258-2262, 1978.

Robinson DL, Goldberg, ME: The visual substrate of saccadic eye movements, in Senders JW, Fisher DF, Monty RA (eds): Eye Movements and the Higher Psychological Processes. Hillsdale, Erlbaum Associates, 1978, pp 3-14.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00043-01 LSR

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cortical Projections of Area 17 in the Rhesus Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leslie G. Ungerleider	Ph.D.	Senior Staff Fellow	LSR	NEI
Other:	Mortimer Mishkin	Ph.D.	Research Psychologist	LN	NIMH

COOPERATING UNITS (if any)

Laboratory of Neuropsychology, NIMH

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The sensory processing and perception of visual information requires the transmission of neural activity across a multisynaptic pathway from striate cortex, or area 17, through several prestriate "association areas." We have begun to explore the circuitry of these corticocortical connections in the primate visual system by the use of both anterograde degeneration and autoradiographic tracing techniques. Our investigations of striate efferents reveal direct projections to at least three separate and topographically organized visual areas within prestriate cortex. The largest projection field is a circumstriate cortical belt which corresponds to von Bonin and Bailey's cytoarchitectonic area OB. A second projection field is located in area OA along the caudal portion of the superior temporal sulcus. Finally, a third projection field, also located within area OA, occupies the anterolateral part of the annectent gyrus and extends forward into the intraparietal sulcus. The corticocortical efferents of these prestriate projection fields are now being examined to determine the pathways by which visual information is relayed from the occipital lobe to visual areas located within the parietal and temporal lobes.

Project Description:

Objectives: Although the projections of lateral striate cortex, the part representing central vision, have been extensively studied in the rhesus monkey, little information exists regarding the projections of posterior and medial striate cortex, parts representing peripheral and far peripheral vision, respectively. We therefore investigated the cortical efferents from all parts of area 17, with the aim of defining the locus, extent, and topographic organization of the entire striate-prestriate projection system.

Methods Employed: One series of monkeys (Macaca mulatta) was prepared with unilateral lesions of lateral, posterior, or medial striate cortex, such that collectively the lesions included all of area 17 with little or no invasion of area 18. Their brains were processed for terminal degeneration by the Fink-Heimer procedure. In a second series, selected striate sites were injected with tritiated amino acids, and the brains processed for authoradiography. Representations of the injection sites ranged from 4° to 25° from fixation in either the upper or lower visual field.

Major Findings: The results indicate that striate cortex projects to at least three separate and topographically organized visual areas within prestriate cortex. The largest projection field is a circumstriate cortical belt which corresponds remarkably closely to area OB of von Bonin and Bailey. It completely surrounds area 17 along the 17-18 border except at the representation of fixation. Within this visual area, the representations of the upper and lower visual fields are entirely separate. Progression from central to far peripheral vision is represented: a) in the lower field, by a progression into the posterior bank and depth of the lunate sulcus, medially along the surface of the buried annectent gyrus into the parieto-occipital sulcus, and then rostrally along the upper lip of the calcarine fissure; b) in the upper field, by a progression into the inferior occipital sulcus, ventromedially into the occipitotemporal and collateral sulci, and then rostrally along the lower lip of the calcarine fissure.

A second, smaller projection field is located in area OA along the caudal portion of the superior temporal sulcus; here, progression from central to far peripheral vision is represented by a progression down the posterior bank of the sulcus and continuing along its floor.

Finally, a third, even smaller projection field, also located in area OA, begins in the anterolateral part of the annectent gyrus and extends forward to occupy the depth of the lateral bank of the intraparietal sulcus. Despite its small size, evidence of a complicated topographic organization within this field raises the possibility that it may contain more than one visuotopic map.

Significance to Biomedical Research and the Program of the Institute: An understanding of the basic mechanisms mediating normal vision is the first step in the prevention, diagnosis, and alleviation of sensory and

perceptual disorders. In particular, a delineation of the neural circuitry involved in the transmission of visual information beyond striate cortex to prestriate "association areas" may promote advances in the treatment of residual visual function following injury, by either disease or acute assault, to the central visual pathways. The combined use of degeneration and autoradiography provides a powerful tool for tracing the neural connections within these central visual pathways.

Proposed Course: The topographic organization of the striate projection fields within prestriate cortex will be further examined in continued autoradiographic studies. By recording the activity of multiple units from the injection needle, tritiated amino acids will be placed into areas of striate cortex representing known parts of the visual field. This recording procedure will also be employed for investigating the corticocortical pathways from prestriate "association areas" into both the parietal and temporal lobes. In addition to the techniques of degeneration and autoradiography for anterograde tracing of neural connections, future experiments will employ horseradish peroxidase for tracing retrograde axonal transport. It is expected that the combined application of these procedures will help to unravel the apparent complexity in the pattern of projections beyond the striate cortex.

NEI Research Program: Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders

Publications:

Ungerleider LG, Christensen CA: Pulvinar lesions in monkeys produce abnormal scanning of complex visual array. Neuropsychologia (in press).

Ungerleider LG, Mishkin M: The striate projection zone in the superior temporal sulcus of Macaca mulatta: Location and topographic organization. J Comp Neurol (in press).

Laboratory of Vision Research

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1978 - September 30, 1979

REPORT OF THE CHIEF, LABORATORY OF VISION RESEARCH

Jin H. Kinoshita, Ph.D.

During the past year, progress has been made in the Laboratory of Vision Research (LVR) in many areas of investigation. This fact is documented by the impressive quality and number of publications by the various members of the LVR. In this report, a summary of some of these studies is presented. I wish to express my appreciation to the section heads and other LVR members who contributed material for this summary.

A possible lead into the process of one of the retinal degenerations has been uncovered by LVR scientists. The Irish setter dog exhibits an inherited form of retinal degeneration that is used as a model for human retinitis pigmentosa. In the affected dog retinas it was found that there is an abnormality in the enzyme that is responsible for cyclic GMP metabolism. Specifically, a switch in cyclic GMP phosphodiesterase (PDE) type fails to occur during early development in affected retinas. This biochemical defect, coupled with abnormally low levels of a PDE protein activator, results in the derangement of cyclic nucleotide metabolism that is characteristic of the disease. Purified brain protein activator has been found to restore PDE activity to that seen in control retinas in vitro thus opening the door for future experiments that we hope will halt or at least slow the progress of the disease in vivo.

Extensive studies of vitamin A are being undertaken because this vitamin plays such an important role in the health of ocular tissues. The functional interrelationships between the pigment epithelium and the visual cells of the retina, with emphasis on the dynamic turnover of membrane lipids, the involvement of vitamin A, and the effects of light, are being investigated. These research projects are designed to contribute information relevant to the diagnosis, treatment, and prevention of certain retinal and choroidal diseases.

The ability of the retinal pigment epithelium to store vitamin A was tested by injecting mice with retinol acetate or retinol palmitate and examining their retinas using fluorescence and electron microscopy. The results showed clear, dose-related increases in the numbers and sizes of vitamin A storing, and lipid droplets in the pigment epithelium but not in other cells of the retina. Such lipid droplets may represent physiological sites of vitamin A storage which are important for the maintenance of photoreceptor cells by the retinal pigment epithelium.

Electron microscopic histochemistry for catalase demonstrated that peroxisomes, like the putative vitamin A-storing lipid droplets, were distributed along the basal and lateral cell surfaces of the pigment epithelium where receptors for plasma retinol-binding protein have been reported. Peroxisomes may play a role in the reactions related to the esterification and sequestering of vitamin A in the retina.

The possible roles of vitamin E (α-tocopherol) in protecting photoreceptor membranes from autoxidation, in influencing pigment epithelial storage of vitamin A, and in retarding the accumulation of lipofuscin (aging pigment) in the retina were investigated. Rats were deprived of vitamin E while receiving marginal or fully adequate amounts of vitamin A for two, five, and eight months. By five months the retinas of vitamin E-deficient rats exhibited altered outer segment membranes, loss of photoreceptor nuclei, and the accumulation in the pigment epithelium of unusually large numbers of intracellular granules showing lipofuscin-specific autofluorescence. Extraordinary accumulations of aging pigment occurred also in extraocular muscle, uterus, heart, liver, and brain. Vitamin A-specific autofluorescence of retinal pigment epithelium and liver were higher in vitamin E-fed compared to vitamin E-deprived rats. Thus, vitamin E probably inhibited the peroxidative loss of vitamin A in both these tissues. In addition, vitamin E protected the photoreceptor cells from membrane disintegration and retarded aging pigment formation in the retinal pigment epithelium.

Other studies have been made of the role of vitamin A in the maintenance of rod and cone photoreceptors and of the ultrastructural changes associated with decreasing levels of rhodopsin and opsin in vitamin A deficient rats reared in low (1.5-2 foot-candles) or high (150-200 foot-candles) levels of cyclic light.

The levels of rhodopsin and opsin and retinal morphology are affected in vitamin A deficient rats reared in low levels of cyclic illumination (1.5-2 foot-candles). Rhodopsin levels decrease in deficient retinas, but 20 percent of normal levels is maintained through 39 weeks on the deficient diet. Opsin levels decrease at a slower rate but reach 20 percent of control levels by 32 weeks. Despite the decrease in rhodopsin levels, obvious deterioration of disc structure is not observed until 16 weeks of deficiency. The disruption of structure is initially localized in discs of the distal third. Degeneration of rod and cone nuclei is also seen. It appears that rod nuclei degenerate at a faster rate than cone nuclei in the central and peripheral retina. The photoreceptor cells are affected by retinol deficiency to a greater extent in the inferior hemisphere than in the superior hemisphere of the eye. Retinol is essential for the maintenance of rod and cone photoreceptors.

Rats exposed to light of moderately high intensity develop abnormal electroretinograms followed by degeneration of photoreceptor cells. Similar changes can be induced in the retina by vitamin A deficiency. However, retinas of rats deficient in retinol are less affected by light than retinas of retinol-adequate rats as determined by electroretinograms. In the retinas of retinol-adequate rats, discs in the distal third of the outer segments are distended and some broken into vesicles after short light exposures. These changes are seen throughout the outer segment length with prolonged exposures. By contrast, the retinol-deficient retinas are less disrupted after short exposures. A smaller number of outer segment discs are disrupted into vesicles. In addition, rod and cone photoreceptors degenerate at a slower rate in the retinol-deficient retinas after exposure to light. This study provides morphological evidence that retinas of vitamin A deficient rats are less susceptible to the damaging effects of light. The decrease in susceptibility to damage is probably related to the decrease in rhodopsin levels.

Vitamin A deficiency can produce xerophthalmia and keratomalacia involving the cornea. It is not clear from the published literature whether inflammation xerophthalmia, or keratomalacia are primary changes which occur as a result of vitamin A deficiency or secondary changes due to infection and poor health. In an effort to obtain information about the primary effects of the deficiency, corneas of retinol-deficient rats maintained on low levels of retinoic acid in a conventional laboratory environment and corneas from retinol-deficient rats receiving no retinoic acid and kept in a germ-free environment have been examined for structural abnormalities before the onset of xerophthalmia. Both groups of deficient rats showed abnormally large numbers of exfoliating epithelial cells, increased density of keratofibrils throughout the epithelial layer, decreased glycogen content, deposits of electron dense particles in the basal lamina region, and accumulation of electron dense bodies in the keratocytes. The corneal stroma of rats fed low levels of retinoic acid became vascularized, but no blood vessels were seen in the corneal stroma of the unsupplemented germ-free rats. This suggests that neovascularization and inflammation may be secondary changes initiated by local or systemic infection.

Further knowledge of the interconnection of photoreceptor terminals in primate has been gained by LVR neurophysiologists. It has become apparent that photoreceptors in the retina are not single isolated photodetectors measuring radiant energy only within the minute domain of their own outer segments, but participate in physiological interaction and make morphological contacts with their neighbors. It has been argued that these interactions, which appear primarily to allow electrical current to flow between neighboring cells, act to improve signal-to-noise ratios at the expense of visual acuity. Inter-receptor contacts have been studied in the primate in regions of high (foveal) and low (peripheral) visual acuity. In peripheral retina numerous specialized contacts were found between photoreceptor terminals. Observations of terminals in the fovea centralis revealed no such interreceptor junctions, a factor which corresponds well with the high acuity of this specialized region.

Anatomical features of retinal neurons, such as size and shape of their dendritic fields, put constraints on their input and output of information and are suggestive of retinal function. Two unusual types of amacrine cells may help to explain "complex" receptive field properties found in ganglion cells. "Asymmetrical" amacrines are unusual in that their dendrites extend away from the cell body in only one direction, toward superior retina. These may be involved in the formation of verticality detectors at the ganglion cell level. "Association" amacrines possess an axon which terminates about half a millimeter from the cell body. Since these cells are spatially polarized they may provide a basis for directionally selective responses recorded from ganglion cells.

A new type of horizontal cell in primate retina has been revealed. Termed H II to distinguish it from the previously identified H I, this cell has a fine axon 0.3 to 0.5 mm in length possessing short collaterals but no terminal aborization. Electron microscopy reveals that dendritic terminals as well as axonal collaterals contact cone pedicles as lateral elements. This is unlike the previously studied H I cell, which has a profuse terminal arborization contacting rods. Calculations of passive electrical spread suggest that a significant portion of signals generated in the cell body may be conducted along the axon, even considering the probable absence of impulse activity.

Consideration of dendritic and axonal overlap with other horizontal cell dendrites leads to speculation that the H II cell may contact blue and green cones.

Another subject under active investigation is the process of disc shedding and renewal. The photoreceptor unit of the retina renews itself by daily formation of new outer segment discs and shedding of packets of older discs at the apical tip. These shed packets are then phagocytized and digested by pigment epithelial cells. LVR biochemists have established that disc shedding and phagocytosis is a light-entrained circadian process in the mammalian retina. The process is complex in that it is controlled by independent oscillators within the retina itself and not by higher centers in the CNS (e.g. pineal) although the CNS does help to synchronize the burst of shedding. A burst of phospholipid synthesis in the outer segments precedes the shedding process and may be the biochemical signal for shedding.

It was found that an animal model for retinitis pigmentosa exhibits a low but definite pattern of disc shedding and phagocytosis, where none has previously been thought to exist. These processes can be increased under appropriate conditions in vitro. These studies may serve to suggest ways to correct the derangement in retinitis pigmentosa.

Studies of the developing eye have revealed the embryological origins of most of the mesenchymally derived tissues of the chicken eye. It was found that the mesoderm contributes all of the vascular endothelium, some vascular smooth muscle, all of the muscle fibers of the extraocular muscles, and some connective tissues and pericytes. The neural crest contributes all of the keratocytes, all of the corneal and trabecular "endothelial" cells, the muscle fibers of the ciliary muscle, all stellate melanocytes, some pericytes, osteocytes of most of the bones of the face and most of the connective tissue. While the neural crest cells are migrating to their final locations, they are guided, in part, by transient adhesions between basement membranes. Such adhesions develop between the basement membrane over the optic vesicle and the lens or overlying ectoderm. These adhesions guide the migration of neural crest cells around the developing eye as the maxillary and frontonasal processes. These studies point out the nature of some of the tissue interactions which control the orderly growth and differentiation of the developing eye. The knowledge gained in these studies may shed light in understanding the nature of human congenital eye abnormalities.

LVR cataract researchers have uncovered a new strain of mice which develops cataract visible to the naked eye five to six weeks after birth. The hereditary cataract in these Philly mice have many similarities to that of Nakano mice. In both cataracts there are major electrolyte changes characterized by an increase in sodium content and decrease in potassium. The alteration in the levels of electrolytes is accompanied by an increase in lens hydration. Thus, in both Nakano and Philly mice the cataracts are the osmotic type. However, the appearance of the cataracts is dissimilar. In the Philly mice the cataract initially involves the anterior region of the lens, while the opacity in the Nakano mice is first found in the posterior region. Moreover, the mechanism

which initiates the cataractous process appears different in the two osmotic cataracts. In the Nakano mice the underlying cause of the cataract appears to be the presence of an inhibitor of the crucial enzyme, sodium-potassium ATPase. The inhibitor has been identified as a polypeptide formed in the Nakano mouse lens. This inhibitor blocks the action of the transport ATPase resulting in the failure of the cation pump mechanism to maintain the normal distribution of cations. This abnormality leads to an increase in sodium accompanied by overhydration. The exact details of the mechanism which leads to the osmotic swelling in the Philly mouse cataract have not been worked out. However, it is distinctly different from the Nakano mouse cataract. In the Philly mouse lens there is no evidence of an ATPase inhibitor. The osmotic change appears to be caused by a defect in membrane permeability.

The study of these hereditary mouse cataracts is useful in that it has the potential for uncovering underlying mechanisms or the genetic defect which may also be involved in hereditary and congenital human cataracts. It may also help in establishing experimental procedures which may be useful in studying human congenital cataracts. A good example of this is illustrated in the study of the Nakano mouse cataract. Once it was established that an ATPase inhibitor was involved in the initiation of the cataractous process in Nakano mice, the question next explored was whether this inhibitor, which reflects the genetic defect in the Nakano lens, could be detected by tissue culturing epithelial cells from a single lens. This would simulate the situation in which the researcher finds himself in the study of human congenital cataracts. He has access to, at best, the epithelial cells of a congenital cataract. Tissue culture is the only possible means by which ample amounts of tissue could be made available to determine the underlying cause of these cataracts. Tissue culture procedures were thus established to grow sufficient cells from the Nakano mouse lens epithelium and to determine whether the ATPase inhibitor could be preserved through successive passage of the cells. The results showed that the Nakano lens cells could be successfully cultured, and adequate amounts of cells were obtained to allow for the isolation of the ATPase inhibitor which was identical to the inhibitor isolated from the Nakano mouse lens. Thus, the possibility exists that human congenital cataracts may also be studied by tissue culture procedures.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00003-07 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cataracts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jin H. Kinoshita	Ph.D.	Chief	LVR	NEI
Other:	Henry N. Fukui	Ph.D.	Research Chemist	LVR	NEI
	Suguru Fukushi	M.D.	Visiting Scientist	LVR	NEI
	Peter Kador	Ph.D.	Staff Fellow	LVR	NEI
	Howard Jernigan	Ph.D.	Research Associate	LVR	NEI
	Lorenzo Merola		Chemist	LVR	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two types of cataracts are currently being investigated. Sugar cataracts are initiated by the action of the enzyme aldose reductase. Effective means of delaying the onset of this type of cataract are being developed.

The second type of cataract studied is the hereditary cataract. We have developed two strains of mice with hereditary cataracts. Both cataracts are of the osmotic type, but subtle differences observed indicate that they are initiated by different mechanisms.

PHS-6040
(Rev. 10-76)

Project Description:

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

Methods Employed: Sugar cataracts can be induced in laboratory animals by making them diabetic with appropriate chemical agents, or by making them galactosemic or xylosemic with a diet enriched with either galactose or xylose. Another approach to studying cataracts is to employ animal models. We have developed Nakano and Philly mouse strains with hereditary cataracts.

Major Findings: Studies of sugar cataracts, diabetic and galactosemic, are being continued. The enzyme aldose reductase (A.R.) is responsible for the initiation of this type of cataract. Considerable effort is being directed to develop inhibitors which would delay if not prevent these cataracts. Currently, there are seven pharmaceutical companies involved in developing A.R. inhibitors. A.R. has been isolated and purified from animal lenses and human placenta. Affinity chromatographic procedures have been developed to purify A.R. from human placenta. The A.R. from human placenta has different properties in its response to inhibitors. For example, the flavonoid, quercitrin, which is very active against rat lens A.R. is only one tenth as active against the human placenta A.R. A chromone carboxylic acid compound, developed in our Laboratory, is the only A.R. inhibitor more active against the human placenta A.R. than it is against the rat lens A.R. Preliminary studies indicate the human placenta A.R. has properties similar to human lens A.R. Obviously, screening A.R. inhibitors for potential clinical use would be more appropriately conducted with human A.R.

The galactose cataract model has been used to study the later stages of cataract. Even though the initiating mechanism is different, many cataracts appear to have in common the terminal stage of nuclear opacification. Prior to the development of nuclear opacity, there is a cessation of lens growth. This phenomenon, observed in sugar cataracts as well as in certain hereditary mouse cataracts, appears related to the decrease in protein synthesis during cataract development. That the altered synthesis of lens crystallin may be associated with changes in the intracellular concentrations of sodium (Na) and potassium (K) ions was examined in rats during the development and reversal of galactose cataracts. Cataracts were produced by feeding an enriched galactose diet; partial reversal of cataracts was effected by removing galactose from the diet after cataract formation. At intervals, groups of experimental and control rats were sacrificed. One lens from each animal was placed in organ culture in the presence of ^{35}S-methionine. Protein synthesis was measured from autoradiographs of electrophoretic gels on which the homogenates were

fractionated. Contralateral lenses were used for the determination of Na and K cations.

A differential effect on protein synthesis was observed. During galactose cataract development the synthesis of lens crystallins was markedly depressed while that of noncrystallin protein was unaffected. This effect correlated with striking changes in the lens cation levels: an increase in Na and a loss of K. During the reversal of the cataract resulting from the removal of galactose from the diet, reactivation of crystallin synthesis occurred concomitantly with recovery of cation levels to normal values. These findings further support the concept that cations influence the synthesis of lens crystallins.

We are continuing the study of congenital and hereditary cataracts in animals. As reported previously, the Nakano mouse cataract appears to involve a Na-K ATPase inhibitor which develops in the lens. The inhibitor adversely affects the crucial cation pump mechanism leading to overhydration of the lens.

We have uncovered a new strain of mice which develop cataracts approximately six weeks after birth. This Philly mouse strain is derived from the Swiss Webster mouse. Although there are some similarities to Nakano mouse cataracts, Philly mouse cataracts are distinctly different.

The gross morphology of the Philly cataract is generally similar, but not identical, to that of the Nakano cataract. In both, the earliest change is a haziness that develops at the posterior pole in the subcapsular region. This cloudiness progresses peripherally but does not extend to the equator. Later, the haziness involves the anterior subcapsular region. These events precede the obvious nuclear lamellar cataract which eventually leads to a total cataract. The changes proceed more rapidly in the Nakano where the nuclear opacity appears at three weeks after birth.

The cataract in the Philly mouse, like that in the Nakano, appears to be osmotic in nature. With the nuclear cataract there is an increase in lens hydration which is concomitant with a sudden increase in sodium and decrease in potassium. Unlike the Nakano cataract, however, there is no evidence of an impairment in the cation pump mechanism. In the early stages of the Philly cataract the uptake of rubidium ions is unaffected. In the Nakano cataract the deficiency in the cation pump activity appears related to the presence of a Na-K ATPase inhibitor. The transport ATPase inhibitor could not be detected in the Philly mouse lens, under the conditions of assay used for the Nakano lens.

Transport studies revealed no significant difference between the Philly and control lens in the accumulation of amino acid. However, when rubidium was substituted for potassium, a decreased accumulation in the Philly lens older than 20 days was correlated with increased rubidium leak-out. This increased leak-out of rubidium appears to reflect the key biochemical change that accounts for osmotic cataract formation, and it suggests the possibility of a defect in membrane permeability.

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

Proposed Course: These projects will be continued. The mode of action of the aldose reductase inhibitors will be studied in more detail in regard to the structure-activity relationships. This study may aid in suggesting what substituents on the inhibitors are needed to increase their potency.

The nature of the Na-K ATPase inhibitor which appears to be the cataractogenic factor in the Nakano mouse lens will be characterized.

NEI Research Program: Cataract--Diabetic Cataract/Congenital, Metabolic, and Genetic Cataracts

Publications:

Tsunematsu Y, Fukui HN, Kinoshita JH: Studies on primary cultures of adult lens cells from normal and cataractous mice. Exp Eye Res 26:671-686, 1978.

Piatigorsky J, Fukui HN, Kinoshita JH: Differential sysnthesis, degradation and leakage of protein in an inherited cataract and in the normal lens cultures with ouabain. Nature 274:558-562, 1978.

Kador PF, Kinoshita JH: Phospholipid effects on the rat lens transport systems Exp Eye Res 26:657-666, 1978.

Kador PF, Merola LO, Kinoshita JH: Differences in the susceptibility of various aldose reductases to inhibition. Doc Ophthalmol Proc Series 18:117-124, 1979.

Kinoshita JH, Fukushi S, Kador PF, Merola LO: Aldose reductase in diabetic complications of the eye. Metabolism 28:462-469, 1979.

Kador PF, Zigler JS, Kinoshita JH: Alteration of lens protein synthesis in galactosemic rats. Invest Ophthalmol Vis Sci (in press).

Kador PF, Jernigan HM Jr, Kinoshita JH: Accumulation and incorporation of radiolabeled choline transport system. Exp Eye Res (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00035-01 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Rod Outer Segments on Cells in Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Igal Gery	Ph.D.	Visiting Scientist	LVR NEI
Other:	Julia Derr	B.A.	Biologist	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Retinal rod outer segments (ROS) inhibit the metabolic activities of cultures of lymphocytes or of other tested cells that grow in suspension. On the other hand, tested cells which attach to the surface of culture containers were not inhibited or were even stimulated by ROS. Preparations of intact ROS discs were less inhibitory than disrupted ones. Yet, disrupted ROS discs do not leak upon incubation any significant inhibitory activity. In addition to vitamin E, other natural antioxidants were found to counteract the inhibitory effect of ROS. These include two enzymes, superoxide dismutase and catalase.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: We have previously shown that ROS inhibit lymphocytes in culture (FY 78). The present study was aimed at further analysis of the ROS effect, mainly with regard to the susceptibility of various cell cultures, the localization of the inhibitory components and their counteraction by various natural antioxidants.

Methods Employed: ROS were fractionated regularly on sucrose gradients; intact discs were obtained from metrizamide gradients provided by Dr. A. Adams (LVR, NEI). Lymphoid cells were obtained from experimental animal spleens or from human peripheral blood. Subcultured mouse lens epithelium, human keratocytes or murine mastocytoma cell line (P 815) were obtained from Drs. P. Russell (LVR, NEI), D. BenEzra (CB, NEI) or G.M. Shearer (IB, NCI), respectively. Macrophages were collected from peritoneal cavities of guinea pigs. All experiments were carried out in microcultures; the activities of the tested agents were measured by their capacity to affect the incorporation of tritiated thymidine by the cultured cells.

Major Findings: Bovine ROS strongly inhibit DNA synthesis in all tested lymphoid cells in culture. Human lymphocytes are less affected than those from mouse or guinea pig. ROS also inhibited cultures of the mastocytoma cell line, P815, which grow in suspension. On the other hand, no inhibition was caused by ROS in cultures of attached cells, namely, monolayers of macrophages, murine lens epithelium, or human keratocytes. Furthermore, lens epithelial cells exhibited significant levels of increased DNA synthesis in the presence of ROS.

Intact ROS discs inhibit lymphocytes much less than disrupted ROS. However, disrupted ROS discs release upon incubation minimal amounts of inhibitory activity. It is assumed, therefore, that disrupted discs increasingly inhibit cell cultures mainly because of the loss of naturally occurring antioxidants, such as vitamin E.

Two enzymes, known to be scavengers for free radicals, were found to counteract the inhibitory effects of ROS. These are superoxide dismutase and catalase. Combinations of vitamin E and these enzymes produced additive counteractive effects.

Significance to Biomedical Research and the Program of the Institute:

This study provides a simple experimental system to examine the damaging effects of ROS and its counteraction by antioxidants. Our results support the notion that potentially damaging ROS components are released during the breakdown of retina in conditions like retrolental fibroplasia, retinitis pigmentosa, or lipofuscinosis. Our data suggest that the outcome of retinal damage may be affected by the local availability and concentration of antioxidants like vitamin E or free radical scavengers. The observed difference between the various tested cells may indicate a relationship between the surface attachability of a cell to its susceptibility to certain inhibitory agents like the ROS components.

Proposed Course: Two aspects in particular will be further studied: (a) the relationship between the surface attachability of cells and their susceptibility to ROS effects, and (b) the capacity of pigment epithelial cells to provide protection for other cells from the damaging effects of ROS. Pigment epithelial cells resemble macrophages in many aspects and the latter cells were reported in our previous report to protect lymphocytes against ROS.

NEI Research Program: Retinal and Chorodial Diseases--Visual Cells and Pigment Epithelium

Publications: Gery I: Inhibition of DNA and RNA synthesis in lymphocyte cultures by rod outer segments and its counteraction by vitamin E and other antioxidants. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00069-02 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Immune Responses to Lens Crystallins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
Other:	Robert Nussenblatt	M.D.	Senior Staff Ophthalmologist	CB	NEI
	Julia Derr	B.A.	Biologist	LVR	NEI

COOPERATING UNITS (if any)

Clinical Branch, NEI

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lens-induced uveitis is attributed to an autoimmune response against crystallins, but the pathogenic mechanism is not known. Rabbits immunized against lens from rabbits or other animals made high levels of autoantibodies, which react with autologous lens antigens, but failed to produce considerable cellular auto-immunity. The dissociated immune responses were measured by a battery of tests in vitro, as well as by skin testing the immunized animals. Rabbit lens antigens induced antibody-mediated Arthus reactions, but no cell-mediated delayed type skin responses. These findings thus support the notion that autoantibodies play the major role in causing the lens-induced uveitis.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Preliminary data, reported in FY 1978, demonstrated a dissociation between the cellular and humoral immune responses to autologous lens antigens. This finding has been further analyzed by including in vivo immunological tests as well as additional immunization combinations.

Methods Employed: Rabbits were immunized by repeated injections of lens extracts, emulsified with complete Freund's adjuvant, from various animals. Serum antibodies were measured by gel precipitation and passive hemagglutination. Lymphoid cells were collected from draining lymph nodes and their responses to antigens were determined according to their increased incorporation of tritiated thymidine. Skin tests were performed by injecting intradermally aliquots of the tested antigens. The Arthus reaction was measured four hours following injection and the delayed type response measured after 48 hours.

Major Findings: The dissociation between the cellular and humoral limbs of the immune response to lens crystallins was found to be sharply defined by the skin reactions of the immunized rabbits. Animals immunized with lens from other species (xenogeneic) showed intense Arthus type skin reactions to lens antigens from all tested animals, including those from the rabbit. On the other hand, delayed type reactions were observed only to the immunizing foreign lens. Arthus reactions are mediated by antibodies, while the delayed type is mediated by sensitized lymphocytes. Most rabbits immunized with rabbit lens extract in an adjuvant emulsion produced autoantibodies to lens crystallins which reacted strongly in the passive hemagglutination test, gave precipitation lines in gel, and produced Arthus reactions of moderate intensity. These rabbits, however, showed no delayed type skin reactions to lens antigens, and their lymphocyte cultures reacted minimally or not at all to these antigens.

Significance to Biomedical Research and the Program of the Institute:

The finding that immunization against lens antigens produces large amounts of autoantibodies but very little or no cellular autoimmune response supports the notion that phacogenic uveitis is caused mainly by antibody-mediated pathogenic processes, similar to those participating in the Arthus reaction. Furthermore, these results provide support for the hypothesis that normally T cells are unresponsive toward autologous lens crystallins, while B lymphocytes are fully reactive toward these antigens. These data may help in selecting medication for phacogenic uveitis; drugs with a selective effect on the B cell compartment should be the appropriate ones for treating this condition.

Proposed Course: The notion that T lymphocytes are normally unresponsive to autologous lens crystallins will be further studied by testing purified preparations of B- or T-cells. The possibility that the selective unresponsiveness is produced by suppressor cells will be examined by using animals in which suppressor cells are selectively absent, namely, mice of the NZB strain, or animals treated with certain doses of cyclophosphamide. The effect of preexposure of the immune system to crystallins on the response to these antigens will be studied by testing the immune reactions to crystallins in animals with cataracts or spontaneous liquification of the lens.

NEI Research Program: Cataract--The Normal Lens; Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Nussenblatt R, Gery I, BenEzra D: Tissue specificity of ocular antigens, in Silverstein AM, O'Connor GR (eds): Immunology and Immunopathology of the Eye. New York, Masson Publishing USA, pp 145-150, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00023-01 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Macrophage Interactions with Ocular Cells and with Storage Lipids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Igal Gery	Ph.D.	Visiting Scientist	LVR NEI
Other:	David BenEzra	M.D., Ph.D.	Chief, Pediatric Ophthalmology	Hadassah Hosp. Jerusalem, Israel
	J. Samuel Zigler, Jr	Ph.D.	Staff Fellow	LVR NEI
	John A. Barranger	M.D.	Chief, Clinical Section	DMNB NINCDS
	Fred Chu	M.D.	Senior Staff Fellow	CB NEI
	Julia Derr	B.A.	Biologist	LVR NEI

COOPERATING UNITS (if any)
Hadassah Hospital, Jersualem, Israel
Clinical Section, Developmental and Metabolic Neurology Branch, NINCDS

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.6	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Macrophages release mediators which affect the metabolic activities of certain ocular cells. Human blood monocytes secrete in vitro a factor which enhances the DNA synthesis of human corneal cell cultures. On chromatography, the factor was located within the molecular range of 40,000-50,000 daltons. It is possible this mediator plays a role in wound healing.

The effects of a lipid storage compound, glucocerebroside (GL_1), were tested on macrophage cultures. This compound, which accumulates in cells of Gaucher's disease patients, stimulates the secretion of a mediator, lymphocyte activating factor (LAF) from both human and mouse macrophages. Moreover, GL_1 in synergy with another stimulant, endotoxin, causes the secretion of extremely high levels of LAF by macrophages. In addition, GL_1 increases the release of lysosomal enzymes by the mouse macrophage cultures. Both LAF and the enzymes may possibly be related to certain features of the Gaucher's disease.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Macrophages participate actively in ocular inflammatory reactions and, conceivably, interact with cells and other components of the eye. These studies were aimed at testing (a) the effects of products of macrophages on the metabolism of ocular cells, in particular those involved in wound healing and (b) the processes in which macrophages are activated by pathological tissue components, mainly those which accumulate in lipid storage diseases.

Methods Employed: Human blood monocytes were cultured in the presence of endotoxin for 24 hours and their supernatants collected and tested for their capacity to stimulate the incorporation of thymidine by human keratocyte subcultures. Chromatography of the supernatants was carried out on Sephadex G-75 columns. The keratocyte cultures were set up in microplates, pulsed with ^{3}H-thymidine and harvested on glass fiber filters.

Levels of activation of macrophage monolayer cultures by glucocerebroside and other agents were determined by measuring in the supernatants the activities of lymphocyte activating factor (LAF) and lysosomal enzymes. LAF activity was assayed according to the capacity of the supernatants to stimulate ^{3}H-thymidine incorporation in mouse thymocyte cultures. The lysosomal enzymes, hexosaminidase, galactosidase, and glucuronidase were measured by Dr. Barranger.

Major Findings: Human monocyte cultures, stimulated with endotoxin, release a factor which markedly stimulates the incorporation of ^{3}H-thymidine by human keratocytes in culture. Chromatographic analysis indicated that this mediator is different from the lymphocyte activating factor (LAF); the latter mediator fractionates with molecules of about 15,000 daltons, while the factor which activates keratocytes elutes at the 40,000-50,000 dalton range.

Glucocerebroside (GL_1), the sphingolipid stored in Gaucher's cells, was found to stimulate profoundly the release of LAF by human or mouse macrophages. Of a particular interest was the finding that GL_1 synergizes with another macrophage stimulant, endotoxin, to yield exceedingly high levels of LAF from the macrophages. In addition, GL_1 stimulates the release of lysosomal enzymes from the mouse macrophages, in particular galactosidase and hexosaminidase.

Significance to Biomedical Research and the Program of the Institute:

The macrophage-made mediator, which affects the growth of corneal cells in culture, may have a regulatory role in the process of wound healing in vivo. Our data may thus provide an additional experimental system for the investigation of the healing mechanism. The chromatographic purification of the mediator may enable testing its effects in cellular reactions both in vivo and in vitro.

The study concerning the effects of GL_1 on secretion of specific products by macrophages is the first known attempt to examine this aspect of the interaction between storage sphingolipids and macrophages. The results, showing that GL_1 molecules activate macrophages, may be related to two features of the Gaucher's disease, namely, a marked increase in the blood levels of hydrolytic enzymes and a high frequency of monoclonal and polyclonal gammopathies. An increased release of enzymes by GL_1-affected macrophages may account for the first feature, while the gammopathies may be related at least in part to the capacity of LAF and other macrophage-made mediators to stimulate proliferation as well as globulin synthesis by lymphocytes. The activities of GL_1 in vivo may be particularly enhanced in the presence of the naturally occurring stimulants, like endotoxin, similarly to the unique synergy observed in vitro.

Pathologic conditions of lipid storage may affect the ocular system, e.g. the Tay-Sachs or Niemann-Pick syndromes, as well as rare cases of juvenile Gaucher's disease. The presented line of research thus provides an experimental system for studying the interactions between sphingolipids and the affected cells.

Proposed Course: The macrophage-made mediator, which stimulates keratocytes, will be further characterized with regard to its biochemical features. Attempts will also be made at analyzing the mode of action of this factor.

Further studies of the interaction between sphingolipids and macrophages will focus on the relationship between the uptake of the lipid, its breakdown by the affected cell and the induced changes in cellular activities. In addition to glucocerebroside, other sphingolipids will be tested and the effects of these compounds will be examined on various other cells.

NEI Research Program: Corneal Diseases--Corneal Transplantation and Stromal Injury and Repair--External Ocular Infectious and Inflammatory Diseases; Retinal and Choroidal Diseases--Developmental and Hereditary Disorders-Inflammatory Disorders

Publications:

Gery I, Davies P: Immunoregulatory products of macrophages, in Cohen S, Oppenheim JJ, Pick E (eds): Biology of Lymphokines. New York, Academic Press, 1979, pp 347-367.

BenEzra D, Gery I: Stimulation of keratocyte metabolism by products of lymphoid cells. Invest Ophthalmol Vis Sci 18:317-320, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00135-07 LVR

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemical Structure of Retina and Pigment Epithelium in Health and Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Helen H. Hess	M.D.	Medical Officer (Research)	LVR	NEI
Other:	David A. Newsome	M.D.	Senior Staff Ophthalmologist	CB	NEI
	Peter Gouras	M.D.	Head, Section on Neurophysiology	LVR	NEI
	Carl Hansen	Ph.D.	Geneticist	VRB	DRS

COOPERATING UNITS (if any)

Veterinary Resources Branch, Division of Research Services; American Histolabs, Inc. (contract).

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Biochemistry

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.15	1.10	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of the project is to study the biochemical composition of pigment epithelium, retina, and rod outer segments in normal circumstances and in retinal and choroidal diseases of experimental or genetic origins. Topics of current interest are: (a) study of the concentration and distribution of inorganic constituents by flameless atomic absorption; (b) localization and physiological function of Ca in retina, pigment epithelium and choroid; (c) possible involvement of Ca, Zn and Cu in retinal and choroidal diseases; and (d) study of hybrids of RCS rats and spontaneously hypertensive (SH) rats to determine whether the slow onset type of retinal degeneration seen in the latter is inherited at the rdy or another gene locus or is due to light damage in an albino animal.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the biochemical composition of retinal photoreceptor, neuronal, glial, and pigment epithelial cells in health and disease, and to explore possibilities for prevention or therapy of retinal and/or choroidal disease when a biochemical abnormality has been identified. Diseases in which pigment epithelium (PE) is involved are of particular interest.

Methods Employed: Retinas, isolated rod outer segments (ROS), and PE of frogs and rats are being analyzed. Samples of urine are being studied in human cases of retinal degeneration. Methods include flameless atomic absorption spectroscopy, microscopy, and a number of standard biochemical laboratory techniques.

Major Findings:

I. Localization of calcium in retina and pigment epithelium:

Our analyses have shown that calcium is abundant in the PE cell of frogs, and it is visibly localized in Bruch's membrane and within the internalized disks of ROS by the technique of staining with K pyroantimonate. The calcium content by analysis is higher in ROS isolated from frog retinas light-adapted in vivo than from retinas dark-adapted in vivo. A higher content of Ca in ROS in the light is consistent with physiological studies pointing to a role for Ca in control of photoreceptor sensitivity (Flaming and Brown, 1979). Higher cytosol Ca is hypothesized to be related to decreased amplitude of photoreceptor response after light adaptation. This would agree with the fact that light-adapted retina, as compared with dark-adapted retina, is inhibited and uses less glucose and oxygen.

II. Trace elements in 24-hour urine specimens from patients with pigmentary retinal degenerations:

This study began as an investigation of a report of Gahlot et al. (1976) that the amount of Cu excreted in a 24-hour urine specimen from patients with primary retinitis pigmentosa (RP) was six times normal. Our early work did not confirm this finding, as all patient values were within a normal range of 5-30 µg Cu/24 hours. Our subsequent analyses, as well as those of two other groups (U.S. and England), have continued to show normal Cu values in 24-hour urines of RP patients. A possibility that such patients may tend to have values in the "high normal" range is being explored. Studies elsewhere in the U.S. have suggested that low dietary Cu may be more common than realized, and a comparison with the Indian diet consumed by the RP patients of Gahlot et al. will be needed.

We determined Zn in the same urines in which Cu was being studied. The normals ranged from 94 to 765 µg Zn/24 hours, with a mean of 625 $\pm$ 84 (SE) for males and 367 $\pm$ 70 for females, in agreement with the literature. Among the group of patients and relatives analyzed so far (38), there were five with abnormally high values, greater than 1000 (1050-1715 µg/24 hours). Because the experiments are being conducted in a masked fashion, the diagnoses in

the group are not all known to the analyst as yet; four of the five urines, however, were from patients with RP and macular degeneration and the other had some type of retinal degeneration.

Information on body weight, dietary pattern, and possible Zn supplements or other pharmacological or alcoholic intake is being collected. Two females (one normal and one in the patient group) had very low values (90-100μg Zn/24 hours) and were on estrogenic medication. Studies elsewhere on Zn excretion in patients on estrogens have indicated that when dietary Zn is low, estrogens have an anabolic effect so that Zn is conserved and not excreted in the urine; with a normal Zn intake of 12.5 mg per day, urine Zn is not affected. Dietary Zn below the recommended daily allowance may occur not infrequently in the U.S.

III. Dietary factors improving reproduction and growth in RCS and congenic control rats:

In our laboratory animal rooms, unprotected from pathogens, the breeding and survival of young RCS rats, especially the congenic control strain, have been serious problems. We have found that feeding a commercial diet with higher concentrations of Zn and vitamins E and A, together with supplements of a natural food (sunflower seeds) having a high content of linoleic acid, results in larger litters, calmer temperament of dams, better milk production, and better growth and survival of pups to weaning and beyond. For the first time, this has enabled us to produce young of specified ages for developmental biochemical studies, including ideal control animals.

IV. Studies of hybrid rats from two strains with retinal degeneration:

Through the collaboration of Dr. Carl Hansen, a geneticist, in the NIH Division of Research Services an F-1 generation from a cross between the RCS dystrophic rat and the SH or spontaneously hypertensive rat was obtained. The black-hooded hybrids (from tan-hooded RCS and albino SH) retained normal retinal structure for up to two years of age, as verified by study of both fresh and fixed stained histopathological material by light microscopy. Since retinal degeneration occurs at a few weeks of age in the RCS rat and by 4 to 12 months in the SH rat, the F-1 hybrid was expected to show changes by 12 months (certainly by two years) if the rdy gene of the RCS rat were in the SH genome, or if a different abnormal gene were at the same locus. Apparently, if the SH rat has a gene for retinal degeneration it is different and present at a different locus than the rdy gene of the RCS rat.

Dr. Hansen also suppled an F-2 generation of rats which were studied at 18-24 months of age by ERG and histopathologic examination. A fourth of the animals had extinguished ERG's, as expected from the autosomal recessive rdy gene. In addition, a fourth of the remaining animals had B waves with amplitudes below 500μV, while the others ranged up to a normal of 1000 μV. Histopathological study confirmed that a fourth of the animals, corresponding to those with extinguished ERG's, were totally devoid of photoreceptors. The remaining specimens, including those from animals with low B waves, con-

tained many photoreceptors. About half of the animals having a poor B wave response were black-hooded and would not have suffered light damage. In his original description of the retinal pathology of the SH rat, Mizuno et al. (1972) reported a subnormal B wave (450 μV or less) in some of the animals, and these had normal retinal architecture when examined by light microscopy, although changes were visible under EM. Further work should be done using a pigmented SH strain, and the pathological and genetic aspects of the disease studied in more detail now that a clear differentiation has been made from the RCS rdy locus. The SH retinal disease could be a model for late-occurring RP.

Significance to Biomedical Research and the Program of the Institute:

Elucidation of the role of calcium in photoreceptors in dark- and light-adaptation could have broad significance for understanding retinal function in health and disease. Ca appears to be related to changes in cyclic nucleotides during illumination, to the level of rod photosensitivity, and perhaps to ROS tip shedding and pigment epithelial phagocytosis of shed tips. If urine or some other readily available body fluid or tissue of patients with pigmentary retinal degeneration can be found to show some abnormality (such as a change in metabolic control of Zn or other metal in 24-hour urine) this could point the way to etiological investigations. The increased reproductive capability of our RCS and congenic control rats on the new diets we have developed has solved our problem in producing young for developmental biochemical studies. Our work on the F-1 generation of the RCS/SH cross showed that the retinal degeneration in the SH strain cannot be from the rdy gene or another mutant gene at the same locus. In the F-2 generation, aside from the expected number of extinguished ERG's from the rdy/rdy dystrophics, several animals showed diminished B waves in the low range reported by Mizuno et al. (1972) in the SH strain. This would be consistent with the SH being a model for late occurring RP, but more study will be required to prove this.

Proposed Course: The project will be continued as part of a new Clinical Branch Section on Retinal and Ocular Connective Tissue Diseases, headed by David Newsome, M.D., with whom collaboration was established this year. The content of calcium in retina, ROS, and PE will be studied further in dark and light in the presence of different media to determine factors that may control changes in level. Relationships of Ca, Zn and other mineral constituents to phagocytic and immunological activities in phagocytic cells, including PE cells will be studied in cultured cells. Additional 24 hour urine specimens from patients with pigmentary retinal degenerations of different types will be assayed for Zn and Cu.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00136-07 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Chemistry and Metabolism of the Lens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul Russell	Ph.D.	Research Chemist	LVR	NEI
Other:	Jin H. Kinoshita	Ph.D.	Chief	LVR	NEI
	Samuel Zigler	Ph.D.	Staff Fellow	LVR	NEI
	Deborah A. Carper		Biologist	LVR	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Biochemical and immunochemical studies of proteins of human and animal lenses and cataracts have been undertaken. An intrinsic protein of the membranes of the human lens was found to be similar to that of the animal lenses. However, with aging a second membrane protein emerges in the human lens. This protein apparently is derived from the principal membrane protein.

As the cataract develops major changes in the lens proteins occur. Aggregation of proteins appears to be an important factor. This process leads to the formation of soluble, heavy molecular weight proteins and also insoluble proteins. The conversion of soluble proteins to insoluble proteins appears as a constant finding in most cataracts. Degradation and leakage of proteins in the lens also occur during cataract formation.

PHS-6040
(Rev. 10-76)

Project Description:

Objective: To understand the factors that lead to cataract formation, it is essential to establish the normal levels of various lens constituents as well as understand the nature and characteristics of the metabolic systems that maintain lens viability. These variables are studied during cataract formation in the hope that a lead to the initiating mechanism may be uncovered. This study is conducted on lenses and cataracts from human and animal eyes as well as on lens cells maintained in tissue culture.

Methods Employed: Various proteins from human and mouse lenses as well as from different cataracts have been studied. They have been analyzed using column chromatography, gel electrophoresis, and immunochemical techniques. In addition, iodination of alpha, beta, and gamma crystallins with isotopic iodine was performed to establish radioimmunoassays for these proteins.

Major Findings: The lens protein has received considerable attention because this is the constituent present in the highest concentration in the lens. Alteration in the crystallins, the class of proteins found in the lens, is thought to account for the opacity observed in some cataracts. Thus, considerable effort has been made to learn about the nature of these proteins in the normal lens and the changes they undergo in cataract formation.

In many cataracts it has been suspected that gamma crystallin disappears from the soluble protein fraction. However, the process by which γ crystallin is lost from the cataract has not been established. We followed the fate of γ crystallin during cataract development in the Nakano mouse. The radioimmunoassay (RIA) technique demonstrated that γ crystallin decreases to a level of 4% in the soluble fraction of the Nakano mouse lens, whereas γ crystallin maintains a level of 32% in the soluble fraction of the normal lens.

The disappearance of γ crystallin can occur by several mechanisms. The loss of this protein can result from degradation from incorporation into the heavy molecular weight (HMW) fraction of soluble protein or into insoluble protein or from leakage into the aqueous. With the sensitivity and specificit of the RIA, we first pursued the hypothesis that γ crystallin may be leaking from the lens. Aqueous humor from normal and Nakano mice was tapped with the aid of a dissecting microscope. When tested by RIA, 1 μl of Nakano aqueous humor had greater than 100 ng of γ crystallin, while the level of γ crystallin in 1 μl of normal mouse aqueous was below the sensitivity of the assay. Over 5% of the total protein in the Nakano aqueous was γ crystallin. Thus, γ crystallin does appear to leak out of the cataract.

The possibility that γ crystallin is lost by other means, as well, is now being investigated. However, the high γ crystallin concentration in the Nakano mouse aqueous conclusively shows that leakage of this protein is one process contributing to the depressed γ crystallin level in cataract.

The water-soluble proteins from human lens have been fractionated by gel filtration into five major fractions--α, β_1, β_2, β_3, and low molecular weight (LMW) crystallins. Each has been purified and partially characterized by immunochemical and physicochemical techniques.

Sodium dodecyl sulfate (SDS) gel electrophoresis suggested that the major β-crystallin polypeptides are common to all three β-crystallin fractions, but that each fraction has a distinct complement of SDS-subunits based on the distribution of other, quantitatively less significant chains. Isoelectric focusing in urea revealed very heterogeneous patterns for each fraction. Consistent with the electrophoretic results, the electrofocusing patterns for the three β-crystallin fractions were very similar to each other. Each β-crystallin fraction contains a group of polypeptides with molecular weight near 43,000. The relationship of these components to the native β-crystallin aggregates has been investigated for each fraction.

Antibodies to each of the five major fractions have been prepared in rabbits and used in immunochemical studies of the composition of the β-crystallins and LMW-crystallins. β_1, β_2, and β_3 -crystallins were shown to be antigenically very similar although some differences were detected. LMW-crystallin was found to contain monomeric β-crystallin as well as γ-crystallin, a 10,000 dalton polypeptide, and at least one other unidentified polypeptide chain.

The predominant intrinsic membrane protein isolated from plasma membranes of chick, calf, and young cow is a polypeptide with an apparent molecular weight of 26,000. SDS-polyacrylamide gel electrophoresis of membrane preparations obtained from stillborn and 9-month-old human lenses also show this 26,000 MW polypeptide to be the major protein component. However, in mature human lenses, a second major membrane polypeptide is found with an apparent molecular weight (MW) of 23,000. Evidence for the emergence of the 23,000 MW membrane polypeptide was seen in a 3-year-old lens and this polypeptide increases rapidly with age, so that in a 24-year-old lens, the amount of the 23,000 MW polypeptide approaches that of the 26,000 MW. The relative abundance of the 23,000 MW and the 26,000 MW membrane polypeptides depends on the region from which the lens fibers were isolated. In mature lenses, membrane preparations obtained from nuclear fibers are richer in the 23,000 MW polypeptide than in the 26,000 MW one. Membrane preparations from cortical fibers of the same lenses show a higher abundance of the

26,000 MW polypeptide. Comparison of some of the physicochemical properties of the 26,000 MW polypeptide with the 23,000 MW one suggest that the 23,000 MW polypeptide is derived from the 26,000 MW polypeptide.

The later phases of the Nakano cataract, where obvious changes in the lens crystallins are taking place, have been examined in regard to lens proteins. Heavy molecular weight proteins have been detected in the lenses of other animals and are thought to be an intermediate in the formation of insoluble proteins. This possibility was examined in the Nakano mouse cataract.

Heavy molecular weight proteins were detected in normal and cataractous mouse lenses. The mouse lens showed an increase in HMW aggregates with age similar to the increases reported with other species. Alpha and beta crystallins were detected in the HMW fractions by immunodiffusion, but the presence of gamma crystallin in the aggregates could not be demonstrated using this technique.

In the cataractous Nakano mouse lens, the polypeptide composition of the HMW fraction was different from that of the normal mouse lens. Sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed a polypeptide of 28,000 MW in the Nakano HMW fraction but not in the normal. In addition, the Nakano aggregates lacked a 16,000 MW band found in the normal HMW material. Although the amount of HMW protein in the Nakano lens was about the same as in the normal lens as a percent of total lens protein, the HMW material of the Nakano lens constituted about 5% of the soluble protein, a much higher value than in the normal mouse lens. One striking difference between the normal and Nakano was the rapid insolubilization of the proteins in the cataractous lens. Ninety days after birth, nearly two thirds of the total Nakano lens protein was water insoluble. The increase in the insoluble protein in the Nakano cataract was more dramatic than the gain in the HMW fraction.

Significance to Biomedical Research and the Program of the Institute: The study of changes within the lens during cataract development is essential for understanding the progression of the opacification. The changes in proteins such as the heavy molecular weight proteins are not well understood. Similarly, changes in the membrane proteins and gap junctions during human cataract formation are not known.

In order to determine the alterations that occur in human congenital cataracts, the use of epithelial cells in long term defined environments is desirable. Tissue culture affords the opportunity to study interaction of different components on the growth of epithelial cells. The identification

and characterization of various defective proteins or other components in human cataractous lenses may be possible by studying the epithelial cells from these lenses maintained in tissue culture.

Proposed Course: Further development of immunochemical techniques such as RIA will be undertaken. These techniques will be used to assess the changes in crystallin levels in various classified human cataracts as well as to detect the possible presence of crystallins in the aqueous humor of individuals with cataracts. The isolation of membrane proteins will be undertaken to determine changes in the amount or composition of the intrinsic membrane proteins during cataract development. Cultures of human lens epithelium as well as animal lens epithelium will be attempted to establish conditions for long term culture of these cells.

Publications:

Russell P, Carper DA, Kinoshita JH: The development and application of a radioimmunoassay to lens crystallins. Exp Eye Res 27:673-680, 1978.

Horwitz J, Robertson NP, Wong MM, Zigler JS, Kinoshita JH: Some properties of lens plasma membrane polypeptides isolated from normal human lenses. Exp Eye Res 28:249-265, 1979.

Russell P, Smith SG, Carper DA, Kinoshita JH: Age and cataract-related changes in the heavy molecular weight proteins and gamma crystallin composition of the mouse lens. Exp Eye Res (in press).

Tanaka T, Russell P, Smith SG, Uga S, Kuwabara T, Kinoshita JH: Membrane alterations during cataract development in the Nakano mouse lens. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00007-05 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Biochemical Pharmacology of the Eye

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hitoshi Shichi	Ph.D.	Research Chemist	LVR	NEI
Other:	Noveen D. Das	Ph.D.	Postdoctoral Fellow	LVR	NEI
	Daniel W. Nebert	M.D.	Chief	DPB	NICHD

COOPERATING UNITS (if any)

Developmental Pharmacology Branch, NICHD

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Polycyclic hydrocarbon-responsive mice were injected every five days with β-naphthoflavone to induce aryl hydrocarbon hydroxylase (Ah) activity and fed acetaminophe (or naphthalene-) impregnated food bars. Lenticular opacities as well inflammation of the ciliary body and iris, developed within a few weeks. Body weight was not affected. Ocular abnormalities were not observed in nonresponsive mice. The genetically well-characterized mouse strains should be useful for studies of ocular toxicity of drugs and environmental chemicals. Gamma-glutamyl transpeptidase (an enzyme involved in mercapturic acid formation) was purified from bovine ciliary body, kidney, liver, spleen, and brain. Enzymes showed different electrophoretic mobilities. After neuraminidase treatment, however, the enzymes migrated at essentially identical rates. Thus, tissue differences in the glycoprotein enzyme are attributed to the varying extent of sialylation.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: We have previously shown that polycydic hydrocarbon-inducible drug metabolizing activities are found in the pigment epithelium and ciliary body. The tissue distribution of drug metabolizing activities is reasonable because as much as 80% of the total blood enters the uveal circulation and because, in these pigmented tissues that receive blood nutrients directly, the drug metabolizing enzyme system will serve as a defense mechanism against harmful drugs and chemicals brought to the eye with nutrients. It is then expected that functional failures (e.g. administration of doses of drugs beyond the metabolizing capacities of tissues) would cause hazardous effects on susceptible tissues such as the lens and retina. One such instance we investigated was the effect of the analgesic-antipyretic compound acetaminophen on the eye of responsive mouse strains. We have observed that following an injection of large doses of acetaminophen into the drug-responsive mice in which aryl hydrocarbon hydroxylase activity had been enhanced by treatment with an inducer (3-methylcholanthrene), lenticular opacity developed in a few hours. In this report I describe experiments in which the effect of feeding low levels of acetaminophen to mice was investigated.

I also describe properties of γ-glutamyl transpeptidase, an enzyme involved in drug detoxification (mercapturic acid formation). Ciliary body is known to be one of the tissues that shows the highest levels of the enzyme.

Methods Employed: Mice (C57bL/6 and DBA/2) were injected intraperitoneally with # β-naphthoflavone to induce aryl hydrocarbon hydroxylase activity. The injection was repeated every other day to maintain induced levels of the drug metabolizing activity. Mice were fed ad libitum food bars previously immersed in corn oil containing acetaminophen or naphthalene. Abnormalities in ocular tissues (e.g. lenticular opacification) were examined in vivo as well as in vitro. Gamma glutamyl transpeptidase of ciliary body and other tissues were solubilized in detergent, purified by column chromatography, and assayed spectroscopically with γ-glutamyl ρ-nitroanilide.

Major Findings: In an experiment in which low concentrations of acetaminophen (or naphthalene) were fed to the mice whose drug metabolizing activities were maintained at high (induced) levels at all times, ocular abnormalities were observed only in responsive (C57 BL/6) mice. Lens opacification, degenerations in the choroid, ciliary body and iris, the presence of inflammatory cells, and anterior synechia were observed. It seems that acetaminophen and its chemically active metabolites caused an inflammatory response in the whole anterior portion of the eye. The location of tissue damage supports the contention that cytotoxic metabolites of the drugs produced either in the ciliary body or in the liver (or both) would be secreted with the aqueous humor and flow into the anterior chamber where they cause damage to surrounding tissues including the lens. This illustrates the importance of the drug metabolizing system of the ciliary body as a defense mechanism and supports the usefulness of the mouse model for studies on ocular drug metabolism.

Glutathione forms a conjugate with metabolically potentiated hydrocarbons. The conjugate is converted by the transpeptidase and other enzymes in a stepwise fashion to the final product mercapturic acid. The enzymes are present in the kidney. We have so far found three (γ-glutamyl transpeptidase, peptidase, and N-acetyl transferase) of the four enzymes involved in mercapturate formation in the ciliary body. One property of the ciliary enzyme deserves a note. Compared to the enzymes from other tissues (e.g. kidney, liver, intestine, brain), gamma-glutamyl transpeptidase from the ciliary body is unique in its high degree of sialylation (brain enzyme has the lowest sialic acid content). Studies of cultured tumor cells and regenerating liver suggest that the extent of sialylation may be correlated to increased cellular proliferating activity. We therefore suspect that the highly sialylated ciliary enzyme may reflect the high proliferating activity (regenerating activity) of the ciliary epithelial cells.

Significance to Biomedical Research and the Program of the Institute:

The present study on acetaminophen and naphthalene metabolism in hydrocarbon-responsive and nonresponsive mice illustrates the importance of the drug metabolizing activity of ocular tissues as a nonhepatic detoxifying mechanism. Little is known about the fate of drugs and enviromental chemicals entering the eye in blood circulation or by topical administration. The mouse model used in this study proves to be useful for biochemical studies of drug metabolism in the eye.

Proposed Course: Gamma-glutamyl transpeptidase and other enzymes involved in mercapturic acid formation in the ciliary body, and a possible genetic regulation of gamma-glutamyl transpeptidase in mice will be investigated.

NEI Research Program: Retinal and Choroidal Diseases--Special Areas of Future Interest (Toxic and Enviromental Disorders)

Publications:

Shichi H, Nebert DW: Drug metabolism in ocular tissues: Extrahepatic Metabolism of Drugs and Other Foreign Compounds, Gram TE, (ed): Spectrum Publications, Inc. Jamaica, NY (in press).

Das ND, Shichi H: Gamma-glutamyl transpeptidase of bovine ciliary body: Purification and properties. Exp Eye Res (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00004-05 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Biochemistry of the Visual Process

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hitoshi Shichi	Ph.D.	Research Chemist	LVR	NEI
	Charles N. Rafferty	Ph.D.	Research Chemist	LVR	NEI
Other:	Robert L. Somers	B.S.	Chemist	LVR	NEI
	Consuelo G. Muellenberg	B.S.	Chemist	LVR	NEI

COOPERATING UNITS (if any)
Department of Biochemistry, Kobe University School of Medicine, Kobe, Japan

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The phosphorylation of rhodopsin seems to be associated with newly formed disks and the plasma membrane of the rod outer segment. Newly formed disks are more susceptible to proteolytic enzymes than old disks.

The chemical structure of the carbohydrate moiety of rhodopsin has been elucidated as N-acetylglucosaminyl β 1-2 mannosyl α 1-3 (mannosyl α 1-6) mannosyl β1-4N-acetylglucosaminyl β1-4N-acetylglucosamine.

GTP binding to rod membranes is stimulated by light. A GTP-binding protein has been solubilized which is distinct from rhodopsin, GTPase and c-nucleotide phosphodiesterase.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objectives of this project are to investigate the light-dark adaptation processes of the retina by means of modern techniques of biochemistry and membrane biology. More specifically, these are (1) identification of a sequence of molecular events initiated by absorption of photons and leading to visual transduction (light process) and (2) elucidation of the biochemical mechanism for regenerating the photosensitivity of photoreceptor membranes (dark process). The investigations presented in this report deal with two aspects of the visual pigment rhodopsin, i.e. (a) elucidation of chemical structure of the oligosaccharide moiety of rhodopsin and the use of structural information for studies of the orientation of rhodopsin in the rod membrane, (b) location of the rhodopsin phosphorylation reaction in the rod outer segment, and (c) isolation and characterization of a GTP binding protein.

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

Major Findings: I. The sugar moiety of rhodopsin.

Purified rhodopsin was subjected to hydrazinolysis and the sugar chains released were reduced with NaB^3H_4 after N-acetylation. The radioactive oligosaccharides thus obtained were fractionated into three components (A, B and C) by paper chromatography. The structures of these components were elucidated as: GlcNAcβ>2 Manα1>3(Manα1>6)Manβ1>4GlcNAcβ1>4GlcNAc, GlcNAcβ1>2Manα1>3 (Man α1-3 or 6Manα1>6)Manβ1>4GlcNAcβ1>4GlcNAc and GlcNAcβ1>2Manα1>3(Manα1>3(Manα1>6) Manβ1>4GlcNAcβ1>4GlcNAc, by sequential exoglycosidase digestion, methylation analysis and endo-β-N-acetyglucosaminidase D digestion. The oligosaccharides A, B, and C were obtained in the yields of 70%, 15% and 15%, respectively. With reference to the proposed processing pathway for the biosynthesis of asparagine-linked sugar chains of glycoproteins, it is concluded that the sugar moiety of rhodopsin has the structure represented by oligosaccharide A. The chemical stucture of the sugar moiety indicates that rhodopsin is capable of binding the plant lectin concanavalin A (Con A). Con A binding studies with rods and disks led us to conclude that the Con A binding sites (i.e. sugar moiety) of rhodopsin are exposed on the external surface of the rod outer segment and on the internal surface of disks. This finding is consistent with the idea that the disks are formed by infolding of the plasma membrane. The conclusion was further supported by the demonstration that incorporation of (^{3}H)-galactose into the sugar moiety of rhodopsin occurs when inverted disks (but not intact disks) were incubated with UDP(^{3}H) galactose and galactosyl transferase. We have previously shown that rhodopsin is phosphorylated when intact disks (but not inverted disks) are incubated with rhodopsin kinase and ATP. These results, taken together, indicate that rhodopsin traverses disk membrane and exposes the carbohydrate moiety on the internal surface and the phosphorylation sites on the external surface.

II Cellular location of the rhodopsin phosphorylation reaction in the rod outer segment.

We have previously investigated phosphorylation of rod outer segments prepared from living frogs in which newly formed disks had been labeled with [^{3}H]-leucine and shown that newly formed disks (hence, the plasma membrane as well that is continuous with the disk infolding) are preferentially phosphorylated. As a continuation of this work, we isolated [^{3}H]-labeled outer segments at different times after injection of [^{3}H]-leucine and phosphorylated. In this way the capability to phosphorylate rhodopsin was scanned along the long axis of rod. We found that the activity of rhodopsin phosphorylation was highest in the basal region (where newly formed disks are located) and decreased gradually to the zero level at the distal region. Birefringence of a frog rod outer segment shows a similar gradient (Kaplan). Disks at the base and at the tip of rod are anatomically different (Andrews and Cohen). Functional differences also exist along the rod longitudinal axis: photosensitivity at the base is higher than at the apical region (Baylor). Although it remains to be seen whether rhodopsin phosphorylation, birefringence, and photosensitivity are related phenomena, there is little doubt that disks in the rod are heterogeneous.

III. Isolation and characterization of a GTP binding protein.

Marked decreases in cyclic GMP in light-illuminated rods are attributed to activation of cyclic nucleotide phosphodiesterase which occurs in the presence of GTP. GTP is an important requirement for hormone stimulation of adenyl cyclase in various tissues. A GTP binding protein has recently been isolated from hormone-responsive erythrocyte membranes. Since there are similarities between the light-stimulation of phosphodiesterase in the rod and hormone-activation of adenyl cyclase in nonphotoreceptor cells, we investigated the possible presence of a GTP binding protein in rod membranes. Bovine as well as frog rod outer segments contain a membrane-bound protein which binds the GTP analog GppNp in the light (the dissociation constant, Kd=0.3M). The amount of GppNp bound is 2.5-3.5 mmole per mol rhodopsin. The binding protein (M.Wt=54,000) can be extracted from rod membranes with detergent and purified on a Agarose column. The chromatographic profile indicates that the binding protein is distinct from rhodopsin, GTPase,or cyclic nucleotide phosphodiesterase. GTP is known to be required in hormone-stimulated adenyl cyclase activation in various systems, in tublin assembly, and in peptide elongation. Light activation of nucleotide-metabolizing enzymes in vertebrate rods provides another example in which a GTP requirement and the presence of a GTP binding protein can be demonstrated. It remains to be seen how GTP, together with GTP binding proteins, exerts its effect on the diverse reactions.

Significance to Biomedical Research and the Program of the Institute:

The sugar moiety of glycoproteins plays an important role in cell recognition, cell-cell interaction and endo-and exo-cytosis. The possibility that the sugar moiety of rhodopsin may serve as a cellular marker for the initiation of phagocytosis of rod membranes by the pigment epithelium has been suggested. The elucidation of the structure of the oligosaccharid moiety of rhodopsin makes it possible to test the idea on a biochemical level. It also helps to

investigate whether the sugar moiety of rhodopsin from dystrophic animals is different from that of normal animals. It is important to relate biochemical functions to structural and physiological functions of the rod outer segment. The present finding on the localized distribution of rhodopsin phosphorylation activity presents the first such example of correlatable biochemical reaction.

Proposed Course: Other aspects of light-triggered biochemicals in the retinal rod will be investigated. This project will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Shichi H, Somers RL: Light-dependent phosphorylation of rhodopsin: Purification and properties of rhodopsin kinase. J Biol Chem 253: 7040-7046, 1978.

Adams AJ, Tanaka M, Shichi H: Concanavalin A binding to rod outer segment membranes: Usefulness for preparation of intact disks. Exp Eye Res 27:595-605, 1978.

Adams AJ, Somers RL, Shichi H: Spatial arrangement of rhodopsin in the disk membrane as studies by enzymatic labeling. Photochem Photobiol 29:687-692, 1979.

Liang CJ, Yamashita K, Muellenberg CG, Shichi H, Kobata A: Structure of the carbohydrate moieties of bovine rhodopsin. J Biol Chem (in press).
Shichi H: Molecular Biology of the Visual Process, Siegel GJ, Albers RW, Katzman R, Aganoff BW (ed): Basic Neurochemistry, ed 3. Boston, Little, Brown and Co. (in press).

Shichi H: Visual pigments and the molecular mechanism of photoreception, in: Vitamins Tokyo Kagaku Dojin Publ. Co. (in press).

Somers RL, Shichi, H: Light-stimulated GTP binding to a membrane protein in rod outer segments. Biochim Biophys Res Comm (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00138-07 LVR

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Visual Cell: Process of Photoexcitation and Restoration

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Yoshikami	Ph.D.	Research Biologist	LVR NEI
Other:	C. Albani	M.D.	Guest Scientist	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Biochemistry

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3		

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The intraocular calcium activity in the fluid between the retina and pigment epithelium was determined from an analysis of the fast photovoltage responses from the retinal rod cells. It is between 10^{-4} and 10^{-3} M, and in darkness this activity causes the rod current of the in vivo retina to be turned on maximally.

Light bleaching of rhodopsin appears to result in the formation of two functionally different forms of opsin involved in the control of the light response of the rod cell. One form of opsin has a finite lifetime and is converted into another more stable form that is active in a different way.

Calcium and other substances which affect cyclic nucleotide metabolism have been shown to affect the kinetics and sensitivity of retinal rod's response to light. They also have been shown to have pronounced effects on the rate of recovery of the rod current following exposure to bright lights.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the nature of the visual cell and determine its physical and chemical means of initiating and sustaining the phenomenon of vision.

Methods Employed: The delicacy, small size, and other properties of the visual cells force novel approaches to their study. A concerted application of a combination of chemical and physical methods involving electrical, biochemical, optical, and anatomial measurements are used to study the retina and its associated ocular tissues.

Major Findings: The lifetime of the fast photovoltage (FPV) from retinal rod cells measured extracellularly is in part dependent on cell membrane conductance. The rod cell membrane conductance in turn can be regulated by varying the extracellular ACa^{++}. Thus, in isolated retinas, the lifetime of the R_2 component of the FPV is to a first approximation reflective of the ACa^{++} of the medium bathing the rod cells (Yoshikami and Hagins, 1973). The lifetimes of the FPV from the eyes of anesthetized albino rats were measured in the dark and light adapted conditions. Comparison of the results obtained in vivo with those in vitro under varying ACa^{++} suggests the ACa^{++} in the fluid surrounding the rods in the in vivo, dark adapted eye is between 10^{-4} and 10^{-5}M. At these ACa^{++} in the in vitro retina, the rod current is fully on. Thus, a Ringer solution with 10^{-5}M Ca^{++} would approximate more closely the in vivo condition than would the Ringer's containing 1-2 X 10^{-3}M Ca^{++} widely used today in experiments involving the retina.

We have developed conditions under which the in vitro retina more closely resembles the in vivo retina by adding liposomes bearing retinol congeners to the perfusing Ringer's solution. With this the light-bleached rhodopsin can be regenerated at will and we are able to determine the role the regeneration of rhodopsin plays in the light control of the rod current.

When 8% of the retinal rhodopsin is bleached in an isolated retina, the rod current is fully suppressed for 10 minutes. It is then gradually restored. Later when the rod current is fully restored, even though there is only 8% less light absorbing rhodopsin, the retinal sensitivity or its signal gain (as assayed by the ability of the retina to half suppress the rod current with a given number of photons) is decreased ten fold. The bleached opsins appear to continue to suppress the signal gain until they are converted to rhodopsin by the addition of 11-cis retinaldehyde. The more rhodopsin bleached, the longer the rod current suppression and the greater the decrease in retinal sensitivity to light. If one allows rhodopsin to regenerate in the in vitro retina following a bleaching exposure, the rod current recovers faster than it otherwise would and its sensitivity to light recovers nearly to the original dark-adapted level. These experiments suggest the existence of two active forms of opsin following photo-bleaching of rhodospin; one (opsin-I) has a finite lifetime and signals the release of the chemical transmitter that suppresses the rod current, and another (opsin-II) a product

of the former is more stable and signals the transmitter release mechanism to reduce its signal gain. Both forms are rapidly converted to rhodopsin if 11-cis retinaldehyde is present.

In addition to the effect regeneration of rhodopsin has on the control of the rod current following the photoexcitation of rhodopsin, we find there are substances that affect the rate of rod current recovery following light exposure. Low calcium and several cyclic nucleotide phosophodiesterase inhibitors accelerate the recovery of the rod current following light exposure, as if they are affecting the lifetime of opsin-I, but they do not alter the ability of opsin II to continue to suppress the retinal light sensitivity.

Significance to Biomedical Research and the Program of the Institute: Our understanding of the causes of, and our ability to prevent and treat, numerous visual disorders depends on a clear knowledge of the processes operant in normal vision. Our finding of the importance of calcium and cyclic nucleotide phosphodiesterase in the control of the visual cell excitation process, and the revelation of the tight coupling between photoexcitation and energy metabolism of this cell, may help us to realize some of the basis for pathology of the retina. The concatenated reactions of retinol in two adjacent tissues, the pigment epithelium and retina, show these tissues are interdependent. Moreover, an understanding of how the retina and the pigment epithelium regulate ionic activities, in particular calcium in the aqueous space between them, has a bearing on the vitality of each tissue. This has special significance to visual pathology when the retina becomes detached from the pigment epithelium.

Proposed Course: How the retina initiates and sustains vision is the focal point of our studies. We will continue to study the physical and chemical processes involved and pay particular attention to the supportive tissue like the pigment epithelium.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00012-01 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Growth of the Retinal Pigment Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Alfred J. Coulombre	Ph.D.	Head, Section on Experimental Embryology	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Embryology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Changes in size and shape of the cells of the retinal pigment epithelium (RPE) of the chick embryo are followed as a function of the expansion in area of the RPE which occurs during the development and growth of the eye. Strips of eye wall are cut from embryos of different ages along known ocular meridians, mounted on microscope slides, transilluminated, and photographed through a compound microscope. Such photographs, calibrated for magnification, not only reveal the three-rayed net formed by appositions of RPE cells, but also permit quantitative study of cell size, shape (sidedness) and nuclearity as functions of position within the eye and of developmental time. Thus far, it has been ascertained that: 1) RPE cells increase in surface area and in volume as a function of age; 2) this expansion is more marked in the fundus of the eye than toward the ora serrata; 3) RPE cells undergo this expansion in response to tensions generated by the expanding vitreous body; 4) a small percentage of cells is binucleate, larger in area than uninucleate neighbors, tends to have more sides than uninucleate cells, and occurs more commonly at the fundus.

Project Description:

Objectives: The RPE and the neural retina remain precisely matched in area throughout their several-hundredfold expansion in area during growth of the eye. This matching of areas is maintained despite the virtual cessation of mitosis in the RPE at quite early stages in development and its persistence in the neural retina until late development. The expansion of the vitreous body has been shown to be responsible for maintaining the match in area. The RPE expands in area (in response to accretion of vitreous substance) principally by cellular hypertrophy and to a far lesser extent by cell division. As RPE cells enlarge, the largest of them are seen to be binucleate. This study seeks to: follow the changes in RPE-cell area as a function of age and of location within the eye; relate cell sidedness to cell size, location within the eye, and the sidedness of nearest neighbors; determine the frequency of binucleate cells as a function of age and of location within the eye, their size, and sidedness and, if possible, their relationship to foci of mitosis; and analyze the expansion of the RPE topologically as a three-rayed net of cell zonulae which is perturbed by occasional cell division.

Methods Employed: Staged embryos of white Leghorn chickens, reared at 37.5°C in a forced-draft incubator, are used in this investigation. Their eyes are removed at different stages of embryonic development and a strip of eye wall is cut along the horizontal ocular meridian (or occasionally, along other meridians) from limbus to limbus. Such strips are laid flat on glass slides, cleared of adhering neural retina, and systematically photographed with transillumination through a compound microscope. The photographic frames, calibrated for magnification, are analyzed in two ways. Some are used to determine RPE-cell density by making cell counts per unit area (the reciprocal of mean RPE-cell area). Others are projected and cell outlines are traced at known magnification in order to determine: the frequency distribution of RPE-cell areas; the frequency distribution of RPE-cell sidedness; the frequency, geometry, and distribution of bincleate RPE-cells; and the spatial relationships among cells of different sidedness. It is planned to use topological methods and computer modelling to simulate perturbations in the three-rayed net formed by the zonulae when a mitotic event does occur in the RPE.

Major Findings: The RPE cells form a low cuboidal, simple epithelium in which the individual cells are joined along their sides to adjoining sides of neighboring cells by zonulae. The zonulae form a three-rayed net on a spheroidal surface (the contour of the eye wall). Thus far, this study has demonstrated several properties of this array. 1) The majority of cells within this net are hexagonal in outline. Smaller numbers of cells are five- or seven-sided. No cells were found with less than five sides and few

with more than seven sides. There is an obligatory pairing (dictated by topological laws) between five- and seven-sided cells. 2) At any given age, cell size is largest in the fundus and smallest toward the ora serrata. 3) In any region of the RPE the mean cell area increases with age. 4) This increase in area is dependent upon tension generated by the expanding vitreous body but is not due to simple stretching of passive RPE cells but to an active hypertrophy (increase in cell volume) which fails to occur in microphthalmic eyes. 5) Some cells (a small percentage of the RPE population) are binucleate, larger in area than their uninucleate neighbors, tend to have more sides than do uninucleate cells, and occur more commonly at the fundus than toward the ora serrata.

Significance to Biomedical Research and the Program of the Institute: The maintenance during embryonic development of contact (at all points and at all times) between the neural retina and the pigmented epithelium is very important for normal development of the retina for several reasons. In a previous study, of this Section it was shown that failure to maintain this contact resulted in metaplasia of the RPE and formation of ectopic neural retina. This abnormality frequently accompanies those types of microphthalmia (e.g. coloboma of the embryonic fissure with aasociated retinal cyst) in which the growth of the vitreous body within the eye is subnormal. Furthermore, the maintenance of congruence of areas between the RPE and the neural retina is important for nutritional reasons (folds of embryonic neural retina which lose contact with the RPE degenerate). Finally, apposition of these two retinal layers is required at all points during the interval (following the fifteenth day of incubation in the embryo of the chicken) when the RPE- cell processes are enveloping the emerging photoreceptor outer segments. Outer segments do not grow in the absence of such contact. The current study carries several steps further our analysis of the mechanisms by which the RPE normally remains precisely tailored in its area to the area of the neural retina, and the ways in which this intimate apposition may be lost teratogenetically.

Proposed Course: Acquisition of data on size, shape, and sidedness distribution of RPE cells, as functions of location within the RPE and of embryonic age, will be extended. The frequency of occurrence of binucleate cells will be determined as a function of location in the RPE and of age. The frequency of occurrence of binucleate RPE cells will be determined at the fundus in normal embryonic eyes and in embryonic eyes rendered microphthalmic by experimental intervention, to determine whether or not the binucleate condition is a consequence of increase in cell size or, alternatively, develops independently of the increase in cell size. The topological consequences of cell division in the RPE will be assessed by modelling the perturbations in the three-rayed net formed by RPE zonules which result from division of cells of different size or sidedness.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Johnston MC, Noden DM, Hazelton RD, Coulombre JL, Coulombre AJ: Origins of avain ocular and periocular tissues. Exp Eye Res (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00177-04 LVR

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Plasma Membrane Composition and Biosynthesis in Chick Lens Fibers and Epithelia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peggy Zelenka	Ph.D.	Geneticist	LVR NEI
Other:	Gloria Devore	Ph.D.	Staff Fellow	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Embryology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project seeks to determine whether the regulation of lens fiber differentiation and maturation is associated with alterations in the plasma membrane. To this end, the principal lipid and protein components of embryonic and adult chicken lens membranes are being identified, and their metabolism is being investigated. Because of the known involvement of phosphatidylinositol turnover in regulatory mechanisms of various other cell types, the initial stages of this study have focused on lens phospholipid metabolism. Computer modeling of the kinetics of ^{32}P incorporation into lens phospholipds in vivo is employed to determine the rates of synthesis and degradation of individual phospholipids. This approach is also being applied to the study of phospholipid metabolism in differentiating explants of embryonic chick lens epithelia in organ culture, thus allowing the possible relationships between phospholipid metabolism and differentiation to be studied under controlled conditions. Studies of lens membrane proteins are being conducted by applying standard techniques of protein chemistry to purified lens membranes.

Project Description:

Objectives: The objectives of this project are: a) to characterize the principal lipid and protein components of plasma membranes from embryonic chick lens fibers and lens epithelial cells; b) to determine whether the differentiation of lens epithelial cells into lens fibers is accompanied by changes in membrane composition; c) to learn whether the differentiation of lens epithelial cells into lens fibers is accompanied by changes in the metabolism of lens plasma membranes; and d) to establish the functional significance of any changes in membrane composition or metabolism.

Methods Employed: ^{32}P-labeled phospholipids are obtained by injecting isotope into six-day-old chick embryos via the choriallantoic circulation. Lens fibers and epithelia are isolated by microdissection of the embryos. Phospholipids are extracted and separated by thin layer chromatography; radioactivity is determined either by scintillation counting or by autoradiography. Rates of synthesis and degradation of individual phospholipids are determined by mathematical modeling, using a single-compartmental model. This model assumes that for any given phospholipid the rate of synthesis is constant and degradation follows exponential kinetics. Precursors of phosphatidylinositol biosynthesis are isolated by thin-layer chromatography after in vitro labeling with ^{32}P, and the kinetics of labeling are compared, using computer curve-fitting, with the kinetics of labeling of the γ-PO_4 of ATP. ATP concentration is determined enzymatically. Digestion of ^{32}P-labeled ATP by ATPase and isolations of the digestion products by thin layer chromatography makes possible calculation of the specific activity of the γ-PO_4 of ATP.

Explants of lens epithelum of six-day-old embryonic chicks are grown under two different culture conditions: 1) Ham's F-10 supplemented with 15% fetal calf serum, or 2) unsupplemented Ham's F-10 for 48 hours followed by Ham's F-10 supplemented with 15% fetal calf serum. Under the first condition the lens epithelial cells differentiate to form lens fibers, while under the second condition they do not differentiate but undergo active mitosis. The turnover of phosphatidylinositol is studied under both conditions by adding ^{32}P to the culture medium for 24 hours to label phosphatidylinositol, and then following the loss of radioactive phosphatidylinositol during a 24 hour chase period. The cell number and the specific activity of the γ-PO_4 of ATP are monitored during the chase period.

For studies of lens membrane proteins, lenses are obtained from chickens at various stages of development and maturation, and the lens membranes are purified by a combination of sucrose gradient centrifugation and centrifugation in citrate-buffered urea. The membrane proteins are separated by SDS-polyacrylamide gel electrophoresis. Individual protein bands are iodinated

with ^{125}I and digested with trypsin; the tryptic peptides are separated by electrophoresis on thin layer plates and located by autoradiography. Cytoplasmic contamination is estimated by preparing the lens membranes in the presence of ^{35}S-labeled -crystallin and high concentrations of cytoplasmic, lens proteins.

Major Findings: Control experiments justify the application of a single compartmental model to the in vivo labeling of phosphatidylinositol in the embryonic chick lens. The kinetics of incorporation of ^{32}P into this phospholipid in seven-day-old embryonic chick lenses is approximately the same as in the six-day-old embryo, indicating that the rate of synthesis of this compound is virtually constant during the sixth day of embryonic life, as required for application of the model. Furthermore, phosphatidylinositol turnover in vitro can be described by exponential kinetics. The precursor of phosphatidylinositol, CDP-diacylglycerol, equilibrates rapidly with the $-PO_4$ of ATP. This allows the specific activity of the $-PO_4$ of ATP, which has been measured in vivo, to be used as an approximation of the specific activity of CDP-diacylglycerol. This information permits calculation of the rates of synthesis and degradation of phosphatidylinositol which give the best agreement between the mathematical model and the observed rate of ^{32}P incorporation into phosphatidylinositol. The results of this analysis indicate that in the lens epithelium of six-day-old embryonic chicks, phosphatidylinositol is snythesized at a rate of about 2×10^{-9} pmole/sec/cell and degraded with a half-life of about four hours. Such rapid turnover of this phospholipid is often correlated with the action of agents which bind to cell surface receptors. In the six-day-old embryonic chick lens fibers, phosphatidylinositol is synthesized at a rate of about 6×10^{-9} pmole/sec/cell and is degraded with a half-life of two to three days. Thus, the differentiation of embryonic chick lens fibers is associated with increased synthesis and decreased degradation of phosphatidylinositol. Both these changes favor accumulation of this phosphalipid in the elongating lens fibers.

Similar changes in phosphatidylinositol turnover accompany in vitro differentiation of explants of embryonic chick lens epithelia. When the explants are cultured under conditions which favor mitosis rather than differentiation, phosphatidylinositol decays exponentially with a half-life of about 12 hours. In contrast, when the explants are cultured under conditions which favor fiber cell differentiation, there is little or no decay in 24 hours. These differences are not due to differences in cell number or specific activity of the precursor pool. Thus, lens fiber differentiation in vitro, as in vivo, is accompanied by decreased turnover of phosphatidylinositol.

Lens membranes prepared by multiple centrifugations through citrate-buffered urea contain only traces of δ-crystallin; furthermore, the remaining δ-crystallin is at least partly due to contamination by cytoplasmic material. The proteins of chicken lens membranes are almost identical in subunit molecular weight and percentage composition to those of calf lens membranes. The principal protein component of chicken lens membranes is a 25,000 molecular weight component which has been proposed by others to be a gap junction constituent. This protein represents only about 10% of the total lens membrane proteins in the 15-day-old chick embryo, but increases during development to nearly 50% in the adult lens membranes. Only one lens membrane protein, a 70,000 molecular weight component, appears to be lost during development.

Significance to Biomedical Research and the Program of the Institute: The plasma membranes of lens cells appear to play important roles in normal development and function of the lens. In addition, they are centrally involved in the genesis and development of several varieties of lens cataract. Despite the widely recognized and important functions of these membranes, work on their composition, turnover, and development has begun only recently. This project focuses on changes in lens cell membranes which are associated with lens fiber differentiation. These results should have broad application in understanding normal lens differentiation and morphogenesis and in attempts to establish etiologies for several types of cataract.

Proposed Course: This project will be continued. The in vivo metabolism of other lens phospholipids will be analyzed by determining the specific activity of the relevant precursor pools. The possibility that phosphatidylinositol turnover is involved in the regulation of lens fiber differentiation will be studied by testing the effects of various agents which promote or prevent in vivo differentiation of cultured explants of embryonic chick lens epithelia. An attempt will be made to determine the fate of degraded phosphatidylinositol in the cultured lens epithelia. In particular, experiments will be done to test for the possible conversion of phosphatidylinositol to polyphosphoinositides, phosphatidylglycerol, and prostaglandins. In addition, phosphatidylinositol metabolism will be studied in cultured embryonic chick lenses under conditions which lead to formation of lens opacities.

NEI Research Program: Cataract--The Normal Lens

Publications:

Zelenka P, Reszelbach R, Piatigorsky J: Developmental changes in proteins of purified membranes of chicken lenses and evidence for contamination by cytoplasmic δ-crystallin. Biochim Biophys Acta (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00032-03 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Moderate Intensity Light on Vitamin A Deficient Retinas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Louvenia Carter-Dawson	Ph.D.	Staff Fellow	LVR	NEI
Other:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR	NEI
	John G. Bieri	Ph.D.	Chief, Section on Nutritional Biochemistry	LNE	NIAMDD

COOPERATING UNITS (if any)

Laboratory of Nutrition and Endocrinology, NIAMDD

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Pathology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of moderate-intensity light (150-200 foot-candles) on the retina of vitamin A deficient (retinol deficient) and vitamin A adequate rats (retinol adequate) were examined. The structure of photoreceptor outer segments remained intact for a longer period of time in retinas of rats deficient in retinol (low levels of rhodopsin) after exposure to light. In addition, more photoreceptor cell nuclei remained after six days of exposure in the retinol deficient rats. These data are consistent with the hypothesis that rhodopsin is involved in initiating the process leading to the structural deterioration of retinal photoreceptors.

PHS-6040
(Rev. 10-76)

Projection Description:

Objectives: Light has been shown to produce deterioration of photoreceptor cells. Rhodopsin (vitamin A + opsin) is thought to be involved in initiating this process. This study was designed to examine the influence of rhodopsin on structural alterations induced by light.

Methods Employed: Albino rats were placed on a retinol deficient diet at weaning. When serum levels of retinol were undetectable, usually between six to seven weeks on the diet, and rhodopsin levels were less than 50% of control values, retinol deficient and retinol adequate rats were exposed to twelve hours of light at 150-200 foot-candles for one to seven days. The retinas were examined by light and electron microscopy.

Major Findings: Microscopic examination showed structural differences between the retinas of retinol adequate and retinol deficient rats after 24 to 30 hours of light exposure. In the retinol adequate retinas, discs in the distal third of the outer segments were highly disrupted. Many were distended and disrupted into vesicles with a maximum diameter of 0.4 μm. These changes often extended into the inner two-thirds of the outer segments. By contrast the deficient retinas contained groups of intact discs interspersed with smaller vesicles in the distal third of the outer segments. Only a small number of vesicles were seen in the inner two-thirds of the outer segments. Also, more photoreceptor nuclei persisted in the retinas of retinol deficient rats after six days of exposure. These data provide evidence that retinas of retinol deficient rats are less sensitive to structural alterations induced by light. This decrease in sensitivity to structural deterioration is probably related to the decrease in rhodopsin levels.

Significance to Biomedical Research and the Program of the Institute:

The underlying mechanisms whereby light produces retinal damage are not clearly understood. Results from these studies provide additional information suggesting that the process leading to light-induced retinal damage is initiated by rhodopsin.

Proposed Course: The central superior quadrant of the retina showed a larger percentage of photoreceptor cell death than other quadrants in retinol deficient and adequate rats after exposure to light. Studies are in progress to provide a quantitative description of cell loss with light exposure and intensity. It is of interest to know whether this region is comparable to the human fovea.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Carter-Dawson 1, Kuwabara T, Bieri JG: Effects of moderate-intensity light on vitamin A deficient retinas. Invest Ophthalmol Vis Sci (in press).

Carter-Dawson L, Kuwabara T, O'Brien PJ, Bieri JG: Structural and biochemical changes in vitamin A deficient rat retinas. Invest Ophthalmol Vis Sci 18:437-446, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00129-07 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Anatomical and Pathological Studies of Ocular Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR	NEI
Other:	Minoru Tanaka	M.D.	Visiting Scientist	LVR	NEI
	Yoshitaka Ohnishi	M.D.	Visiting Scientist	LVR	NEI
	Shigeru Uga	M.D.	Visiting Scientist	LVR	NEI
	Carole Latker	Ph.D.	Guest Worker	LVR	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Pathology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Histopathological studies were conducted on numerous human and animal eyes by transmission and scanning electron microscopy, histochemistry and histological sectioning. Studies conducted on the cornea, lens, and aqueous humor are all related to certain clinical problems.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Clarification of the normal structure and function of each cell of the eye is fundamental to understanding the pathophysiology of various eye diseases. Also, a systematic study of the eye with naturally occurring diseases may directly lead to the elucidation of the pathogenesis involved.

Methods Employed: A large number of clinicopathological specimens sent to this laboratory from various eye research centers throughout the world were studied. Details on individual experiments on animals are described under Major Findings.

These eye tissues were fixed in glutaraldehyde solution and processed for transmission and scanning electron microscopy. Depending on specific diseases, various types of histochemical reactions were applied on cryo-, frozen, paraffin and plastic sections.

Major Findings:

I. Cornea

New insights of the detailed structure of cells of the stroma and epithelium have been revealed. Electron microscopic study of the keratocytes has demonstrated that the cell has several cytological characteristics which are somewhat different from those of the fibrocyte. The keratocyte is capable of retracting cell processes and of migration. The keratocyte and the collagen lamellae are not attached to each other and there is a potential space between them. The space serves as the intracorneal channel for transportation of fluid and particles, and also wandering cells take this channel for their invasion. The corneal stroma contains a relatively large numer of wandering cells in the normal condition. Both exogeneous and endogenous foreign bodies in the stroma are phagocytized mainly by the wandering cells and not by the keratocyte.

Studies by the freeze-fracture technique showed that the general appearance of the split cell membrane of the corneal epithelium was similar to that of other membranes. Besides the characteristic structure of the fractured cell membrane at desmosomes, gap junctions and hemidesmosomes, the cell membrane demonstrated many "bump and hollow" type configurations measuring 70-80 nm in diameter. This structure is known to be commonly associated with micropinocytotic vesicles and cellular pores. Careful examination of transmission electron micrographs of animal corneas revealed that the number of micropinocytotic vesicles was far greater than previously noted. Pinocytosis was most abundant in the basal cell and decreased toward the superficial layers. Cluster distribution of pinocytotic vesicles was frequently demonstrated in the basal cell. In addition, abundant pinocytosis was present in the cell membrane surrounding nerve fibers which occasionally reached the superficial layer. It was found that numerous nerve fibers pierce through the cytoplasm of the basal cell, in addition to running through the normal intercellular spaces.

Endothelial wound healing studies revealed the events that occur in the healing of prick wounds of the posterior surface of the cornea. By a sliding process the endothelial cells move into the wound defect and rapidly re-cover the damaged posterior surface. The endothelial cells, which accumulate and fill the wound, transform into keratocyte-like cells. These cells gradually disappear, but the fibrous tissue which is produced by these cells remains in the wound area. No mitotic activity is detected in the endothelial cells during wound healing.

Since the origin of both the corneal stroma cells and endothelium is considered to be neurocrest rather than mesenchymal cells, the unique process of wound healing may be due to the specific embryonal nature of this tissue.

II. Lens and Cataracts

Lentoid bodies, lens-like aggregations of the cultured lens epithelial cells, of normal and Nakano cataractous strain mice were studied by transmission and scanning electron microscopy. Although the appearances of cells constituting lentoid bodies of both normal and cataractous mice were similar, electron microscopic histochemistry revealed that there is a marked difference in the Na-k ATPase activity. The lead particles which were produced by ATPase were abundantly present in the vicinity of cell membranes of lentoid body cells of the normal mouse, whereas they were absent in cells in Nakano strain mice. This histochemical evidence confirms the biochemical findings which revealed that the deficiency in the Nakano cataract appears to involve Na-K ATPase.

A large number of human lenses of various age groups was studied by light and electron microscopy. The earliest histologic change related to age was a decrease in number of the lens epithelial and bow cells. Electron microscopy revealed that epithelial cells of lenses of middle-aged persons began to show abnormalities. Many epithelial cells of this age group became irregular in shape and contained increased numbers of microorganelles including swollen mictochondria and abundant rough endoplasmic reticula. Moreover, cells at the bow area frequently showed retained microorganelles, even though the background cytoplasm appeared normal. These findings indicate that the differentiation of the epithelial cell is retarded considerably and this abnormality precedes the degeneration of lens fibers.

A new cataractous Philly mouse strain, which is derived from the Swiss-Webster mouse, was studied clinically and by light and electron microscopy. The hereditary cataract of this strain occurred as an anterior subcapsular opacity on about the 10th postnatal day and slowly progressed until the whole lens became opaque on approximately the 40th day.

Several histologic abnormalities were demonstrated in the early stage of cataract formation. Instead of elongating in a normal fashion, the epithelial cells became taller in situ; degenerating cells and intercellular spaces were occasionally present in the superficial bow cortex; and many of the cells retained their nuclei. The lens fibers in the opaque posterior subcapsular zone showed marked irregularity in their arrangement and swelling of their

posterior ends, resulting in wide intercellular spaces at the posterior suture. Numerous microorganelles persisted in the cytoplasm of the lens cells in the opaque anterior cortex region. Markedly electron-dense bodies, consisting of amorphously aggregrated fine granules, accumulated within the anterior portion of the lens cell. The dense bodies measured about 015-1.0μm and small ones were often present in the remaining mitochondria. Studies of the whole-mount flat preparations of the epithelium of the cataract-developing lenses revealed that the number of mitotic figures was markedly reduced. These findings suggest that one of the causative factors of this cataract of the Philly mouse results from retarded function in proliferation and differentiation of the lens cells.

Freeze-fracture study revealed that the cell membrane of the normal mouse lens has an extremely large number of gap junctions. Sizes of individual gap junctions of the Nakano cataractous lens cell were considerably smaller than normal and the intramembranous granules in these small junctions were sparse. The granules became absent in cells of seven- month-old Nakano mice. Biochemical analysis performed by Dr. Russell demonstrated that the amount of the 26,000 mw polypeptide, which is closely associated with the cell membrane, had decreased significantly in cataractous lenses. The structural changes in gap junctions and decrease of the membrane associated polypeptides in cataract forming lenses may suggest that certain abnormalities in the cell membrane are one of the factors causing cataract in the Nakano mouse.

III. Glaucoma

The anterior optic nerve and the macular region of the retina of glaucomatous eyes of five rhesus monkeys have been examined by light and electron microscopy. The experimental glaucoma had been induced by argon laser treatment of the anterior chamber angle. Severe degenerative changes were seen in eyes with higher intraocular pressure. Longer duration of glaucoma produced changes sharply localized to the axon bundles in the scleral lamina cribrosa. Accumulation of mitochondria and dense bodies occurred anterior and posterior to collagenous septae. The location of these changes is in agreement with the localization of the block of axoplasmic transport identified by autoradiographic studies. It is speculated that these cytologic changes reflect blockage of axoplasmic flow in the optic nerve of eyes with glaucoma.

It is generally known that following paracentesis, plasma proteins enter the anterior chamber, but the junctions between the pigmented and nonpigmented ciliary epithelium remain intact. However, during experiments in which the aqueous humor of Rhesus monkeys was exhaustively drawn three or four times within one hour, it was shown that marked cellular damage of the epithelium occurred specifically at the most anterior portion of the pars plicata. This portion is usually free from the zonular attachment, and the nonpigmented cells often contain melanin granules. This localized damaging effect prompted us to look into the regional difference of the junctions of the ciliary epithelium. The present study revealed that tight junctions of the nonpigmented epithelium in the general area of the pars plicata formed netlike configurations consisting of many randomly arranged junctional strands,

while the junctions in the anterior-most zone consisted of sparse strands which were arranged in a parallel direction. The junctions in the pars plana were of a mixed form. This observation suggests that the function of the ciliary epithelium may differ by locations, presumably due to strength of the movement of the ciliary muscle to the zonule, and that leakage of serum following paracentesis may occur at the anterior-most portion of the pars plicata, where the junctions appear to be weaker than in other parts.

Some types of glaucoma may be related to the abnormal development of the aqueous outflow channels. Thus, a study was initiated to learn more of the development of the trabecular meshwork in hamsters and monkeys. At an early stage of development of the cornea, cells which have a similar appearance to the developing endothelial cells proliferate in the rudimental angle zone at a late stage of the general development of the ocular tissue. At the period when the photoreceptor cells differentiate (the first postnatal week of the hamster, and the 120th fetal day of the monkey), the meshwork cells become distinguishable from the corneal endothelium. The meshwork cells develop numerous intracellular vacuoles which become large intercellular spaces. The cells of the cavernous meshwork tissue begin to produce random patches of the trabecular connective tissue consisting of basal lamina substance, filaments, collagen fibers and elastica. No direct connection between the trabecula and the scleral connective tissue is observed during the development. The angle is formed by cleavage of the meshwork tissue. However, the splitting does not occur in the generally believed mesenchymal tissue of the angle. The thin processes of the prominently spongy meshwork cells separate from each other. The vertex of the angle and the anterior surface of the iris are covered with a thin single layer of cells which have neither basal lamina nor cellular junctions. The Schlemm's canal is formed at the tissue space between the meshwork tissue and the sclera.

Significance to Biomedical Research and the Program of the Institute: The staff of this section is able to pursue a multidisciplinary study to attack problems which are directly related to clinical ophthalmology. Further clarification of the normal and abnormal structure and function of ocular tissues and cells is a significant part of eye research.

Proposed Course: Similar projects are actively ongoing and will be continued in the next fiscal year.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders/Inflammatory Disorders/Uveal Tract; Corneal Diseases--Corneal Edema, Dystrophies, and Inherited Disorders/Corneal Transplantation and Stromal Injury and Repair/Tumors and Other Lid, Conjunctival, and Orbital Problems; Cataract--The Normal Lens/Cataract Induced by Drugs and Radiation and Occurring Secondary to Other Eye Disorders; Glaucoma-- Etiology of Glaucoma (Primary Open-Angle Glaucoma/Secondary Glaucomas)

Publications:

Kuwabara T: Current concepts in anatomy and histology of the cornea. Contact & Intraocular Lens Med J 4:101-132, 1978.

Wiggert B, Mizukawa A, Kuwabara T, Chader GJ: Vitamin A receptors: Multiple species and possible compartmentalization in retinal photoreceptors. J Neurochem 30:653-659, 1978.

Avendano J, Tanishima T, and Kuwabara T: Ocular cryptococcis. Am J Ophthalmol 86:110-113, 1978.

Robison WG, Jr, Kuwabara T: A new, albino-beige mouse: Giant granules in retinal pigment epithelium. Invest Opthalmol Vis Sci 17:365-370, 1978.

Kuwabara T, Sakuragawa M, Iwata K: Lamellar inclusion bodies in non-neural ocular tissues induced by a hypochelesteremic drug AY9944. Metabolic Ophthalmol 2:85-95, 1978.

Gaasterland D, Tanishima T, and Kuwabara T: Axoplasmic flow during chronic experimental glaucoma. I. Light and electron microscopic studies of the monkey optic nervehead during development of glaucomatous cupping. Invest Ophthalmol Vis Sci 17:838-846, 1978.

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

Kuwabara T: Photic damage of the retina. Excerpta Medica (in press).
Carter-Dawson L, Kuwabara T, O'Brien P, Bieri JG: Structural and biochemical changes in vitamin A deficient rat retinas. Invest Ophthalmol Vis Sci 18:437-446, 1979.

Kuwabara T, Okisaka S: Photic changes in the pigment epithelium, in Zinn K, Marmor M (eds): The Retinal Pigment Epithelium, Cambridge, Harvard Press (in press).

Kuwabara T: Age-related changes of the eye, in Moncrieff N.J., Weeks L (eds): Proceedings of Symposium on Biology of Special Senses in Aging. Ann Arbor, Institute of Gerontology (in press).

Huang FL, Russell P, Kuwabara T: Fine structure of lentoid bodies derived from normal and cataractous mouse lenses. Exp Eye Res (in press).

Tanaka M, Russell P, Smith S, Uga S, Kuwabara T, Kinoshita JH: Membrane alterations during cataract development in the Nakano mouse lens. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00149-06 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Ultrastructure and Function of the Pigment Cells of the Eye

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Gerald Robison, Jr.	Ph.D.	Geneticist/Cell Biologist	LVR	NEI
Other:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR	NEI
	John G. Bieri	Ph.D.	Chief, Section on Nutritional Biochemistry	LNE	NIAMDD

COOPERATING UNITS (if any)

Laboratory of Nutrition and Endocrinology, NIAMDD

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Pathology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The interrelationships of vitamin E and vitamin A in the maintenance of retinal structure were studied in rats deficient in both vitamins. After 35 weeks, vitamin E deficiency resulted in a 16% loss of photoreceptor cells; vitamin A deficiency resulted in a 30% loss; whereas combined vitamin E and A deficiency resulted in a 90% loss. This accelerated retinal damage in doubly deficient (-E, -A) retinas probably resulted from accelerated oxidative loss of tissue vitamin A when the antioxidant effects of vitamin E were missing.

The pigment epithelium of -E, +A rats had massive accumulations of lipofuscin granules (aging pigment). However, in -E, -A rats the pigment epithelium had much less lipofuscin than expected, perhaps because vitamin A is involved in lipofuscin formation directly, or because it influences phagocytosis of photoreceptor membranes which become peroxidized to form lipofuscin.

In summary, the roles of vitamins E and A are closely linked in the retina. The lack of either leads to irreversible structural damage and the lack of both accelerates the damage and results in unusual aging pigment formation.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the structural and functional interrelationships that exist between the pigment epithelium and the visual cells of the eye. We are examining how the pigment epithelial cells are involved in the maintenance of photoreceptor cells and what specific functions are lacking in various experimental and pathological cases.

Methods Employed: Female Sprague-Dawley rats were fed a basal diet free of vitamins E and A. Within a week of weaning the rats were divided into four diet groups. The +E, +A group received a supplement of 250 mg α-tocopherol and 2 mg retinol per kilogram of diet. The -E, +A rats received only the retinol. The +E, -A rats received α-tocopherol plus retinoic acid, which maintains most tissues in a healthy state but results in a vitamin A-deficient retina. The -E, -A rats received only the retinoic acid. Retinas were examined in frozen section for the autofluorescence specific to lipofuscin and were examined by conventional light microscopy to count changes in cytoplasmic inclusions and photoreceptor cell number. Electron microscopy was utilized to assess intracellular changes.

Major Findings: Irreversible damage to the retina was significantly greater in rats deficient in vitamins E and A than in rats deficient in vitamin E alone or in vitamin A alone. Photoreceptor cells are lost in vitamin A deficiency probably because retinol normally provides an important structural component of their membranes. Photoreceptor cell loss in vitamin E deficiency probably derives from autoxidation of photoreceptor membranes when deprived of this important tissue antioxidant. Retinal cells are lost sooner when deprived of both vitamins simultaneously, not only because the effects are additive, but because in the absence of the antioxidant properties of vitamin E the vitamin A stores in the retina are left vulnerable to oxidation which can accelerate the loss of vitamin A from photoreceptor cells and lead to an acceleration of their structural damage.

The amount of lipofuscin which accumulated in the retinas of vitamin E deficient rats was much less in rats simultaneously deficient in vitamin A. Therefore, vitamin A may be a component of lipofuscin or may influence factors such as phagocytosis that are involved in its formation.

Significance to Biomedical Research and the Program of the Institute:

Although the roles of vitamin A in retinal structure, function, and disease have been studied rather extensively, the roles of vitamin E and its interrelationships with vitamin A have received little attention. Vitamin E, like vitamin A, is a lipid and has been found in relatively high concentrations in the photoreceptor membranes of the visual cells. Although vitamin E alone influences the accumulation of aging pigment in other tissues, vitamin A and vitamin E are both involved in the retina. Studies of the roles of vitamin E in influencing retinal vitamin A levels and in protecting against oxidation of the photoreceptor membranes should contribute to our understanding of the ocular effects of malnutrition, of diseases involving limited lipid absorption, and of nervous system diseases and other disorders where aging pigment accumulates prematurely.

Proposed Course: In order to distinguish the roles of dietary retinol and phagocytosis in lipofuscin formation in the retina, we will use the same four diet groups involving vitamin E and vitamin A deficiencies in studies with rats which lack photoreceptor outer segments due to hereditary factors or light damage. Also, rats will be deprived of vitamin E and maintained under various light cycles and intensities to see how light damage to the retina is altered without the protective influence of α-tocopherol. Vitamin A levels will be varied and carefully monitored.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Robison WG Jr, Kuwabara T, Bieri JG: Vitamin E deficiency and the retina: Photoreceptor and pigment epithelial changes. Invest Ophthalmol Vis Sci 18:683-690, 1979.

Bieri JG, Tolliver TJ, Robison WG, Kuwabara T: Lipofuscin in vitamin E deficiency and the possible role of retinol. Lipids (in press).

Robison WG, Kuwabara T, Bieri JG: Deficiencies of vitamins E and A in the rat: Retinal damage and lipofuscin accumulation. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00005-07 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Electrophysiology, Morphology, and Structure of Mammalian and Avian Retinas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ralph Nelson	Ph.D.	Physiologist	LVR	NEI
	Avery Nelson	Ph.D.	Senior Staff Fellow	LVR	NEI
	Andrew Mariani	Ph.D.	Guest Worker	LVR	NEI
Other:	Helga Kolb	Ph.D.	Biologist	LNP	NINCDS
	Margaret Robinson		Physical Science Aid	LVR	NEI

COOPERATING UNITS (if any)

Max-Planck Institut für Physiologishe and Klinishe Forschung, Bad Nauheim, F.R.G.; Department of Ophthalmology, Columbia University College of Physicians and Surgeons, New York; Laboratory of Neurophysiology, NINCDS

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Neurophysiology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.25	3.00	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We study intracellularly the physiology of single retinal neurons in cats and pigeons. Intensity response curves, input from rods and different cone types, receptive field properties, and response waveforms are assesed. Individual neurons, stained through the microelectrode with horseradish peroxidase (HRP), are identified according to anatomical schemes. Using the resolution of the electron microscope, we can then identify synaptic contacts made between the stained cells, which have electron opaque profiles, and other retinal neurons such as photoreceptor, horizontal, bipolar, amacrine, and ganglion cells. The morphology of individual neurons in monkey, cat and pigeon retinas is also studied at the light microscopic level using Golgi impregnation. The size, shape, and orientation of pigeon amacrine cells and primate horizontal cells suggest their specific functional roles. In outer plexiform layer of primates, electron microscopy of Golgi stained horizontal cells reveals the contacts made with different classes of photoreceptors. Material fixed for ultrastructure demonstrates contacts between photoreceptor terminals and their variation in density and type with retinal location. Changes in the synaptic ultrastructure of rods and cones are correlated with their physiological state of adaptation; activity of synapses in response to varying stimuli is assayed by uptake of HRP into synaptic vesicles.

Project Description:

Objectives: To understand the functional, structural, and ultrastructural organization of mammalian and avian retinas, to discover the synaptic interconnections among neurons and the functional pathways between them, to examine synaptic ultrastructure and observe the modifications produced by stimulation, to relate this information to disease states of the retina.

Methods Employed: We use Golgi impregnation to study the morphology of retinal neurons, to classify them, and to gain insight into function in the retinas of cat, monkey, and pigeon. Electron microscopy of Golgi impregnated cells of the outer plexiform layer provides information about connections with photoreceptors; use of Rall's modification to the cable equations allows the inference, from structural measurements, of signal propagation within cells. Electron microscopy of photoreceptor terminals indicates their interconnections; in conjunction with the extracellular application, endocytosis and compartmentalization of horseradish peroxidase (HRP), the normal functioning of the photoreceptor synapses can be studied at the ultrastructural level.

We characterize the response properties of neurons in the cat and pigeon retinas by intracellular recording of their transmembrane potentials and extracellular electroretinographic (ERG) recording during photic stimulation Viable retina-eyecup preparations are maintained in vivo through perfusion of the ophthalmic arteries with synthetic media. HRP, injected into neurons through the electrodes, fills their axons and dendrites, and after incubation with appropriate reagents, the morphology of individual, physiologically studied neurons is revealed in the light and electron microscope. In the light microscope cells are drawn and classified according to analogy with their Golgi counterparts; in the electron microscope the synapses forming the input and output of the unit can be identified by the ultrastructural features of the neighboring unstained processes. Thus the synaptic relationship of the physiologically studied cell with other retinal neurons can be known and the retinal pathways along which visual information travels can be elucidated. Monochromatic stimuli produced from calibrated optical benches allow the measurement of rod and different classes of cone input in the responses of individual cells. Movable bar stimuli allow the characterization of receptive field properties in terms of a single number, the space constant, which relates to the electrical interactions with neighboring cells, and thus to anatomically observable gap junctions.

Major Findings:

I. Receptive field properties of pigment epithelial (PE) cells:

In our experiments with the effects of patterned, multispot and multibar stimuli on the ERG we have likened the spatial properties of the c-wave component to those of a linear photodiode. It was thus of interest to extend this analogy to the sources of the c-wave as measured in the intracellular responses of PE cells. The penetration of a PE cell was signalled by a

large (about 60 mV negative) resting potential near the external limiting membrane and a monotonous stereotyped waveform, in response of a low-pass filter of light, whose major component resembled the response of a low-pass filter of time constant about 0.8 second. The receptive field properties were measured with long narrow slits as a function of wavelength, slit width, and energy. Receptive fields were measured two ways. In the first a narrow slit was physiologically centered and the amplitudes in response to slits of different widths were measured; in the second a narrow slit of fixed width was flashed at different positions in the receptive field and the responses measured. If the PE cells can be modelled as a linear homogeneous electrical syncytium, the voltage (V) for a given width is predicted to be given by $V_{max}(1-e^{-a/2\lambda})$ where 'V_{max}' is the response amplitude for a slit wide enough to produce saturated responses, 'a' is the width of the slit and 'λ' is the syncytial space constant. In a total of 28 such measurements obtained in 5 PE cells such curves fit the data, within limits imposed by electrode noise, in all cases, indicating that pigment epithelial cells are an electrically linear, and thus spatially linear system. The space constant was found to vary with the energy of the stimulus, as measured by V_{max} ($P>.999$). This varied from about 25 μm for V_{max}=7mV to an extrapolated least squares fit of 92 $\pm$ 13 μm S.D. for V_{max}=0 mV. For the second receptive field measure, where slits for fixed width were flashed at different positions, data were also fit by theoretical curves for a homogeneous linear system. In a total of 31 such measurements on 7 PE cells space constants were here found to be only weakly correlated with stimulus energy, as measured by the response to a centered slit ($P= .95-.98$). The space constant, extrapolated to a threshold measurement, was found to be 96 $\pm$ 24 S.D. μm. Thus PE cells and photodiodes share in common the property of linear electrical summation of signals originating in different parts of their receptive fields; so the c-wave also shares in this property of linear spatial summation. The linear spatial properties of the PE cells combined with their tiny summing area (about .03 mm^2), make them useful devices for optical calibration in the distal retina. The problem of diffuse straylight confounds interpretations of many physiological data. Using the PE cells as optical probes has allowed us to calculate that within 0.5mm of a projected slit, diffuse straylight is about 2.5 log units less intense than the stimulated area. These cells provide a reliable index of the state of rod function.

I. Physiology of the in vitro, perfused pigeon eyecup: The pigeon retina, owing to its great regularity of structure both in the highly organized layering of its inner and outer plexiform layers and the large variety of yet highly stereotyped neuronal morpholgies, provides an exciting frontier for the correlation of function with structure in single retinal neurons. This structural interest is rivaled at the ganglion cell level where a variety of extremely complex receptive fields are found. In order to facilitate intracellular recordings in this retina we have developed an in vitro eyecup preparation perfused through the ophthalmotemporalis artery. This artery feeds the short and long ciliary arteries and the pecten arteries, which provide for retinal nutrition. The absence of pulsation in the vascular flow as well as the absence of other movements and vibrations in this preparation, and the probability the electrode-dulling vitreous body

should greatly enhance the probability and stability of intracellular recordings. Electroretinograms, with the full complement of a-b and c-waves have been reported for several hours in the eyecup and perfused whole eye preparations, indicative of viable retinal function in vitro. Interestingly, the spectral properties of the c-wave appear to peak in the red and may be driven by long wavelength cones. This is unlike mammal retinas where the c-wave is a purely rod driven phenomenon. Work is currently in progress in obtaining intracellular recordings from this retina and in identifying the morphology of physiologically characterized cells through the technique of HRP injection.

III. Ultrastructure and interconnections in photoreceptor terminals:

It has become apparent that photoreceptors are not single isolated photodetectors measuring light energy only in the minute domain of their own outer segments, but participate in physiological interactions and make morphological contacts with their neighbors. Physiologically, it has been argued that these interactions, which appear primarily to allow electrical current flow between neighboring cells, act to improve signal to noise ratio while reducing acuity. It is thus of interest to study such contacts ultrastructurally in the primate retina in regions of high (foveal) and low (peripheral) visual acuity. In peripheral retina specialized contacts have been found between the lateral surfaces of cone pedicals as well as between the scleral surfaces of cone pedicals and the vitreal surfaces of rod spherules. This latter junction differs from similar junctions found in cat retina in that primate cones do not employ extensive teleodendria to make them. Both types of junctions appear to be small gap junctions ultrastructurally. Junctions interconnecting rod spherules in the primate remain elusive. Small areas have been found between adjacent rod spherules where Muller cell processes cease to intervene. These areas of apposition are extremely punctate and the membrane profiles turn obliquely, frustrating efforts to determine their nature and whether specialized contacts occur. Further observation will be needed to clarify this point since physiological data on rods, obtained in amphibia and reptiles, demands electrical junctions. Observations of photoreceptor terminals in the fovea centralis revealed no interreceptor junctions, a factor which is likely related to the high acuity of this specialized area.

The manner in which the release of transmitter containing vesicles at the cone terminals is modulated by photic stimulation remains obscure. Previous work on primate using HRP failed to reveal large differences in the rate of vesicle recycling in light and dark adaptation. One interpretation of these results is that, over the three hour period of light exposure in the previous experiments, the cones became adapted to the ambient illumination and returned their mean rate of vesicular release to the previous dark level at the same time readjusting their intensity response characteristic towards higher energies. In order to test for such a slow readjustment the experiment will have to be performed much faster, on the order of minutes rather than hours. This is more likely to be possible in the perfused cat eyecup, where reagents have ready access to the retinal surface, than in the intact monkey eye, where they must diffuse indeterminately long through the viscous vitreous. Work is in progress, then, to study rapid changes in vesicular recycling in the terminals of cat cone.

IV. Amacrine cells of the pigeon retina:

Anatomical features of retinal neurons, such as size and/or shape of their dendritic fields, probably put certain constraints on their input and output of information. Using Golgi impregnation, the form and spatial distribution of retinal neurons is being studied. Certain features of two unusual types of amacrine cells appear to be related to reported "complex" receptive field properties. "Asymmetrical" amacrines are unusual in that their dendrites extend in only one direction away from the cell body. This results in an elongate dendritic field which is always oriented in a dorso-ventral direction (i.e. vertically). "Association" amacrines have dendrites which are radially organized with respect to their cell bodies, but they possess an axon which usually arises directly from the cell body, may have collaterals and terminates in a varicose arborization 350-600 μm from the cell body. In contrast, "ordinary" amacrines, which are diverse in their morphology and may be vertically stratified, always display a radial symmetry when viewed in flat preparations. Due to the unique orientation of the "asymmetrical" amacrines, it is possible that they are involved in formation of verticality detectors, which are responses recorded from ganglion cells sensitive to only moving or stationary vertical edges. The "association" amacrines are spatially polarized, and thus may provide a basis for directionally selective responses recorded from ganglion cells. Since this cell type has an axon, it is clearly consistent with the physiological concept of lateral inhibition extending in only one direction which then determines the null-preferred axis.

V. A new type of horizontal cell in the macaque retina:

Using Golgi impregnations of Macaca mulatta and Macaca arotoides, we have found a new type of horizontal cell in the primate retina. This new type of horizontal cell is termed HII in contrast to the previously described horizontal cell which is termed HI. HII's in the primate retina have wavy multibranched dendrites which form loose clusters of dendritic terminals. In addition they possess a 300-500 μm axon which is fine (0.5 μm diameter) and wavy, may have short collaterals, and bears single terminals or clusters of terminals along its length. Golgi-EM shows that the dendritic clusters of HII's contact cone pedicles and form lateral elements at the ribbon synaptic complex, while the terminals born by the axon also contact cone pedicles as lateral elements. In contrast, the previously described HI's contact cones with their dendrites, but rods with their axon terminal arborization. This new type of primate horizontal cell is therefore distinctly different, not only in morphological appearance, but also in its connections with photoreceptors. Furthermore, it bears little resemblence to the axonless (A type) horizontal cell described in other mammalian retinas.

While previous work has shown that the long axon of the axon bearing horizontal cells in mammals (B type in cat, HI in primate) electrically isolated separate rod and cone integrating units, our calculations of passive conduction of electrical signals along the axon of the new macaque HII's

indicates that a significant portion of a signal generated in the cell body may be conducted along the axon. Thus purely structural considerations suggest that these long processes without terminals may be functionally axons even considering the probable absence of impulse activity.

Some of the anatomical aspects of this project are described in Project No. ZO1 NS 02152-04 LNP; Dr. Helga Kolb, PI.

Significance to Biomedical Research and the Program of the Institute:

In diagnosing and treating the diseases of the eye it should prove useful to understand retinal function at the cellular level and the pathways through which visual signals travel and are processed. In this regard it is interesting that our repertoire of intracellularly studied and stained neurons now includes several from cats afflicted with central retinal degeneration (CRD). These are not in sufficient quantities to draw definite conclusions concerning modifications of retinal pathways. Our recent discovery in the cat retina of the extreme sensitivity of the ERG b-wave to the spatial pattern of the stimulus has recently been demonstrated also in humans by Zrenner and Diehl and may have clinical value. Many disease states involve dysfunction at the cellular level and treatments have as their targets particular classes of cells. A knowledge of what classes of neurons the retina contains and what their physiological properties and roles in vision may be provides a necessary substrate for interpreting and treating retinal dysfunction.

Proposed Course: This project will be continued along lines indicated in the project description. Emphasis will be given to obtaining stable intracellular recordings for the first time in pigeon retina and to the use of HRP injection to identify the morphology of the recorded cell. Comparisons will continue to be made between homologous cell types in the retinas of different species using the Golgi staining technique. Particular emphasis will be given to obtaining information concerning the ultrastructural characteristics of photoreceptors which may correlate with their electrophysiological responses and the changes in ultrastructure that occur with stimulation. We plan to continue using HRP to assay activity for cone synapses, modifying the preparation and stimuli to obtain a light/dark difference in uptake sufficient to characterize the spectral classes of terminals. Existing data supplemented by additional data currently being obtained on axon terminals of horizontal cells, bipolar and amacrine cells in the cat retina will be organized and made ready for publication. Studies of new aspects of the spatial and spectral properties of horizontal cell bodies of cat retina will be completed.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaptation

Publications:

Nelson R, Zrenner E, Gouras P: Patterned stimuli reveal spatial organization in the electroretinogram, in Tazawa Y (ed): Proceedings

of the XVIth ISCEV Symposium. Morioka, Jpn J Ophthalmol (in press).

Gouras P, Zrenner E: The blue cone mechanism. Excerpta Medica (in press).

Gouras P, Zrenner E: Enhancement of flicker by color opponent mechanisms. Science (in press).

Zrenner E, Gouras P: Blue cones of cat produce a rod-like electro-retinogram. Invest Ophthalmol Vis Sci (in press).

Kolb H, Gallego A, Mariani A: A second type of horizontal cell in monkey retina. J Comp Neurol 1979 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00066-02 LVR

PERIOD COVERED

October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Neurotransmitter Chemistry of Retinal Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Barbara-Anne Battelle	Ph.D.	Senior Staff Fellow	LVR	NEI
Other:	None				

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are underway to identify neurotransmitters in photoreceptor cells and other retinal neurons. The objective of these studies is to understand the neurochemical circuitry responsible for processing visual information in retinas. Particular emphasis is given to the role of biogenic amines in visual systems. The visual system being studied currently is that of the American horseshoe crab Limulus polychemus. The anatomy and physiology of this visual system is well understood; virtually nothing is known of its neurochemistry. Studies conducted in this Laboratory have focused on the possible role of the putative neurotransmitters octopamine and serotonin. Biochemical techniques have been employed to study the synthesis, accumulation and metabolism of these amines. Electrophysiological techniques are being developed to study the role of these amines in visual function.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In order to understand the processing of visual information in retinas it is necessary to know the morphological, electrophysiological, and neurochemical organization of retinal neurons. The morphological organization and electrophysiological properties of retinal cells have been studied extensively and are in part understood. The neurotransmitter chemistry of retinal cells is largely unknown. The general aim of my research is to determine the neurotransmitters used by identified retinal neurons and to examine neurochemical mechanisms involved in processing visual information.

Methods Employed: High voltage paper electrophoresis and other chromatographic techniques are employed to study the synthesis, accumulation, and metabolism of putative neurotransmitters from radioactively labeled precursors. Sensitive enzymatic assays are being used to measure endogenous levels of amines in various parts of the visual system. In order to facilitate interpretation of results, the relatively simple visual system of Limulus polychemus has been chosen as an experimental model. This visual system contains relatively few types of neurons, and the anatomy and electrophysiology of the system has been described.

Major Findings: The putative neurotransmitter octopamine is synthesized by virtually all parts of the Limulus visual system so far tested. This includes the lateral, ventral, and median eyes as well as optic ganglia. Significant levels of endogenous octopamine have been measured in lateral, ventral and median eye preparations. Levels of endogenous octopamine are particularly high in optic ganglia which contain the terminals of photoreceptor cells. Evidence is accumulating that octopamine may be the neurotransmitter of ventral eye photoreceptors and that this amine has an important function in the lateral eye as well. Several unknown metabolites of octopamine have been found. Initial experiments have been conducted to identify these metabolites.

Other laboratories have presented evidence that serotonin may have a significant role in modulating the sensitivity of Limulus lateral eye and in mediating the well-known phenomenon of lateral inhibition. Biochemical studies of tryptophane metabolism in lateral eyes performed in this laboratory suggest that some other molecule similar to serotonin--but not serotonin--is the endogenously active biogenic amine. Serotonin synthesis is not detected in Limulus lateral eye.

Significance to Biomedical Research and the Program of the Institute:

Knowledge of neurotransmitters in retinas is vital to the understanding of how visual information is processed. The identity of the neurotransmitters used by photoreceptor cells of vertebrate and invertebrate eyes is completely unknown. Limulus eyes are relatively simple and are composed principally of photoreceptor cells. Because of this simplicity likelihood it is highly likely that the identity of Limulus photoreceptor cell neurotransmitters will be determined conclusively. By studying relative, simple invertebrate preparations, new insights will be gained into the neurochemical properties of photoreceptor cells. The Limulus visual system is particularly rich in biogenic amines. Therefore, it is a system well-suited for study of the role of biogenic amines in changes in photoreceptor cell sensitivity, adaptation, and retinal circuitry.

Proposed Course: Studies of neurotransmitter synthesis in Limulus ventral, lateral, and medial eyes will continue. Considerable effort will be directed toward the identification of the unknown metabolites synthesized from tyramine and tryptophane. Experiments will be done to test if octopamine, serotonin, or their metabolites can be released from photoreceptor cells with light or high potassium stimulation.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaptation

Publications:

Battelle BA, Kravity EA, Stieve H: Neurotransmitter synthesis in Limulus ventral nerve photoreceptors. Experientia 35:778-780, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00148-06 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cyclic Nucleotides and Vision

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Gerald J. Chader	Ph.D.	Research Chemist	LVR NEI
Other:	R. Theodore Fletcher	M.S.	Chemist	LVR NEI

COOPERATING UNITS (if any)
1) Clinical Pharmacol. Branch, NCI, 2) Lab. Chem. Pharmacol, NHLBI, 3) Section Ophthamol., School of Vet. Medicine. Univ. Penn., Philadelphia, PA

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cyclic nucleotides, especially cyclic GMP, are important in the visual process and in other functions of the retina and pigment epithelium. Deficiencies in the enzymes of cyclic nucleotide metabolism may also be responsible for diseases related to retinal dysfunction. In a dog model for retinitis pigmentosa it appears that a switch in cyclic GMP phosphodiesterase (PDE) fails to occur during development in retinas of affected animals. This, coupled with low levels of a PDE Protein Activator in affected retinas, appears to result in derangement in cyclic nucleotide metabolism that is characteristic of this hereditary degenerative disease.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the role of cyclic nucleotides in normal vision and in retinal disease.

Methods Employed: Retinas from test animals are dissected; photoreceptor units are isolated by sucrose density gradient ultracentrifugation and the activities of the enzymes of cyclic nucleotide metabolism are assayed by standard techniques. Cyclic nucleotides concentrations are measured by immunochemical titration after initial purification by column chromatography.

Major Findings: A defect in enzyme activity in the retinas of Irish setter dogs with inherited retinal degeneration has been found. The phosphodiesterase (PDE) enzyme which metabolizes cyclic GMP is deficient in affected animals. Moreover, the concentration of the Protein Activator of the PDE enzyme is low in affected retinas. This enzymic deficit apparently leads to the high cyclic GMP levels that are characteristic of this disease.

Significance to Biomedical Research and the Program of the Institute: If the above finding is correct and is found to be similar in humans, the cause of at least one form of retinitis pigmentosa will be understood. This then could subsequently lead to rational modes of treatment of the disease.

Proposed Course: Studies on the PDE enzyme will continue in other animal models of retinal degeneration. We will also begin a regimen of therapy in the affected dogs in hopes of stopping or at least slowing down the course of the disease.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Aguirre G, Farber D, Lolley R, Fletcher RT, Chader GJ: Rod-cone dysplasia in Irish setters: A defect in cyclic GMP metabolism in visual cells. Science 201:1133-1144, 1978.

Fletcher RT, Chader GJ: Cyclic nucleotide and protein kinase systems in the developing chick retina and pigment epithelium. Biochim Biophys Acta 544:45-52, 1978.

Tamai M, Chader GJ: The early appearance of disc shedding in the rat retina. Invest Ophthalmol Vis Sci (in press).

Liu L, Krishna G, Aguirre G, Chader GJ: Cyclic GMP phosphodiesterase activator: Involvement in a hereditary retinal degeneration. Nature (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00041-01 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Phospholipid Synthesis; A Biochemical Correlate to Circadian Disc Shedding in the Rat Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter Dudley	Ph.D.	Research Associate	LVR	NEI
Other:	James P. Alligood	B.S.	Biologist	LVR	NEI
	Paul J. O'Brien	Ph.D.	Research Chemist	LVR	NEI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.7	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The shedding of rat rod outer segment (ROS) discs, by a process of phagocytosis, is a circadian phenomenon characterized morphologically by the release of ROS discs into the pigment epithelium. This process occurs over a period of a few hours beginning about two hours after the start of the light period. Prior to the appearance of phagosomes in the pigment epithelium and coincident with onset of the daily light period, there is increased phospholipid synthesis, particularly phosphatidyl choline and phosphatidyl ethanolamine. This occurs when animals entrained to 12 hours dark, 12 hours light, are kept in the dark. Phosphatidylinositol synthesis appears to increase when animals are exposed to light at the initiation of the light period. An in vitro assay system employing labelled glycerol, incubation of retinas for 20 minutes, and separation of phospholipid classes by two-dimensional thin layer chromatography have led to the detection of this event which may be correlated with shedding.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Biochemical events surrounding disc shedding have not been documented. The objective of this project is to determine the labeling of phospholipid classes in rat retina to see if lipids are synthesized in response to the initiation of the daily light cycle.

Methods Employed: Ordinary biochemical techniques were employed such as incubation of retinas, extraction of lipids, and separation of phospholipid classes by two dimensional thin layer chromatography.

Major Findings: Increased phosphatidyl choline and phosphatidyl ethanolamine synthesis occurs in rat retina at the time of light-onset when retinas are incubated in the dark. This indicates that this event is circadian in nature and not light-stimulated. When animals are kept on an altered lighting schedule for six days such that the light period begins at 9:00 P.M. instead of 7:00 A.M., there was increased phospholipid synthesis at 9:00 P.M. The shift in the peak of phospholipid synthesis by alteration of lighting schedule helps to correlate the biochemical event with disc shedding.

Significance to Biomedical Research and the Program of the Institute:

The detection of a biochemical event associated with disc shedding would allow the closer study of events associated with visual cell-pigment epithelium interactions. Some retinal degenerations are characterized by the inability of the pigment epithelium to phagocytize ROS disc membranes. This may be due to the lack of the retina's ability to control the lipid composition of ROS discs, especially those nearest the pigment epithelium, as well as the plasma membrane, which interacts with the pigment epithelium.

Proposed Course: Autoradiography will be used to determine if certain portions of the visual cells, especially those adjacent to the pigment epithelium, significantly increase their level of lipid incorporation during the time of light-onset.

NEI Research Program: Retina and Chorodial Diseases--Visual Cells and Pigment Epithelium

Publications:

Dudley P, Anderson RE: Phospholipid transfer protein from bovine retina with high activity towards retinal rod disc membranes. FEBS Let 95: 57, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00179-04 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Ultrastructural and Biochemical Correlates in the Vertebrate Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Arnold I. Goldman	Ph.D.	Staff Fellow	LVR	NEI
	Paul J. O'Brien	Ph.D.	Research Chemist	LVR	NEI
	Paul S. Teirstein	Ph.D.	Medical Student	LVR	NEI

COOPERATING UNITS (if any)

Mount Sinai Hospital and Medical School
New York, New York

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies have been conducted on the control of shedding of outer segment tips and their phagocytosis by the pigment epithelium. Animals were killed, the eyes processed for electron microscopy, and phagosomes were counted by light microscopy. The dynamics of the circadian rhythm of shedding were studied by maintaining albino rats in complete darkness, constant light (CL), or under different environmental light cycles. CL inhibited shedding but did not erase the underlying rhythm controlling shedding. The minimum dark period required for shedding was determined. A series of experiments was conducted in which bilateral optic nerve section was employed to determine if the CNS participated in the control of the shedding. The shedding rhythm continued after the optic nerves were severed, although it could no longer adapt to altered lighting cycles. The surgery had no effect on the response of the eyes to CL exposure. Finally, a burst of shedding could be triggered at any time of the day by exposing the animal to CL followed by two hours darkness and one and one-half hours light, although the magnitude of the induced shedding depended upon the time of the day.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: It has been established that animals which have been maintained under conditions of cyclic lighting exhibit a daily burst of shedding and phagocytosis of outer segment tips within two hours of the onset of light. This occurs on schedule even if animals are placed in constant darkness after entrainment. The experiments in this project were designed to search for the site of control of this phenomenon and the mechanisms by which this control is expressed. It is hoped that knowledge of the pathways of control will lead to a better understanding of the etiology of retinal diseases in which these control mechanisms may malfunction.

Methods Employed: Light microscopy was used to observe patterns of shedding and phagocytosis. Animals were treated by modification of lighting schedules and surgical ablation of the optic nerves.

Major Findings: Changes in the lighting cycles to earlier onset of light (phase advances) produce changes in the shedding rhythm more quickly than do phase delays. Furthermore, large advances change the shedding more quickly than small advances. The shedding rhythm can be maintained in darkness for up to two weeks, although there is a broadening in the shedding peak as well as a decrease in the magnitude of this peak. If animals are maintained in constant light (CL) for one to two days, shedding occurs on schedule the next day. A minimum of two hours of darkness in a 24 hour cycle is necessary for the shedding to occur. Shedding can be triggered at times other than that dictated by the circadian oscillator by placing the animal in CL for more than 20 hours, followed by two hours dark and one and one-half hours light, although the magnitude of shedding is greatest during the expected time of the circadian peak.

When animals with severed optic nerves were studied, it was determined that the circadian shedding rhythm was maintained for a least one week, and that all of the responses to CL (inhibition, expression of the rhythm after CL had terminated light-triggered shedding) were unchanged by the surgery. Nevertheless, when the optic nerves were severed, the shedding rhythm would no longer shift in response to altered lighting conditions, indicating that synchronization of the shedding rhythm under the direct neural control of the central nervous system.

From these experiments we conclude that shedding is controlled by a complex series of events: Light stimulates the photoreceptors and signals a "synchronizer" in the central nervous system. The "synchronizer" in turn entrains each retinal oscillator separately to the ambient lighting conditions. At the appropriate time, the oscillator initiates a train of events which prepares the outer segment tips for shedding. These events require darkness and are at least two hours in duration.

Significance to Biomedical Research and the Program of the Institute:

The mechanisms of regulation of shedding of discs and their subsequent phago-

cytosis by the pigment epithelium are crucial in the understanding of photoreceptor renewal processes. The knowledge gained in this way can be instrumental in the diagnosis and treatment of various retinal degenerations where the balance between synthesis and degradation of photoreceptor membranes may be disturbed.

Proposed Course: This project will be terminated when Arnold Goldman, the principal investigator, leaves NIH. He will continue to pursue this problem, however, at the Medical College of Wisconsin in collaboration with Dr. Paul O'Brien. The next area of research is to elucidate the biochemical basis of the control processes outlined above. In addition, further experiments are planned to study in greater detail the response of the shedding rhythm to various manipulations of the ambient lighting conditions.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Goldman AI, O'Brien PJ: Phagocytosis in the retinal pigment epithelium of the RCS rat. Science 201:1023-1025, 1978.

Goldman AI, O'Brien PJ, Masterson E, Israel P, Teirstein P, Chader GJ: A quantitative system for studying phagocytosis in pigment epithelium tissue culture. Exp Eye Res 28:455-468, 1979.

Goldman AI: A computational technique for prediction of the number of visible phagosomes from data on uptake of radiolabeled outer segment material. Exp Eye Res 28:469-474, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00068-02 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Physiology of the Pigment Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eileen Masterson	Ph.D.	Staff Fellow	LVR	NEI
Other:	Gerald J. Chader	Ph.D.	Research Chemist	LVR	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Glucose transport in cultured pigment epithelial (PE) cells was characterized. Transport is of the facilitated diffusion type. Permeation of the hexose is stereo-specific and exhibits competition by structurally similar solutes.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study and characterize glucose transport of the pigment epithelium in culture.

Methods Employed: Pigment epithelial cell cultures were maintained using standard cell culture techniques. Cells at confluency were studied as to their transport (uptake) of radiolabeled hexoses. Energy poisons and other metabolic inhibitors were used in the incubation medium to characterize the type of transport exhibited by the cells.

Major Findings: There is preferential uptake of D-glucose vs. L-glucose in PE cells indicating that transport is stereospecific. Incubation of PE cells in energy poisons did not affect hexose uptake but did inhibit amino acid uptake. Thus, the process for hexoses is not energy-dependent. Counter-transport can be demonstrated as can competitive and noncompetitive inhibition by various analogs, all of which points to facilitated diffusion. Kinetics of 3-O methyl glucose and 2-deoxyglucose transport revealed a K_m of 4.5 mM and 2.7 mM respectively and Vmax of 22.8 and 21.8 nmol/mg protein/min respectively; these values are in line with those seen in other cultured cell systems. These studies demonstrate conclusively that glucose transport in cultured PE cells is of the facilitated diffusion type.

Significance to Biomedical Research and the Program of the Institute: Retinal photoreceptor cells are highly metabolically active and dependent on a ready glucose supply. The pigment epithelium supplies the major portion of the nutrients of the photoreceptor. Knowledge of the mechanism of glucose uptake and transport in PE cells is thus important for understanding the energy requirements of the visual process. Moreover, such study may lead to an understanding of how derangement in glucose uptake and metabolism could lead to diseases of the retinal-PE unit.

Proposed Course: Glucose transport and metabolism in PE cells will be studied in further detail. Uptake and transport of other important nutrients (e.g. amino acids) will be also investigated.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Masterson E, Israel P, Chader G: Pentose shunt activity in developing and cultured retina and pigment epithelium. A switch biochemical expression in cultures of pigment epithelial cells. Exp Eye Res 27:409-416, 1978.

Israel P, Redfern N, Chader G: Neural retinal-pigment epithelial interactions. Morphological characteristics of cells grown together

in culture. Ophthalmic Res 125:125-130, 1978.

Wiggert B, Masterson E, Israel P, Chader G: Differential retinoid binding in chick pigment epithelium and choroid. Invest Opthalmol Vis Sci 18:306-310, 1979.

Goldman AI, O'Brien PJ, Masterson E, Israel P, Teirstein P, Chader G: A quantitative system for studying phagocytosis in pigment epithelium tissue culture. Exp Eye Res 28:455-467, 1979.

Israel P, Masterson E, Goldman AI, Wiggert B, Chader G: Retinal epithelial cell differentiation: Influence of culture medium in vitro. Invest Ophthal Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 EY 0067-02 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Studies on the Developing Cornea

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eileen Masterson	Ph.D.	Staff Fellow	LVR	NEI
Other:	Gerald J. Chader	Ph.D.	Research Chemist	LVR	NEI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Glucose metabolism was studied in the embryonic cornea during the period of transparency development. Results indicate that much of the glucose taken up in this period is utilized for protein synthesis which appears to be especially important at this time.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This study focuses on determining the significance of various metabolic pathways during corneal development. The embryonic chick cornea between stages 40 and 45 transforms from an opaque tissue to a transparent one; the biochemical basis for this is unknown. The data obtained from these studies are aimed at evaluating the basic biochemical processes which occur during this critical period.

Methods Employed: Corneal CO_2 formation and glucose uptake were studied using uniformly labeled [^{14}C]-glucose incubated in an in vitro system. Incorporation and uptake of radiolabelled sugars and amino acids were evaluated in the same system.

Major Findings: Stages 40 to 45 constitute a critical period of corneal deturgescence and development of transparency in the chick embryo. Glucose uptake, incorporation, and oxidation were studied in the chick cornea during this period and the effects of several metabolic inhibitions were tested. Corneal $^{14}CO_2$ formation is constant between stages 38 and 45 but rises after hatching. Glucose uptake and incorporation increases between stage 38 and 40, is constant between stage 40 and 45, and increases again after hatching. Both DON (an inhibitor of proteoglycan synthesis) and actinomycin D (an inhibitor of RNA synthesis) had little effect on glucose uptake and incorporation by cornea at stages 40 and 45. Puromycin (an inhibitor of protein synthesis), on the other hand, significantly decreased glucose incorporation by the cornea at stages 40 and 45. The results indicate that much of the glucose taken up is utilized for protein synthesis which appears to be especially important during this period of corneal development.

Significance to Biomedical Research and the Program of the Institute:

This project is directed at elucidating some of the basic biochemical events which occur during corneal transparency development. Exploration of the mechanisms which permit the cornea to transform itself into a transparent tissue (which occurs both in the chick and in mammals) may aid in the understanding of the process which permits transparency in the adult and be important in understanding diseases which involve corneal opacification.

Proposed Course: Biochemistry of corneal transparency will be further probed.

NEI Research Program: Corneal Diseases--Corneal Edema, Dystrophies, and Inherited Disorders

Publications:

Masterson E, O'Brien PJ, Chader GJ: Metabolic inhibitors and glucose oxidation in developing chick cornea. Exp Eye Res 28:121-127, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00016-12 LVR

PERIOD COVERED
October 1, 1978, to September 1979

TITLE OF PROJECT (80 characters or less)

The Biochemistry of Normal and Dystrophic Retinas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul J. O'Brien	Ph.D.	Research Chemist	LVR NEI
Other:	James P. Alligood	B.S.	Biologist	LVR NEI

COOPERATING UNITS (if any)
School of Veterinary Medicine, University of Pennsylvania

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The content of opsin was lower in rod outer segment preparations from miniature poodles carrying the gene for an inherited progressive retinal atrophy than it was in normal dogs. This deficiency was found in very young poodles even before electroretinogram or ultrastructural differences could be observed. Opsin synthesis in the affected dogs appeared normal, however. These determinations were made by gel electrophoresis of outer segment proteins following intravitreal injection of radioactive leucine.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The renewal of photoreceptor cell outer segments is a continuous process which is impaired in some pathological conditions such as progressive degeneration or developmental anomalies of the retina. The purpose of this project is to elucidate the biochemical events involved in photoreceptor renewal, especially the synthesis of protein, in the retinas of both cow and dog.

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

Major Findings: Protein synthesis and outer segment renewal was demonstrated in both normal and affected littermates produced by the crossing of an affected and a carrier miniature poodle. Intravitreal injection of radioactive leucine resulted in the production of labeled opsin which was visualized as a migrating band by autoradiography and identified as opsin by gel electrophoresis.

One of the littermates had a greatly reduced level of opsin in the outer segment preparation made from the single eye enucleated at an early age. The electroretinogram was normal as were the ultrastructure and autoradiographic labeling pattern. Only after a year was an electroretinogram abnormality detected in the remaining eye.

Significance to Biomedical Research and the Program of the Institute: A very sensitive biochemical marker has been found enabling an early detection of retinal atrophy. This disease progresses very slowly, and heretofore, it was thought that electroretinogram changes were the first sign of a defect. With an early detection of this biochemical difference, it becomes possible to examine other markers such as cyclic nucleotide levels or phospholipid composition and synthesis at a time when few other defects are apparent. Unlike the more rapidly progressive animal models of retinal degeneration, the poodle may be much better suited to determine which biochemical defect is the first to appear and thus to elucidate the etiology of this disease which so closely mimics human retinitis pigmentosa.

Proposed Course: Both miniature poodles and Irish setters with inherited retinal degenerations will be studied further to determine the earliest sign of a defect, be it protein, lipid or carbohydrate synthesis or composition of photoreceptors.

Publications:

Tamai M, O'Brien PJ: Retinol dystrophy in the RCS rat. In vivo and in vitro studies of phagocytic action of the pigment epithelium on the shed rod outer segments. Exp Eye Res 28:399-411, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00015-14 LVR

PERIOD COVERED

October 1, 1978, to September 1979

TITLE OF PROJECT (80 characters or less)

The Cell Biology of the Vertebrate Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul J. O'Brien	Ph.D.	Research Chemist	LVR NEI
Other:	James P. Alligood	B.S.	Biologist	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.1	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Rat retinas were incubated with radioactive leucine, glucosamine, and mannose at various times prior to and following the scheduled onset of light. All three exhibited a stimulation of incorporation into opsin prior to the onset of light when compared to other proteins of the retina. Constant light abolished this specific stimulation of opsin synthesis. These observations suggest that the stimulation of opsin synthesis is a circadian event related to the circadian shedding of rod outer segment membranes that follows the scheduled onset of light.

PHS-6040
(Rev. 10-76)

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 and in the process of shedding and phagocytosis of disc membranes.

Methods Employed: Ordinary biochemical techniques were used, such as incubation of retinas with radioactive precursors, cell fractionation, SDS gel electrophoresis, and scintillation counting.

Major Findings: Leucine, glucosamine, and mannose were all incorporated into rat opsin in vitro, as well as into other proteins of the retina. A selective stimulation of opsin synthesis was observed in the morning hours prior to the scheduled onset of light. This stimulation was abolished by constant light.

Significance to Biomedical Research and the Program of the Institute: The circadian shedding of photoreceptor outer segment membranes in the rat requires at least two hours of darkness prior to the onset of light. Light, in turn, signals the onset of shedding. It appears that elevated opsin synthesis is at least one of the dark reactions taking place. This provides a specific biochemical event that can be measured in both normal and diseased retinas. It is an event intimately associated with photoreceptor renewal and shedding processes, either of which could be defective in certain retinal degenerations. An increasing array of biochemical tests for normal retinal functions will increase the opportunities for identifying the lesions in human retinal degenerative processes.

Proposed Course: The addition of other sugar residues to opsin will be studied as a function of the phase of the shedding cycle. Sugars such as galactose, fucose, and N-acetylgalactosamine may be added to opsin in the plasma membrane and play a role in retinal-pigment epithelium interactions.

Publications:

O'Brien PJ: Rhodopsin: A light-sensitive membrane glycoprotein, in Greaves MF, Cuatrecasas P (eds): Receptors and Recognition, vol 6, series A. London, Champman and Hall, Ltd, 1978, pp 107-150.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00070-02 LVR

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Vitamin A and Ocular Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Barbara Wiggert	Ph.D.	Research Chemist	LVR	NEI
Other:	Helen Sampson	B.S.	Chemist	LVR	NEI
	Paul Russell	Ph.D.	Research Chemist	LVR	NEI
	Gerald J. Chader	Ph.D.	Research Chemist	LVR	NEI

COOPERATING UNITS (if any)

Howe Laboratory of Ophthalmology, Harvard Medical School,

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.4	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Uptake and localization of ^{3}H-Retinol and ^{3}H-Retinoic acid by Y-79 human retinoblastoma cells in culture were studied. Specific binding proteins ("receptors") for both retinoids were observed in the cytoplasm but only ^{3}H-retinoic acid was bound and specifically compartmentalized in cell nuclei. Thus retinoic acid (a normal vitamin A metabolite) may act in the nucleus at the genetic level in these cells while retinol appears to play a role in the cytoplasm, perhaps in glycoprotein biosynthesis.

Retinoid receptors are also found in other ocular tissues (e.g. pigment epithelium) where they appear to mediate the action of retinoids.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To elucidate the mechanism of action of retinoids in ocular tissues.

Methods Employed: Sucrose density gradient centrifugation, autoradiography, gel filtration, thin layer chromatography, polyacrylamide gel electrophoresis, and isolectric focusing on thin layer gels were employed in studying vitamin A metabolism and retinoid receptors.

Major Findings: Autoradiographic studies of Y-79 human retinoblastoma cells demonstrated a selective accumulation of radiolabel in nuclei following incubation with ^{3}H-retinoic acid but not ^{3}H-retinol. This is in agreement with our earlier biochemical studies which showed the presence of a nuclear receptor for retinoic acid but not for retinol in these cells. The cytosol and nuclear retinoic acid receptors have identical electrophorectic mobilities and isoelectric points. Thin layer chromatographic studies of extracts of Y-79 cells incubated with radiolabeled retinoids indicated some conversion of ^{3}H-retinol to a metabolite with TCL mobility similar to ^{3}H-retinoic acid. There was no apparent metabolism of ^{3}H-retinoic acid either in whole cells or in nuclei.

Incubation of the cytosol fraction of freshly dissected pigment epithelial tissue or pigment epithelial cells grown in tissue culture with ^{14}C-retinylpalmitate showed that retinylpalmitate was not bound to the 2S cytosol protein which binds retinol but was instead bound to another cytosol protein which sediments at about 5.5-6.0S on sucrose gradients.

Gel filtration studies showed that the 7S protein which binds retinol and is apparently compartmentalized in retinal rod outer segments is a large molecule having a molecular weight of between 600,000 and 1,500,000.

Significance to Biomedical Research and the Program of the Institute: Vitamin A is necessary for the normal growth and development of most cells of the body. It also plays a special role in the visual process. Thus, elucidating its mechanism of action in ocular tissues not only is important in understanding how ocular tissues (e.g. retina) function normally, but should also contribute to a better concept of how ocular diseases involving vitamin A metabolism may be prevented or treated.

Proposed Course: Further studies are planned on the role of retinoid binding proteins ("receptors") and on the metabolism of retinoids in both fresh ocular tissues and in cells grown in tissue culture.

NEI Research Program: Retinal and Chorodial Diseases--Developmental and Hereditary Disorders

Publications:

Russell P, Wiggert B, Chader GJ: Separation of retinoid receptors from cultured retinoblastoma cells. Biochim Biophys Acta 543:586-589, 1978.

Wiggert B, Derr J, Fitzpatrick M, Chader GJ: Vitamin A receptors of the retina: Differential binding in light and dark. Biochim Biophys Acta 582:115-121, 1979.

Wiggert B, Masterson E, Israel P, Chader GJ: Differential retinoid binding in chick pigment epithelium and choroid. Invest Ophthalmol Vis Sci 18:306-310, 1979.

DeLuca L, Adams S, Bhat P, Sasak W, Silverman-Jones C, Akalonsky I, Frot-Coutez J, Fletcher R, Chader G: Recent developments in studies on biological function of vitamin A in normal and transformed tissues. Pure and App Chemistry 51:581-591, 1979.

Russell P, Wiggert B, Derr J, Albert D, Craft J, Chader G.J: Nuclear uptake of retinoids: Autoradiographic evidence and metabolic conversions in retinoblastoma cells in vitro. J Neurochem (in press).

DATE DUE